Induction, Repair and Biological Consequences of Clustered DNA Lesions

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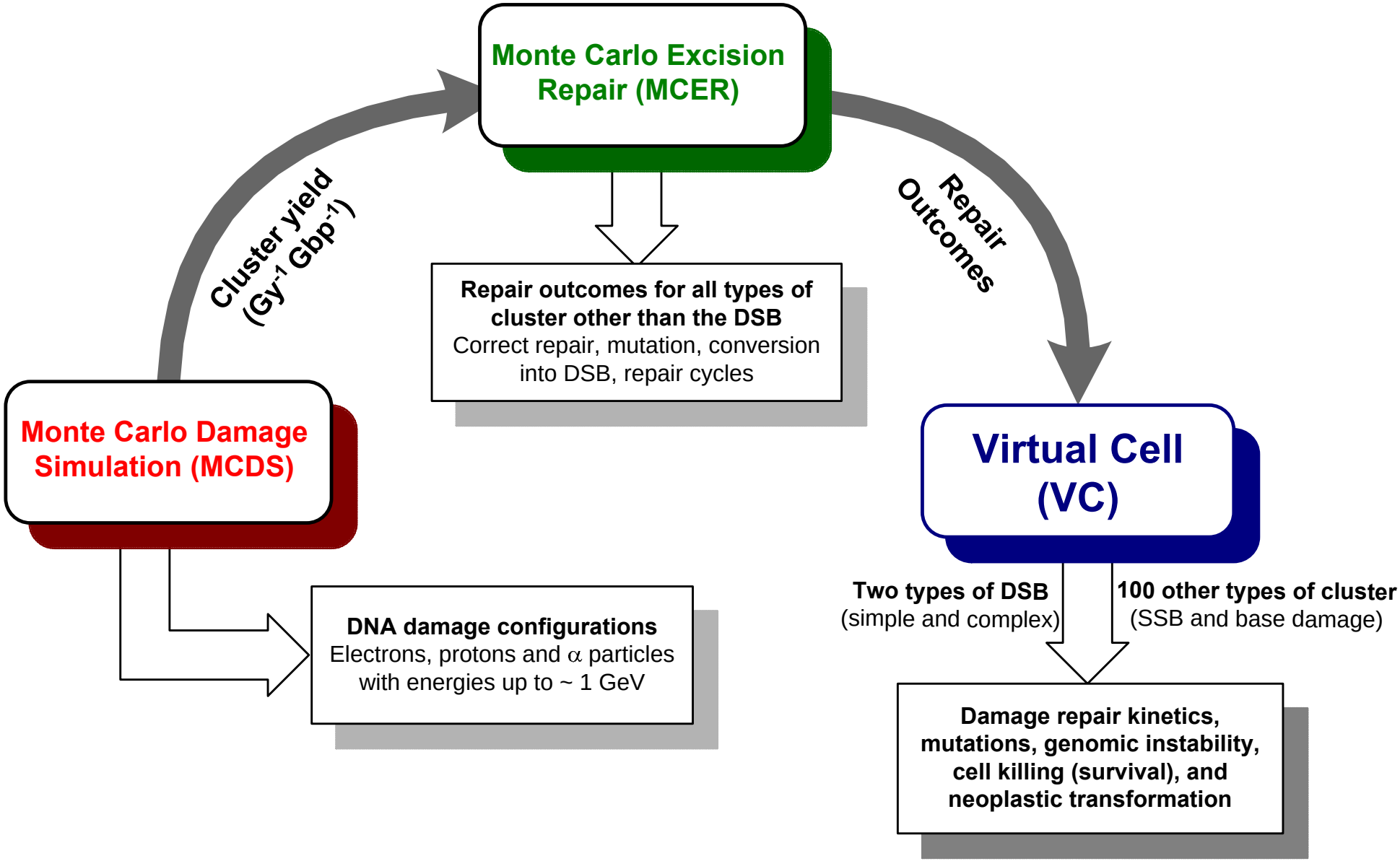
Continuing Education Lecture (CEL-8)
Thursday, June 29
7:00-8:00 a.m., Ballroom B

PURDUE
UNIVERSITY

DNA damage induction and repair

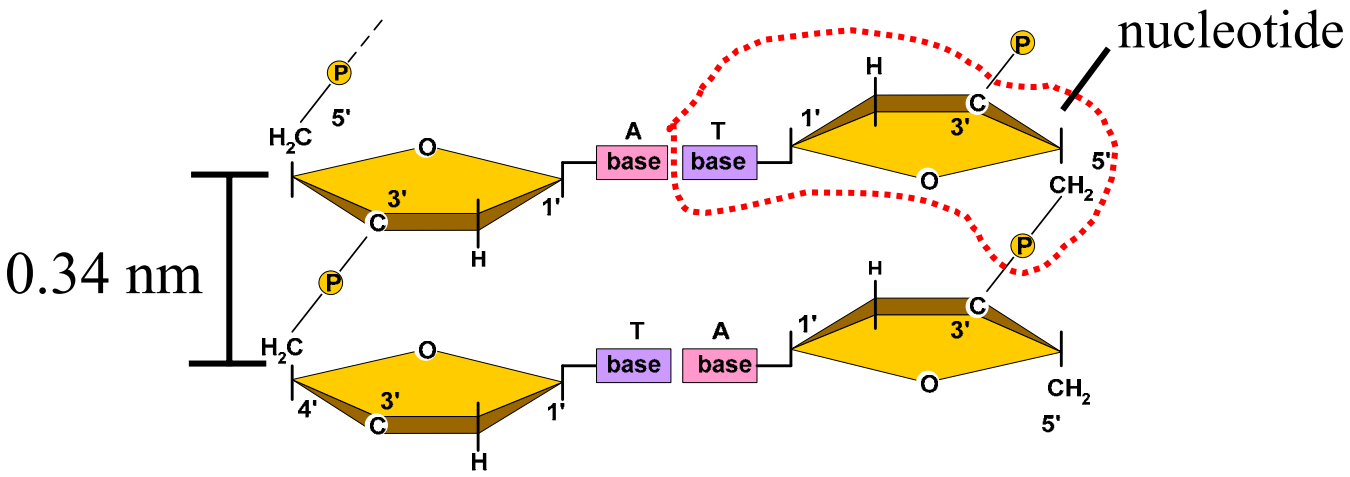
- Monte Carlo Damage Simulation (MCDS)
  - V.A. Semenenko and **R.D. Stewart**. A fast Monte Carlo algorithm to simulate the spectrum of DNA damages formed by ionizing radiation. *Radiat Res.* **161**(4), 451-457 (2004).
  - V.A. Semenenko and **R.D. Stewart**. Fast Monte Carlo simulation of DNA damage formed by electrons and light ions. *Phys. Med. Biol.* **51**(7), 1693-1706 (2006).
- Monte Carlo Excision Repair (MCER)
  - V.A. Semenenko, **R.D. Stewart**, E.J. Ackerman. Monte Carlo Simulation of Base and Nucleotide Excision Repair of Clustered DNA Damage Sites. I. Model Properties and Predicted Trends. *Radiat. Res.* **164**, 180-193 (2005).
  - V.A. Semenenko and **R.D. Stewart**. Monte Carlo Simulation of Base and Nucleotide Excision Repair of Clustered DNA Damage Sites. II. Comparisons of Model Predictions to Measured Data. *Radiat. Res.* **164**, 194-201 (2005).

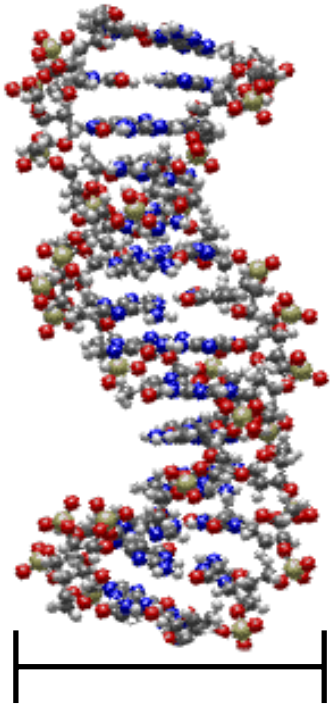
Multiscale Modeling of Biological Responses



Deoxyribonucleic acid (DNA)

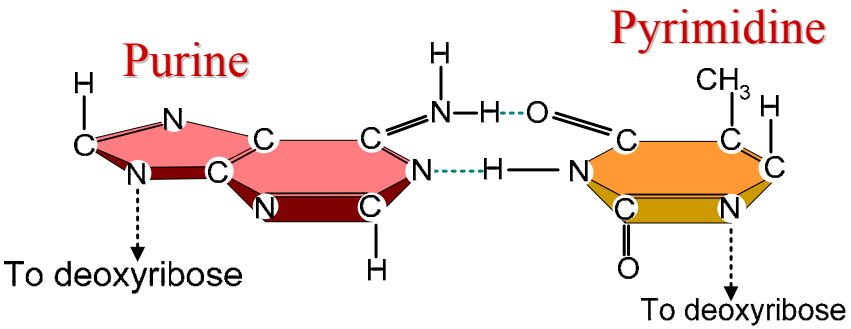
- Nucleotides consists of three parts:
  - Deoxyribose sugar
  - Phosphate group (acts as a bridge between adjacent deoxyribose sugars)
  - Nitrogen-containing pyrimidine or purine organic base





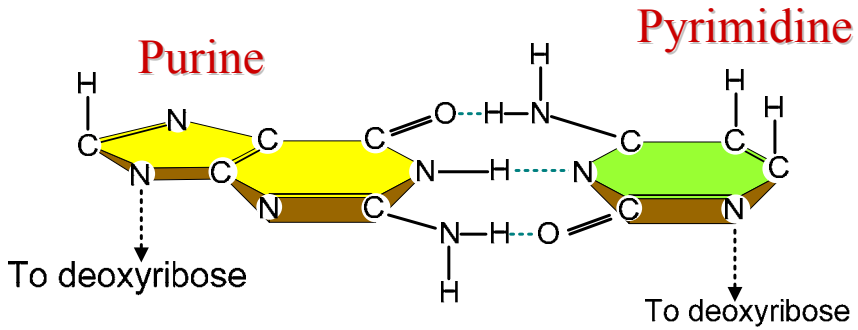
Depends on DNA conformation
(A, **B**, C, D, E, and Z)

Base pair (bp)



adenine thymine

AT base pair
(2 hydrogen bonds)



guanine cytosine

GC base pair
(3 hydrogen bonds)

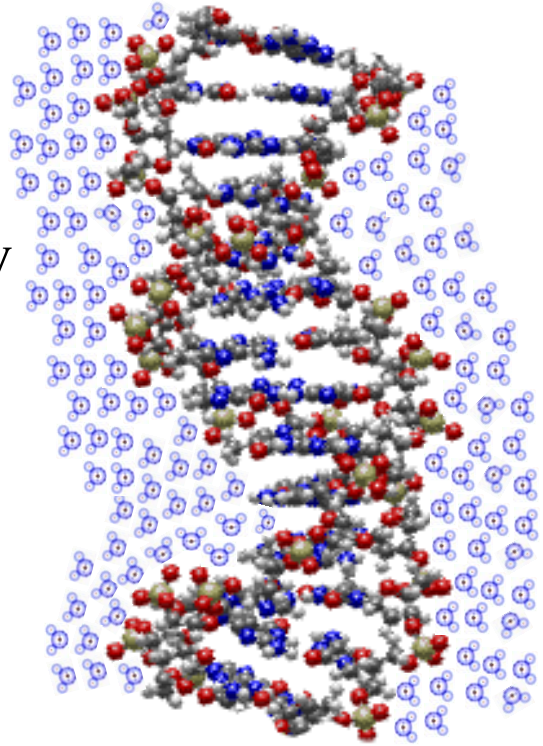
Hydrogen bond (0.087-0.22 eV per bond)

$H^{+} \cdots O^{-}$

Electron spends more time near oxygen, nitrogen or sulfur so that bases becomes polarized and attract each other

Stability of the DNA helix

- Hydrogen bond (~ 0.5 eV/bp)
- van der Waals forces (~ 0.05 eV/bp)
  - Rapid unequal sharing of electrons among covalently bonded atoms
- Hydrophobic interactions (~ 1 eV/bp)
  - Bases in the interior of the DNA helix exclude water and form a nonpolar environment



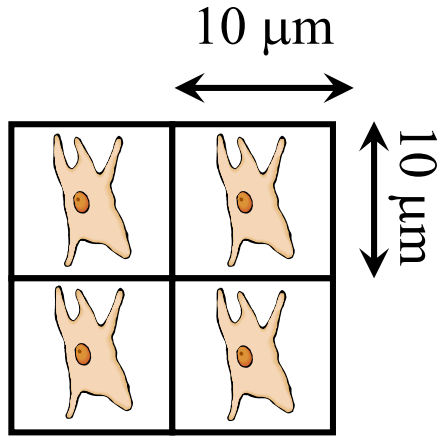
For comparison… elements with the lowest and highest ionization potential are cesium (3.89 eV) and helium (24.6 eV), respectively.

Below about 37º C, DNA segments at least 10 bp in length are stable. Stability tends to increase as length of segment increases. But above 50º to 60º C, DNA becomes destabilized and unwinds (denatures or melts).

DNA content, chromosome and cell size

- Diploid human cell contains 46 chromosomes and about 6 billion base pair (bp) of DNA (6 Gbp)
- Each chromosome is a single DNA molecule
  - Min. chromosome length = 24.65 Mbp = 8381.3 μm (= 0.33 in.)
  - Avg. chromosome length = 130.43 Mbp = 44,348 μm (= 1.75 in.)
  - Max. chromosome length = 310.21 Mbp = 105,471 μm (= 4.16 in.)

For comparison, a typical mammalian cell has a diameter ~ 10 μm. Cell nucleus is ~ 5 μm.



Mass ~ 1 ng/cell
Volume about 10^3 μm^3/cell

Maximum of 10^9 cell cm^{-3} ←

Tracks formed in water by 70 keV/μm ions

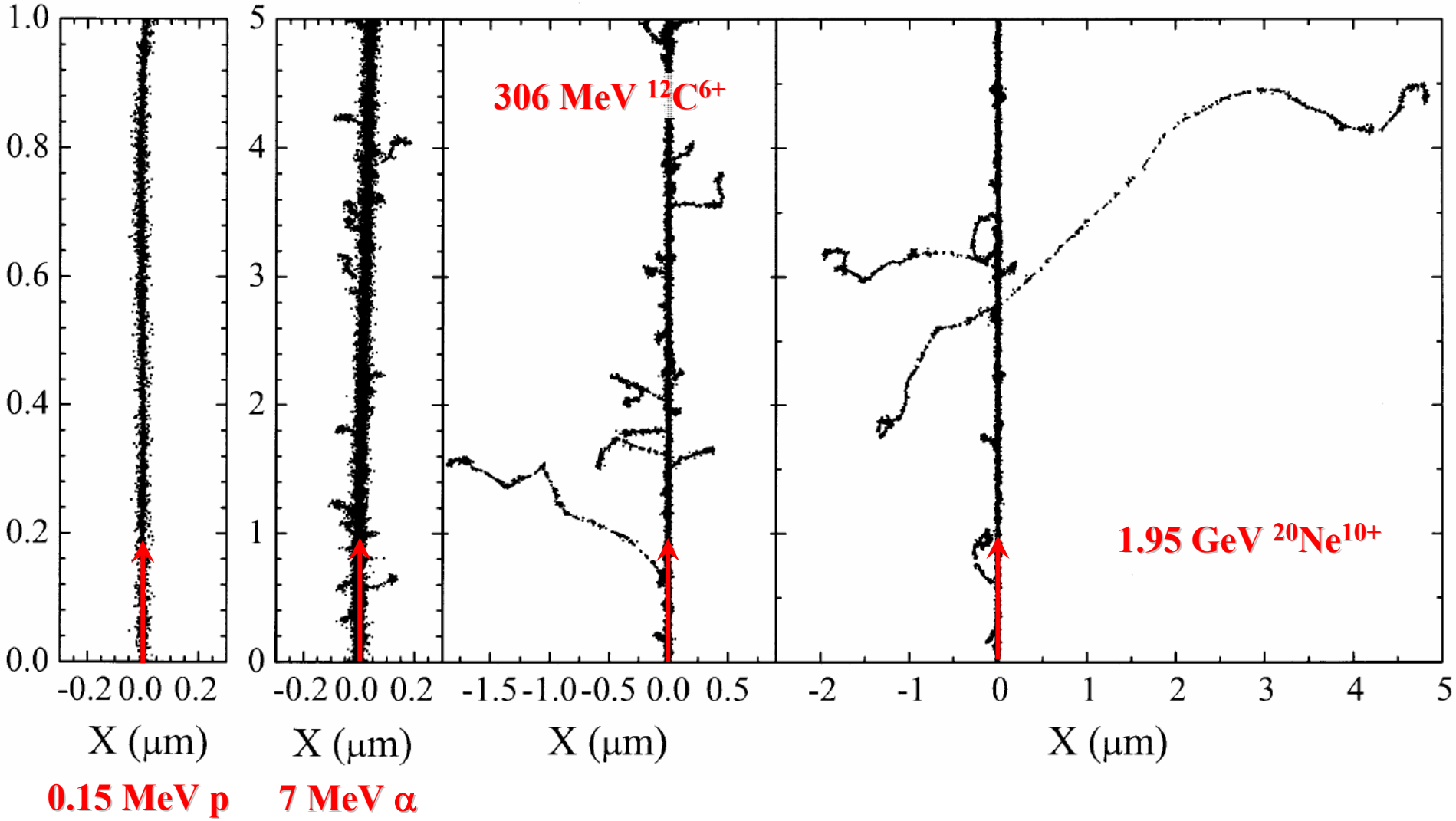
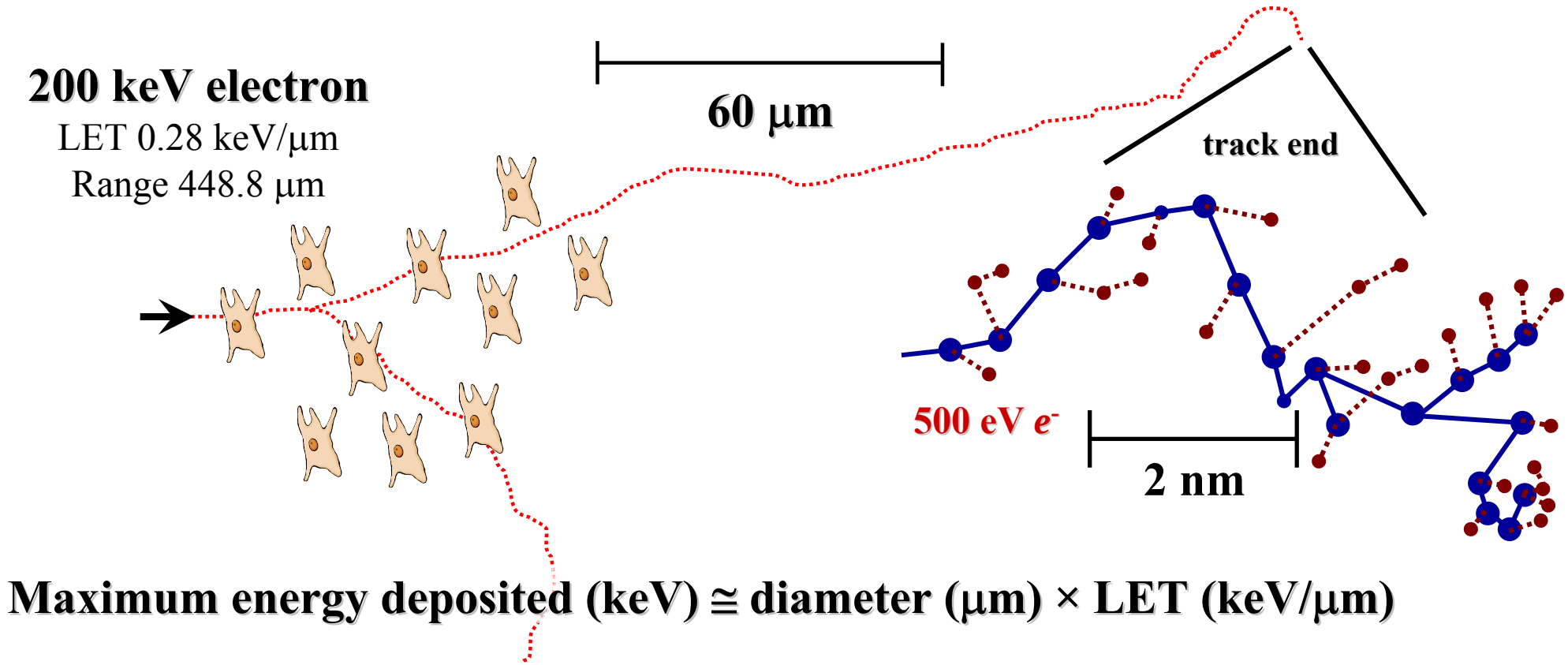


Image adapted from Muroya Y, Plante I, Azzam EI, Meesungnoen J, Katsumura Y, Jay-Gerin JP. High-LET ion radiolysis of water: visualization of the formation and evolution of ion tracks and relevance to the radiation-induced bystander effect. *Radiat Res.* **165**(4), 485-491 (2006).

Energy deposited per charged particle



Maximum energy deposited (keV) ≅ diameter (μm) × LET (keV/μm)

5 μm × 0.28 keV/μm = 1.4 keV (1400 eV)

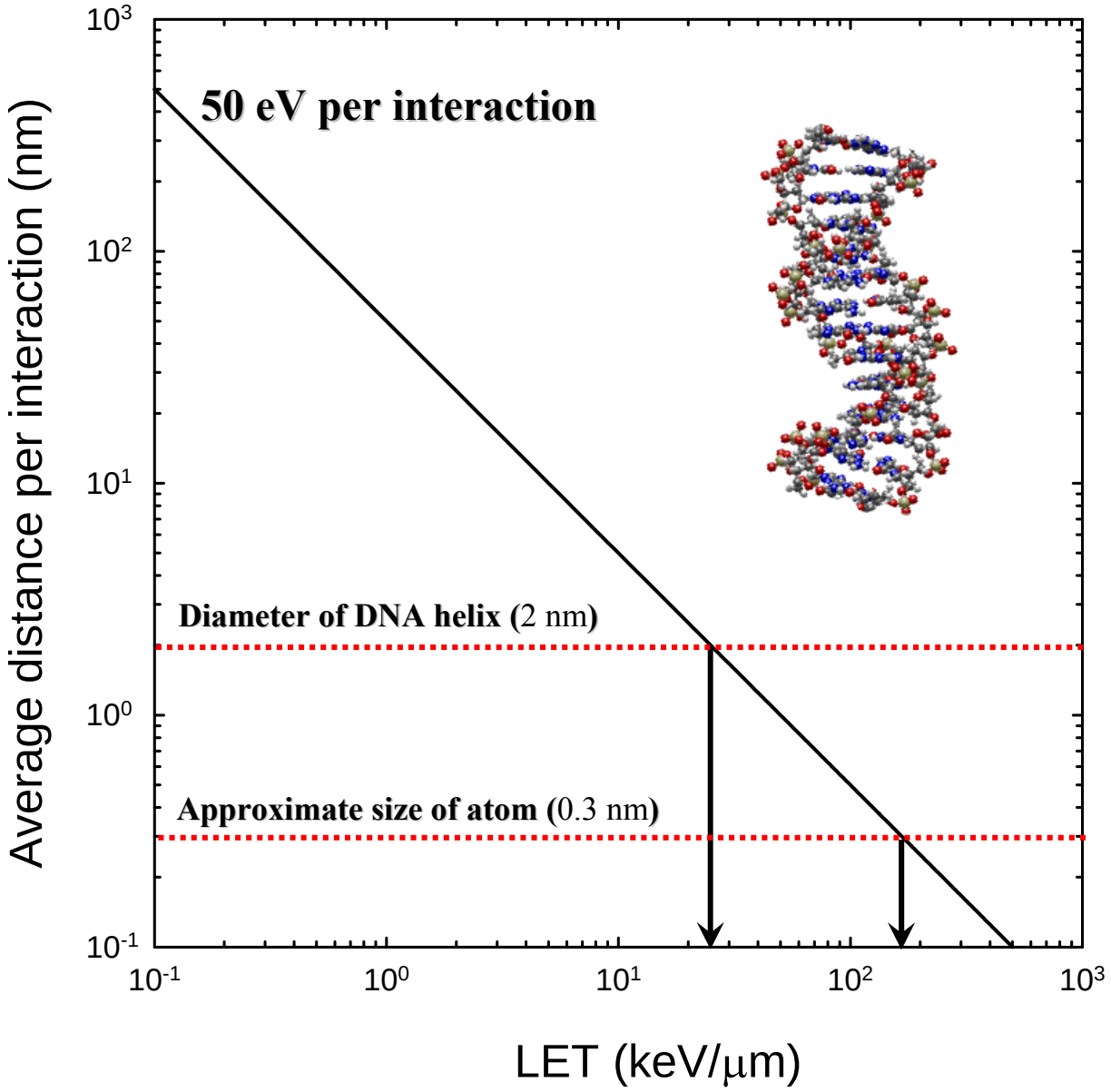
1400 eV × interaction/50 eV = 28 elastic or inelastic interactions

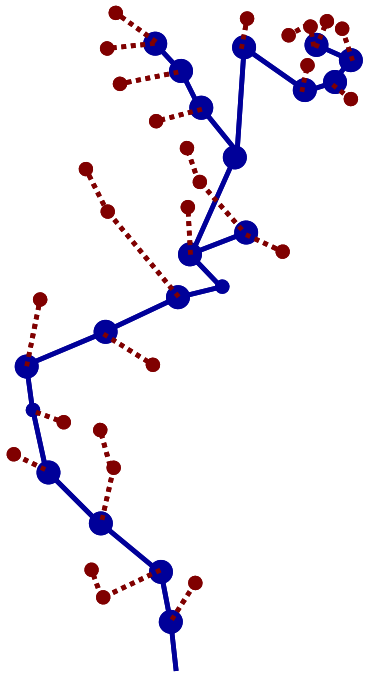
Distance per 1 interaction = 5 μm ÷ 28 collisions = 0.176 μm

= 176 nm ⟶ **Large compared to diameter of DNA helix (~ 2 nm)**

For energetic electrons in water, average energy transfer per interaction ranges from about 44 to 78 eV (36-60 eV inelastic and 13-13.5 excitation)

Distance between interactions

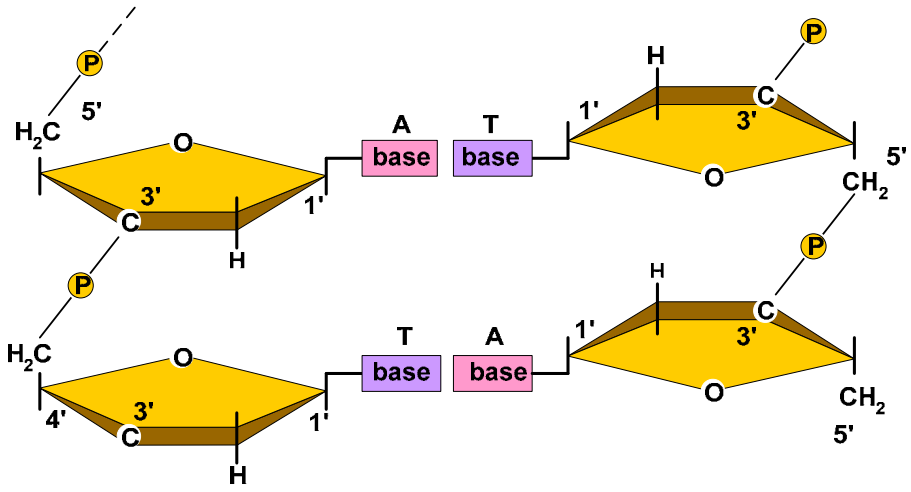




Direct effects of ionizing radiation

	% Volume of Nucleus	Mass (pg)	MeV per Gy	Number 50 eV events
cell	-	1,000.00	6.2422	124,843.9
nucleus	100.00	125.00	0.7803	15,605.5
human DNA	4.90	6.13	0.0382	764.8
base (A, T, G or C)	2.06	2.58	0.0161	321.8
phosphate (PO_4)	1.51	1.89	0.0118	236.3
deoxyribose sugar	1.32	1.66	0.0103	206.8
sugar+phosphate	2.84	3.55	0.0222	443.0

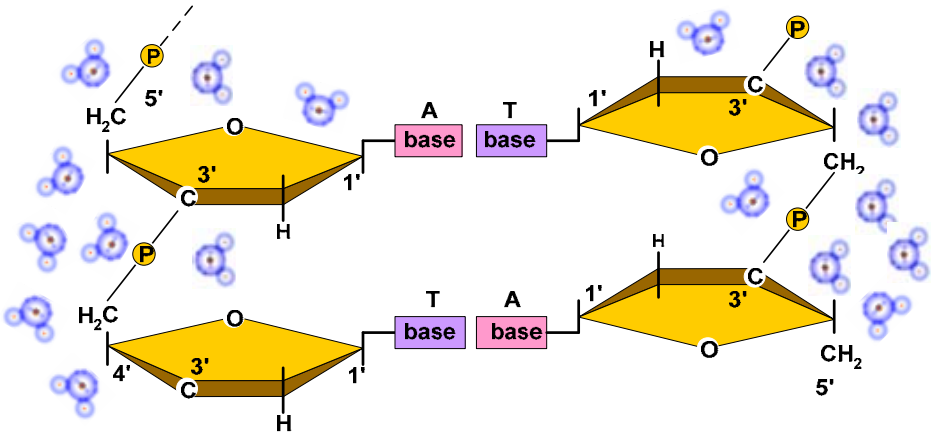
energy deposited ∝ mass



Indirect Effect

- Reactive chemical species diffuse and interact with the DNA
  - Cell and cell nucleus is about 70% water
  - Primary hydration layer contains about ~ 20 water molecules per base pair of which 12-15 are tightly bound to DNA ("**quasi-direct effect**")
  - Hydroxyl radical (·OH) is believed to be the main culprit

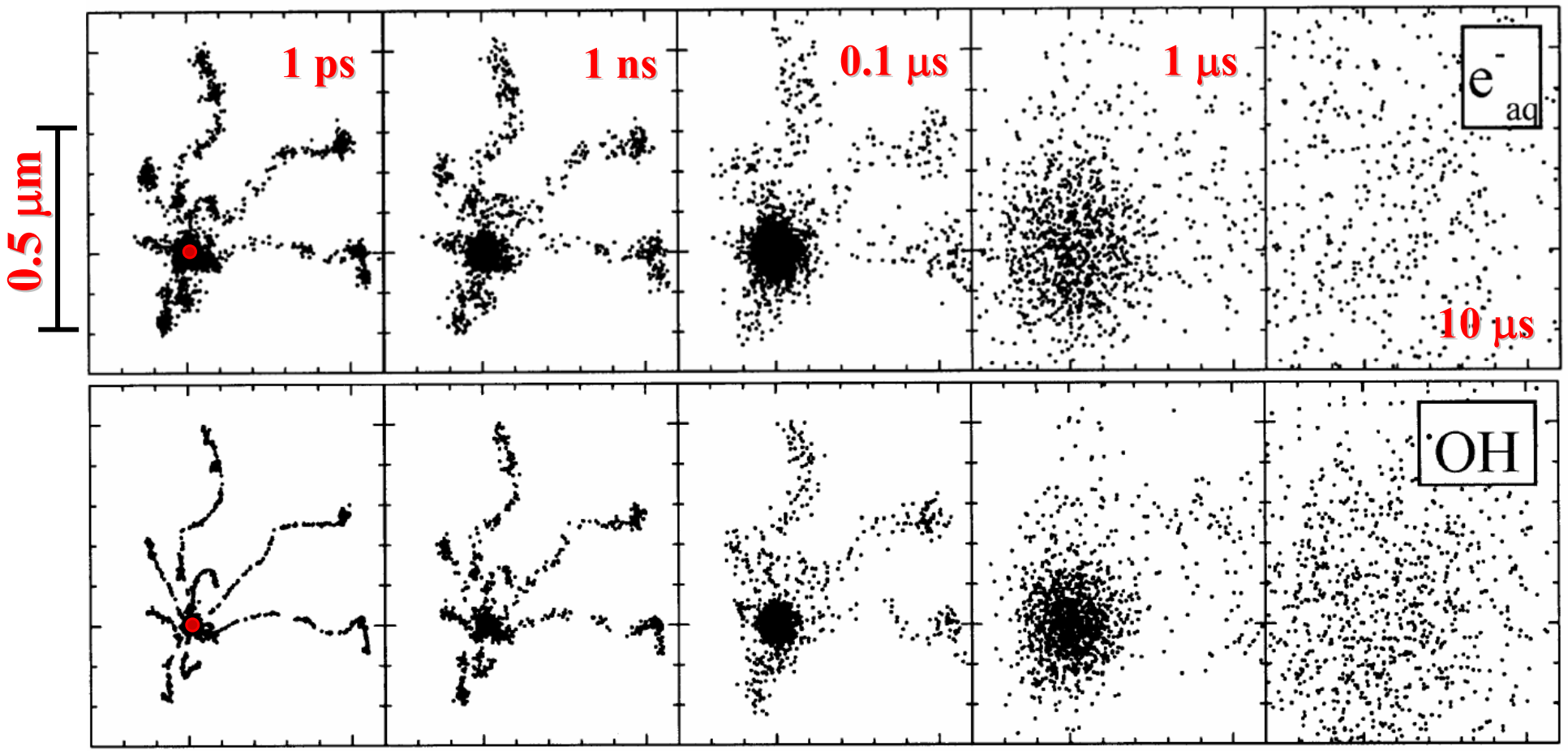
$$H_2O \rightarrow H_2O^+ + e^- \qquad H_2O^+ + H_2O \rightarrow \cdot OH + H_3O^+$$



Disproportionate number of H_2O near phosphate group

For more on the radiation chemistry of water, see J.W.T. Spinks and R.J. Woods, Introduction to Radiation Chemistry, 3rd edition John Wiley & Sons, Inc. New York, NY 1990.

Radical diffusion

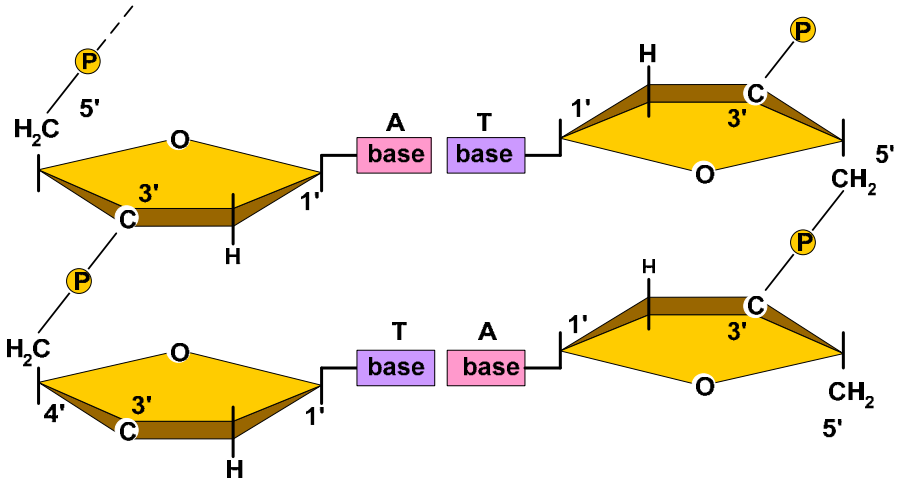


Spatial distributions of ·OH and e^-_{aq} in liquid water. Red dot indicates location of a 1 µm segment of a 24 MeV $^4He^{2+}$ ion (26 keV/µm) directed into the image

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).

Damage from hydroxyl radicals (·OH)

·OH readily interacts with hydrogen (hydrogen abstraction) bound to the 3′, 4′, and 5′ carbons of deoxyribose sugar. Some evidence for preferential attack on 4′ C.



In a cellular milieu, estimated ·OH radical diffusion distance is about 6-9 nm (Roots and Okada 1975)

Radicals formed in cytoplasm (*outside nucleus*) cannot easily reach the DNA*

·OH also readily interacts with purine (A and G) and pyrimidine (T and C) bases

*** Caveat: ·OH + ·OH → H_2O_2 (hydrogen peroxide). H_2O_2 can diffuse much greater distances and may be able reach the DNA and interact with a DNA-bound metal to an form ·OH radical (Fenton reaction) in close proximity to the DNA.**

Roots R and Okada S. Estimation of Life Times and Diffusion Distances of Radicals Involved in X-ray Induced DNA Strand Breaks or Killing of Mammalian Cells. *Radiat. Res.* **64**, 306-320 (1975).

Direct and indirect damage

	% Volume of Nucleus	Mass (pg)	MeV per Gy	Number 50 eV events
cell	-	1,000.00	6.2422	124,843.9
nucleus	100.00	125.00	0.7803	15,605.5
human DNA	4.90	6.13	0.0382	764.8
base (A, T, G or C)	2.06	2.58	0.0161	321.8
phosphate (PO4)	1.51	1.89	0.0118	236.3
deoxyribose sugar	1.32	1.66	0.0103	206.8
sugar+phosphate	2.84	3.55	0.0222	443.0
water	70.00	87.50	0.5462	10,923.8

Direct vs. Indirect

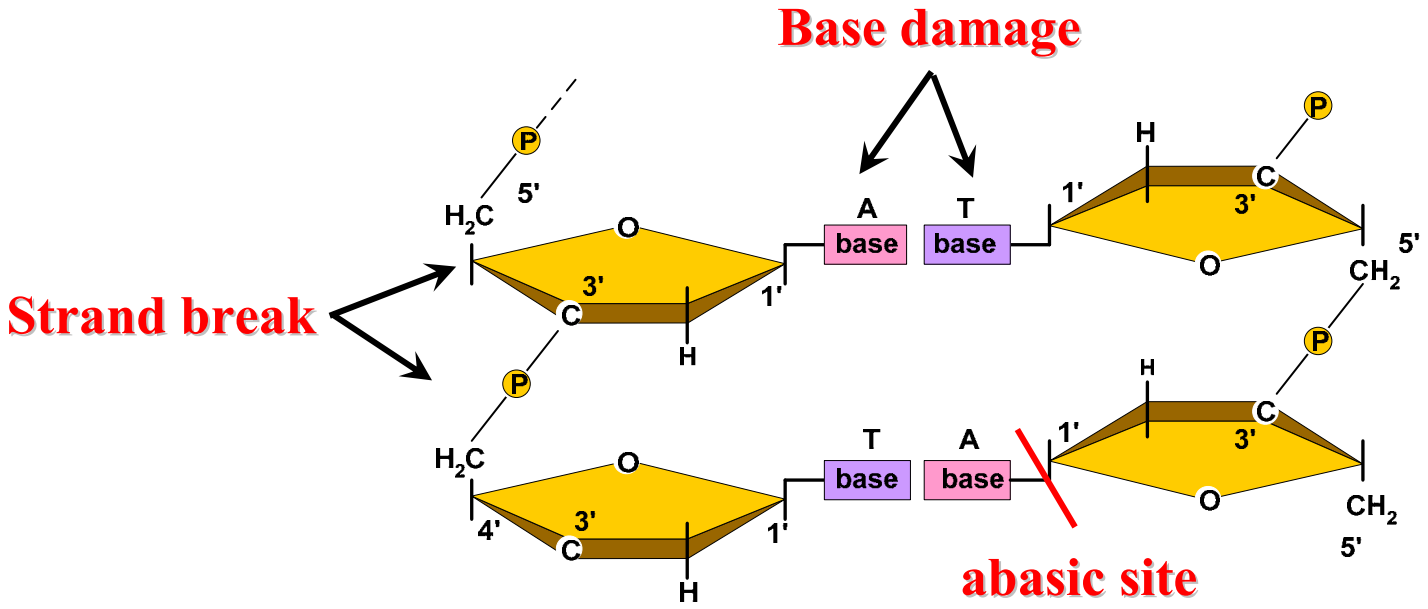
% indirect $\leq$ 93.46%

% direct $\geq$ 6.54%

A maximum of about 11,700 nucleotides out of 10^{10} may be damaged by a 1 Gy dose of radiation (1 in 10^6)

Lesions formed by radiation

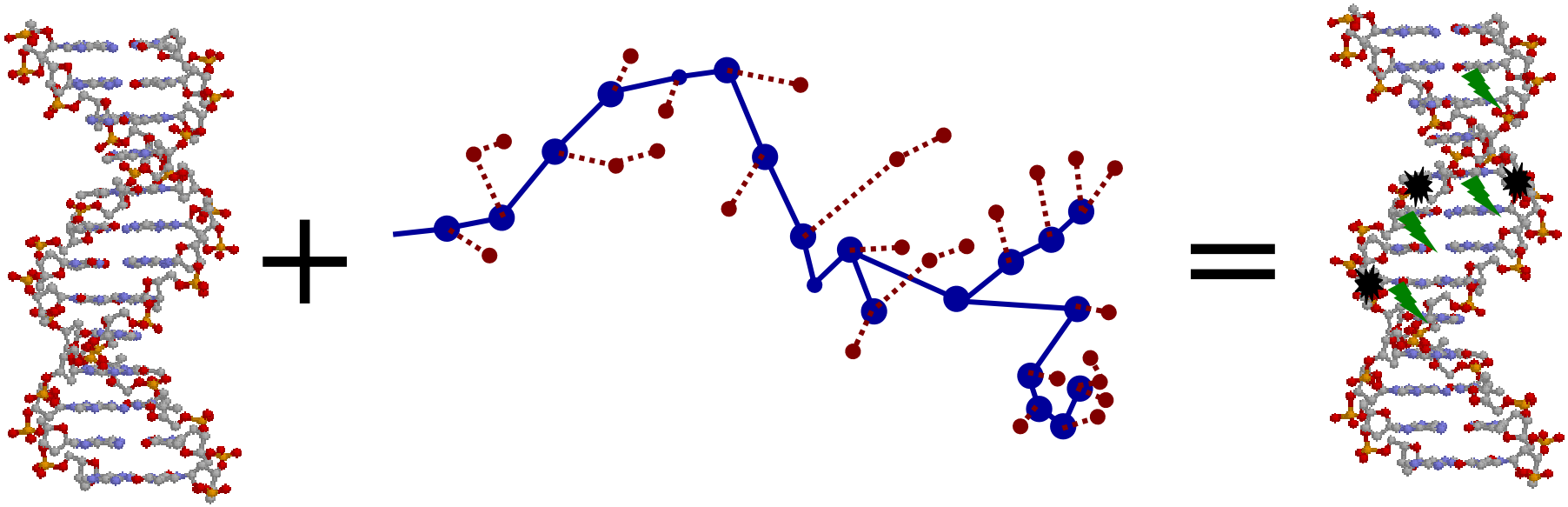
- Damage to a single nucleotide is often (but not always) referred to as a *lesion*
- Types of lesions (damaged nucleotides) include
  - Abasic or AP (apurinic/apyrimidinic) sites = base loss
  - Base damage (A, T, G or C)
  - Strand breaks (damage to sugar or phosphate), usually accompanied by base loss



Strand breaks and base damage are formed through the direct and indirect mechanisms

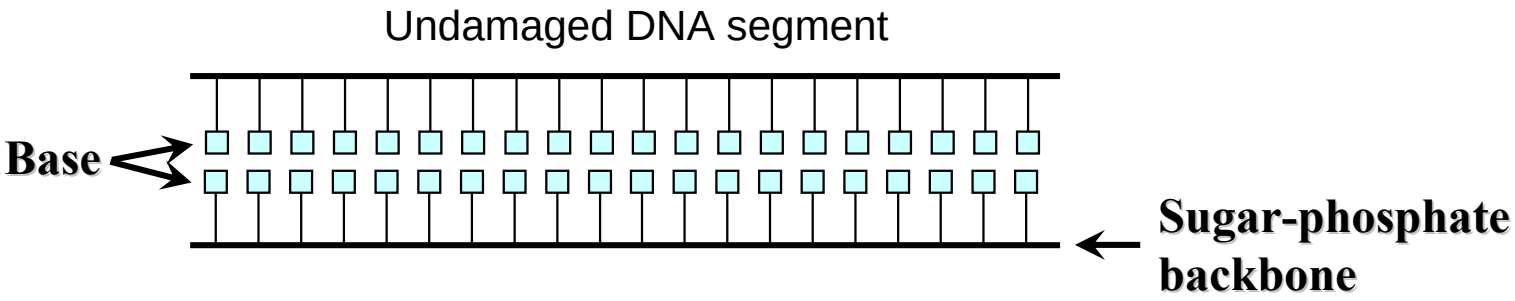
Lesion clustering

One of the more unique characteristics of ionizing radiation is its ability to produce several lesions within one or two turns of the DNA, i.e., *clustered lesions**

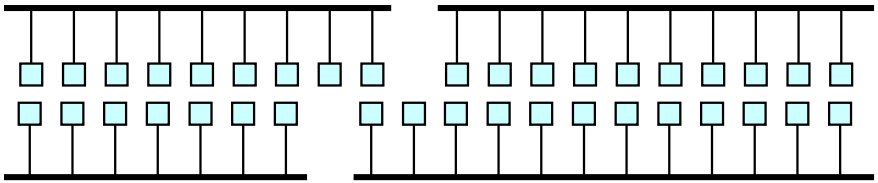


* "Clustered lesions" are also referred to as *locally multiply damaged sites* (LMDS) and *multiply damaged sites* (MDS)

Double strand break (DSB)



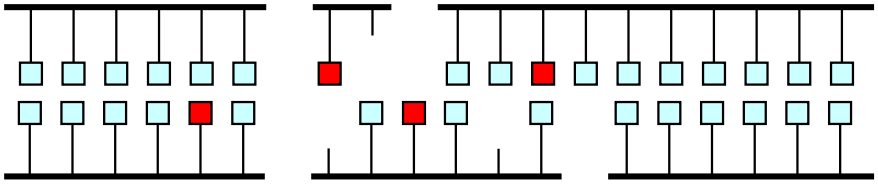
A DSB is a cluster that contains at least two strand breaks on opposing strands within ~ 10 bp of each other



Simple DSB (2 lesions)

Strand breaks formed by radiation are chemically reactive ("sticky")

Number of lesions forming a cluster is a measure of "complexity"

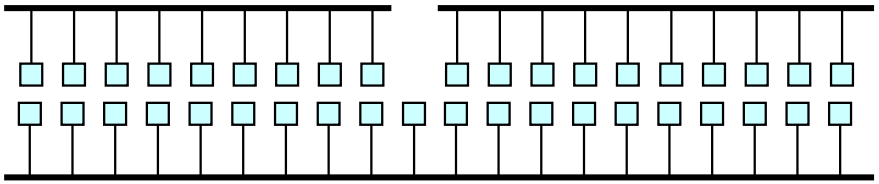


Complex DSB (11 lesions)

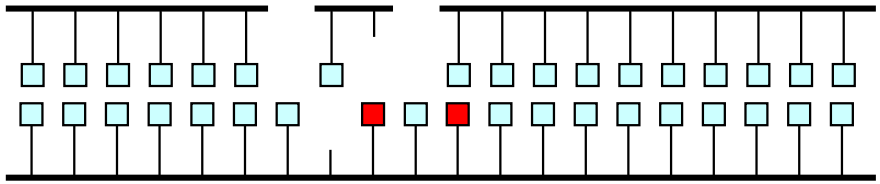
Red square (■) denotes base damage

Single strand breaks and base damage

SSB (single strand break) denotes the *family* of all types of cluster other than the DSB that contain *at least* one strand break

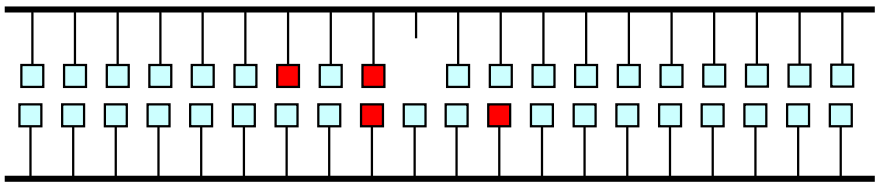


Simple SSB (1 lesion)



Complex SSB (6 lesions)

Clusters that do not contain any strand breaks are referred to as “base damage”



Base damage (5 lesions)

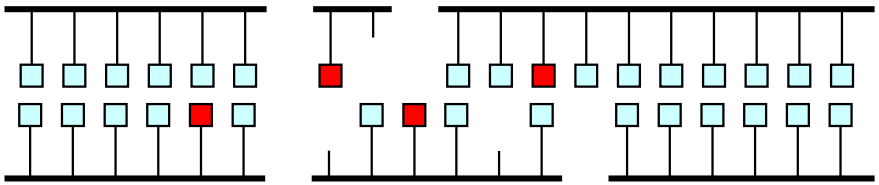
Damage yield ∝ absorbed dose

Damage yield per cell is proportional to dose up to at least a few hundreds or thousands of Gy

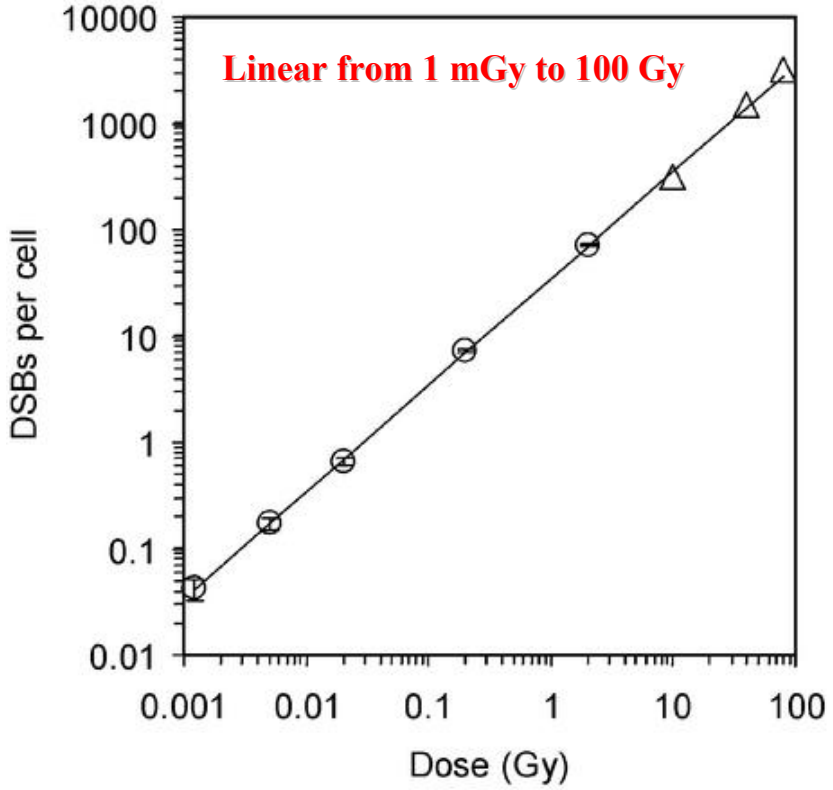
- **Low and high LET radiation**
- **All types of damage (SSB, DSB, …)**

↓ implies

All types of damage, including DSB, are formed through *one-track* mechanisms



All of these lesions were formed by the *same* radiation track

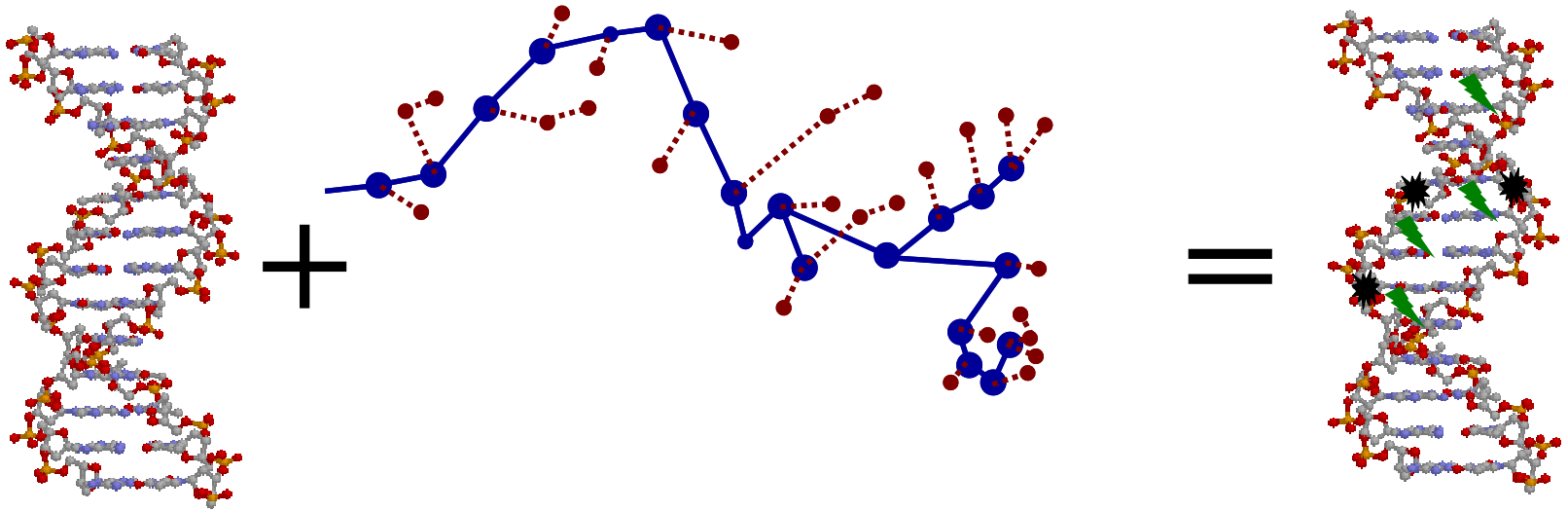


DSB induction in non-dividing primary human fibroblasts (MRC-5) irradiated by 90 kVp x-rays (Rothkamm and Lobrich 2003)

Rothkamm K, Lobrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci* USA. **100**(9), 5057-5062 (2003).

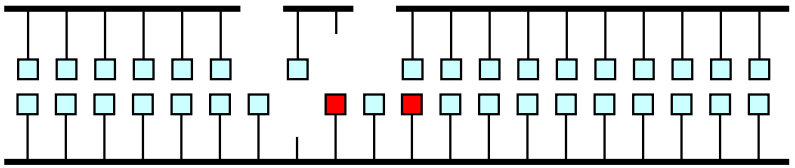
Track structure simulations

- Analog Monte Carlo simulation of primary and secondary elastic and inelastic collisions down to ~ 1 to 10 eV
- Explicit simulation of direct and indirect damage mechanisms
  - Location of energy deposits superimposed on higher-order models of DNA in an aqueous environment



Track structure simulations (pros)

- Predict from first principles *nucleotide-level maps* of cluster configurations
  - Experimental assays provide very limited information about the local complexity of clusters (overall SSB and DSB yield)



Required for DNA repair simulations

- Effects of particle LET on cluster yield and complexity related to track structure (physics)
  - Relative biological effectiveness (RBE)
- Provide approximate agreement with measured SSB and DSB

Track structure simulations (cons)

- Computational requirements are substantial
  - Model spans physical and chemical processes occurring across an enormous time scale (ps to μs) and large spatial scales (nm to cm)
- Highly simplified model of a very complex cellular environment
  - Few if any cell-specific features
  - Ultimately relies on at least 2-4 adjustable parameters that must be determined from measured data (parameters are presumably independent of LET)
- Models (*still*) not fully validated
  - Differences between measurements and calculations not fully resolved even for the most widely studied type of damage (i.e., the DSB)
  - Are nucleotide level maps of damage correct?

Opportunities for future research! Funding?

Monte Carlo Damage Simulation (MCDS)

Radical departure from the analog Monte Carlo approach.
Three step procedure used to model cluster formation:

1. Determine number lesions formed in the DNA
2. Place lesions within the DNA (group them into clusters)
3. Classify clusters according to properties and complexity

Step 3 is not specific to the MCDS algorithm

Three adjustable parameters **determine the induction and clustering of damage.** ***Two parameters are independent of LET*** **(Step 1). One parameter varies with particle type and energy (Step 2)**

Step 2 is the "radical departure" from track structure simulations

http://rh.healthsciences.purdue.edu/mcds/

Strand break and base damage induction

- Cluster yield is proportional to absorbed dose
  - Negligible chance more than one track will deposit energy "close" to a small section of DNA (~ 10 to 20 bp)
  - After 1 Gy, fewer than 1 in 10^6 nucleotides are damaged
- Lesion induction $\propto$ energy deposited in nucleus
  - Because (energy deposited) = (mass) × (absorbed dose), lesion induction is also proportional to absorbed dose

Strand break: $\sigma_{Sb} = 1300\ \text{Gy}^{-1}\ \text{cell}^{-1}\ \ (216.67\ \text{Gy}^{-1}\ \text{Gbp}^{-1})$

Base damage: $\sigma_{Bd} = f\sigma_{Sb} = 3900\ \text{Gy}^{-1}\ \text{cell}^{-1}\ \ (650\ \text{Gy}^{-1}\ \text{Gbp}^{-1})$

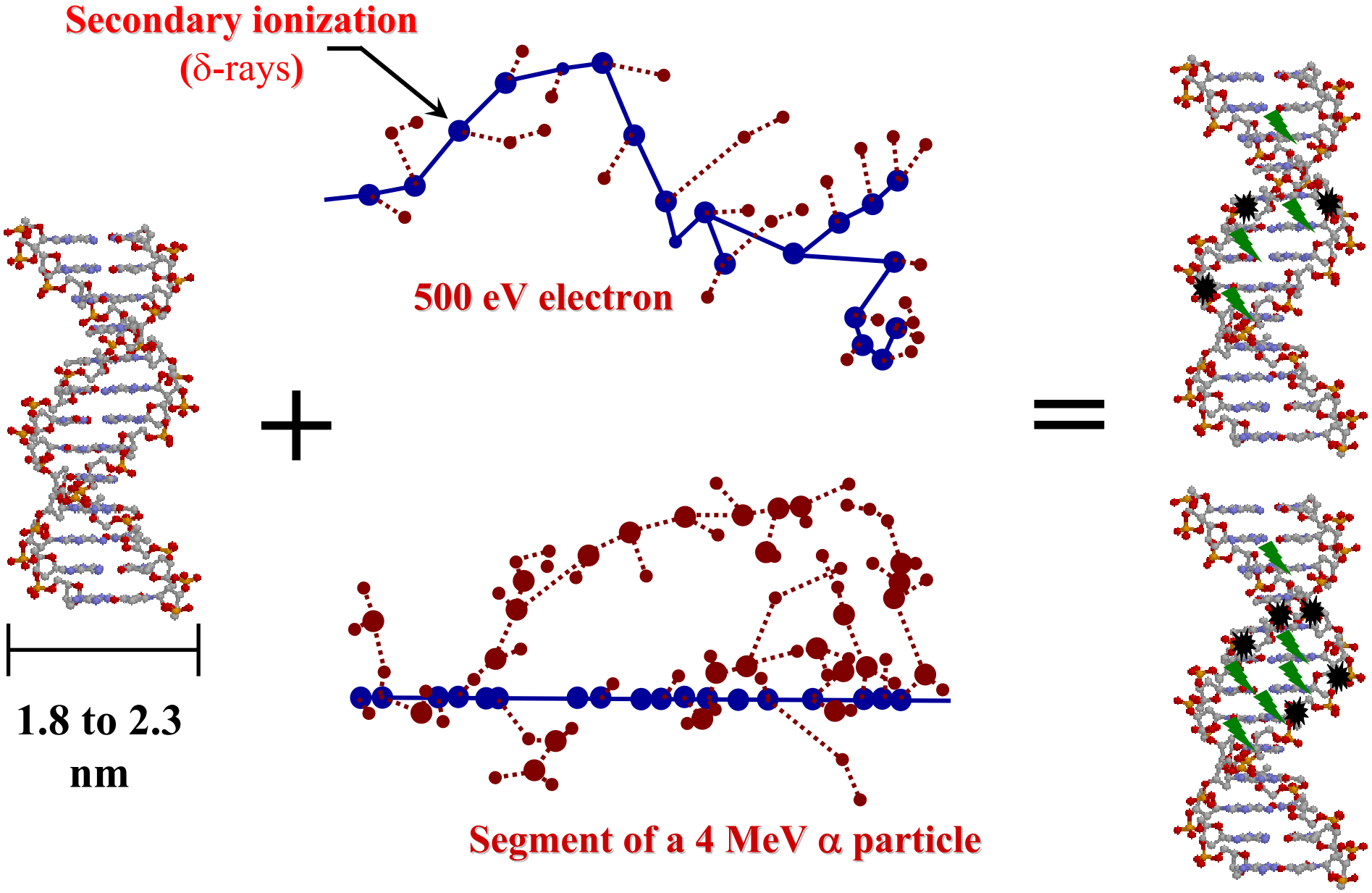
→ **3 damaged bases per strand break (*f* ~ 2-4)**

Total lesions: $\sigma = \sigma_{Bd} + \sigma_{Sb} = (1+f)\sigma_{Sb}$

$= 5,200\ \text{Gy}^{-1}\ \text{cell}^{-1}\ (867\ \text{Gy}^{-1}\ \text{Gbp}^{-1})$

Compare to rough estimate of less than 11,700 Gy^{-1} cell^{-1} (764 Gy^{-1} cell^{-1} direct).

LET effects (*clustering @ DNA level*)



LET effects (*clustering @ cellular level*)

- Lesion induction is the same for low and high LET radiation
  - (number of lesions) $\propto$ (energy deposited) $\propto$ (mass of nucleus) $\times$ (dose)

$$\sigma_{\text{low LET}} = \sigma_{\text{high LET}} = 5200\ \text{Gy}^{-1}\ \text{cell}^{-1}$$

- Number of lesions per unit fluence (particle traversal) is larger for high LET radiation than for low LET radiation

$$D \propto \frac{LET}{\rho}\Phi \qquad \text{lesion per unit fluence} = \sigma\frac{D}{\Phi} \propto LET$$

ρ = density (g cm^{-3}) **Φ = fluence (cm^{-2})**

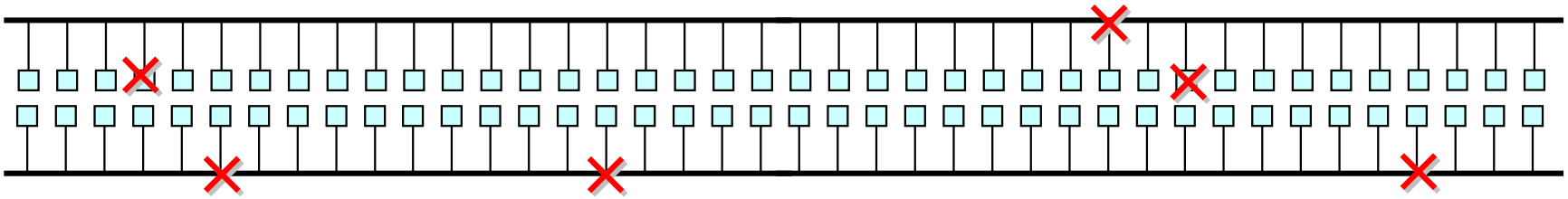
LET = unrestricted linear energy transfer (keV μm)

As particle LET increases, same number of lesions are distributed among fewer cells (constant absorbed dose**)**

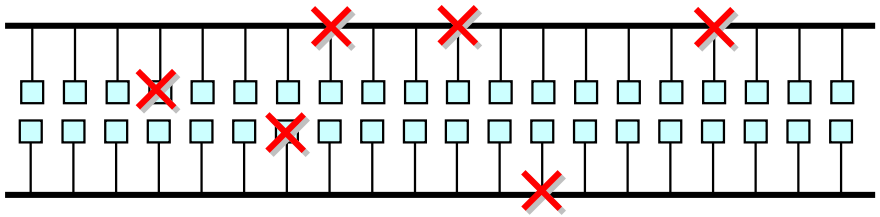
MCDS clustering algorithm

Number of lesions: $N_{Sb} = D\sigma_{\mathrm{Sb}} = 1300, \quad N_{Bd} = Df\,\sigma_{\mathrm{Sb}} = 3900$

Insert lesions into a segment of DNA at random (no overlap)



Increase clustering by *decreasing* the DNA segment length



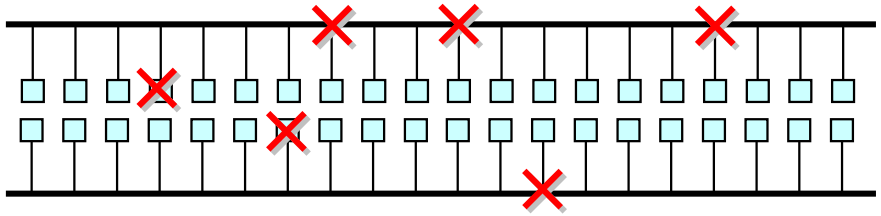
DNA segment length (n_{seg}) treated as a purely *ad hoc* adjustable parameter that depends on particle type and energy

Effects of particle type and energy on n_{seg}

Length of DNA segment (n_{seg}) decreases as Z_{eff}/β^2 (LET) increases

$$n_{seg}(x) = 149,200 - \frac{123,600x}{x+267} \text{ bp Gy}^{-1}, \quad 1 \le x \equiv Z_{eff}^2/\beta^2 \le 3200$$

large KE (~ GeV) and low LET ← → small KE and high LET



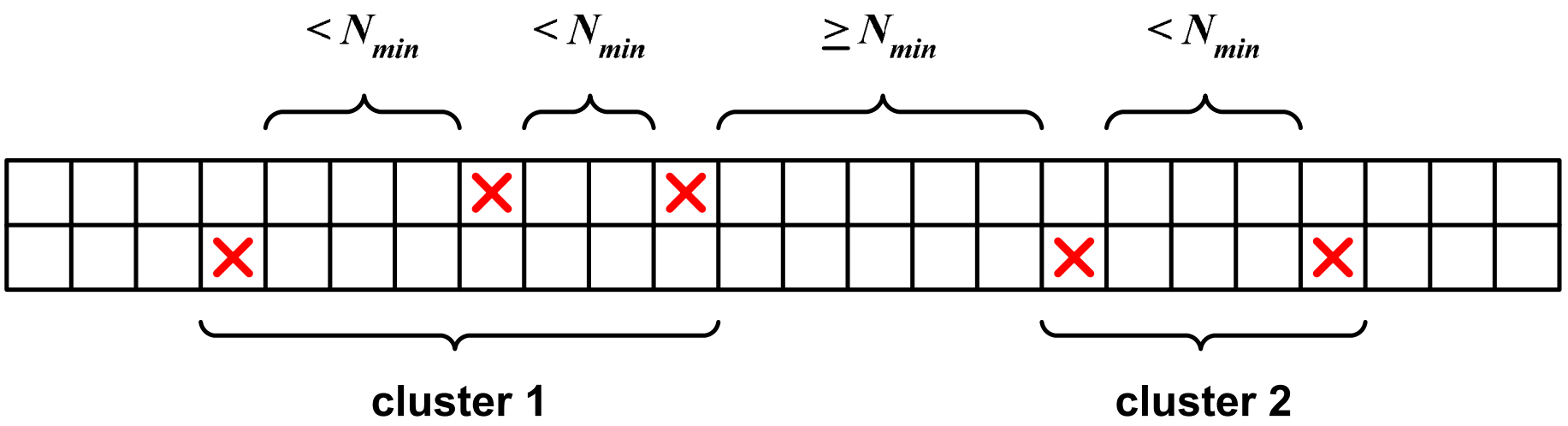
Clustering increases as n_{seg} decreases

Low LET n_{seg} ($x = 1$): 148.74 kbp (28.6 bp per lesion)

High LET n_{seg} ($x = 3200$): 35.12 kbp (6.75 bp per lesion)

NOTE: n_{seg} is much smaller than average size of chromosome (130,430 kbp)

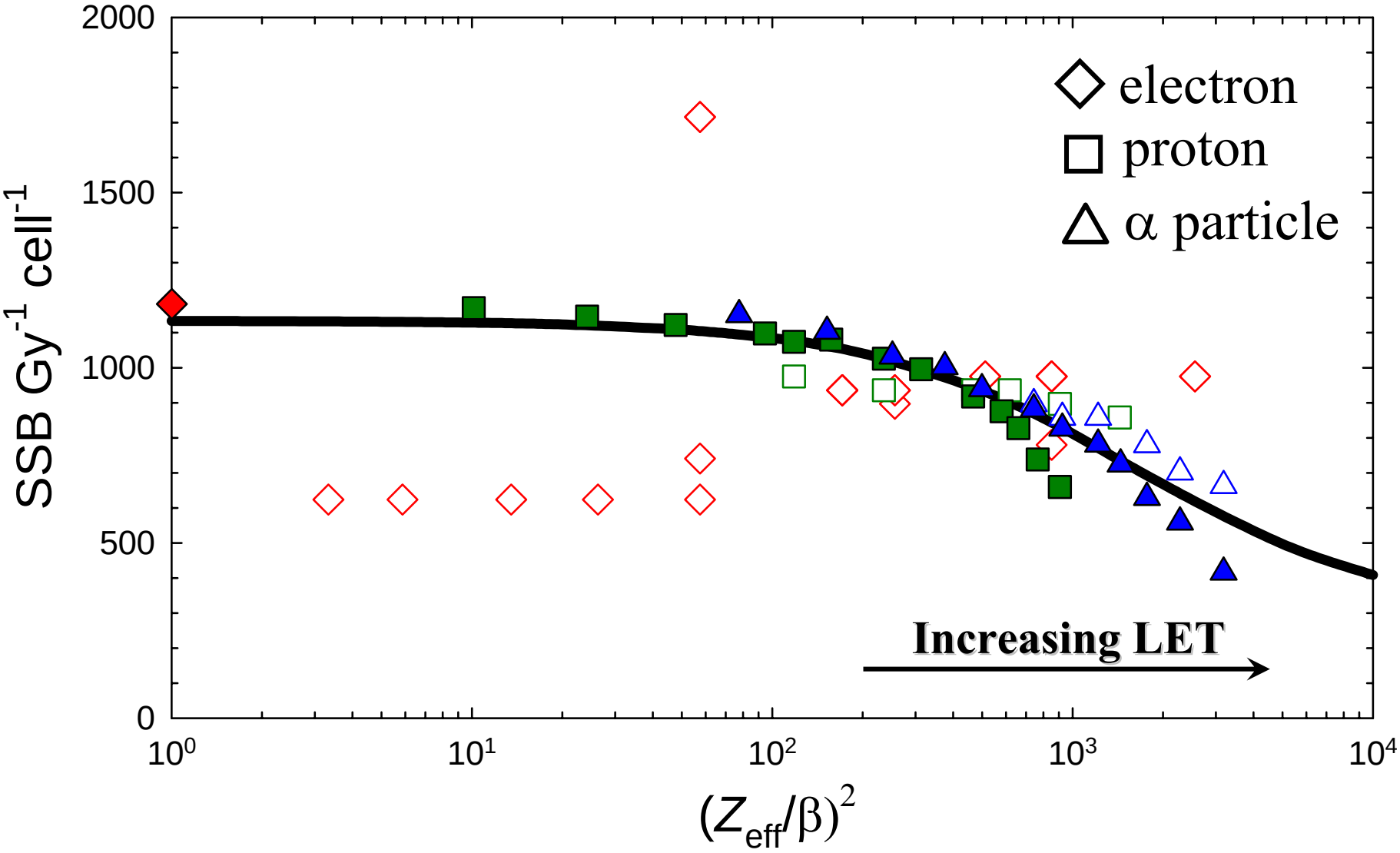
Cluster classification



Scan along DNA segment and group all lesions within $N_{min} = 9$ bp of each other into a cluster.

Analyze properties of cluster (length and number of lesions). Classify as SSB, DSB or base damage

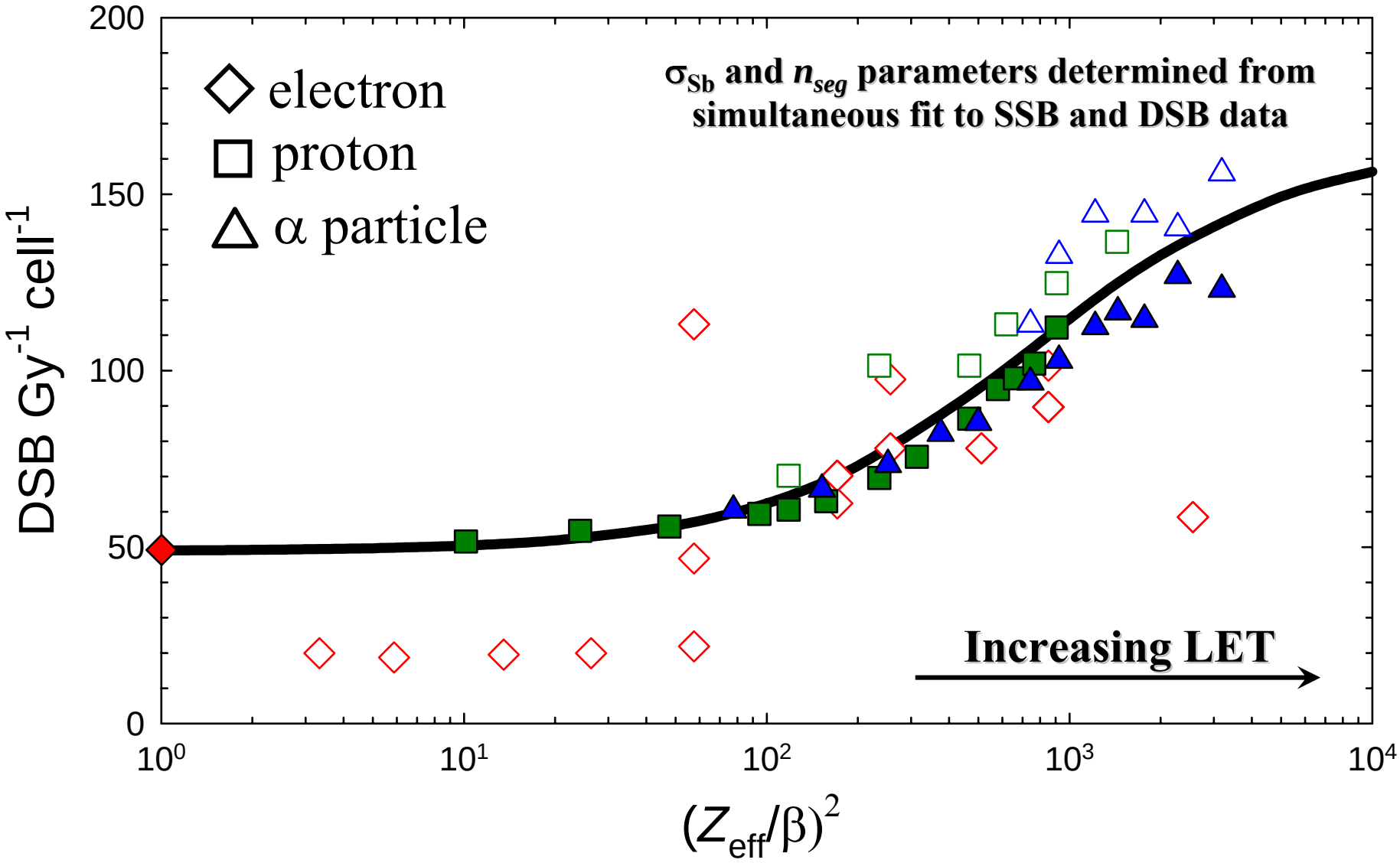
SSB yield (MCDS vs. track structure)



Filled symbols: Friedland *et al* 2003, 2005

Open symbols: Nikjoo *et al.* 1994, 1997, 1999, 2001, 2002

DSB yield (MCDS vs. track structure)

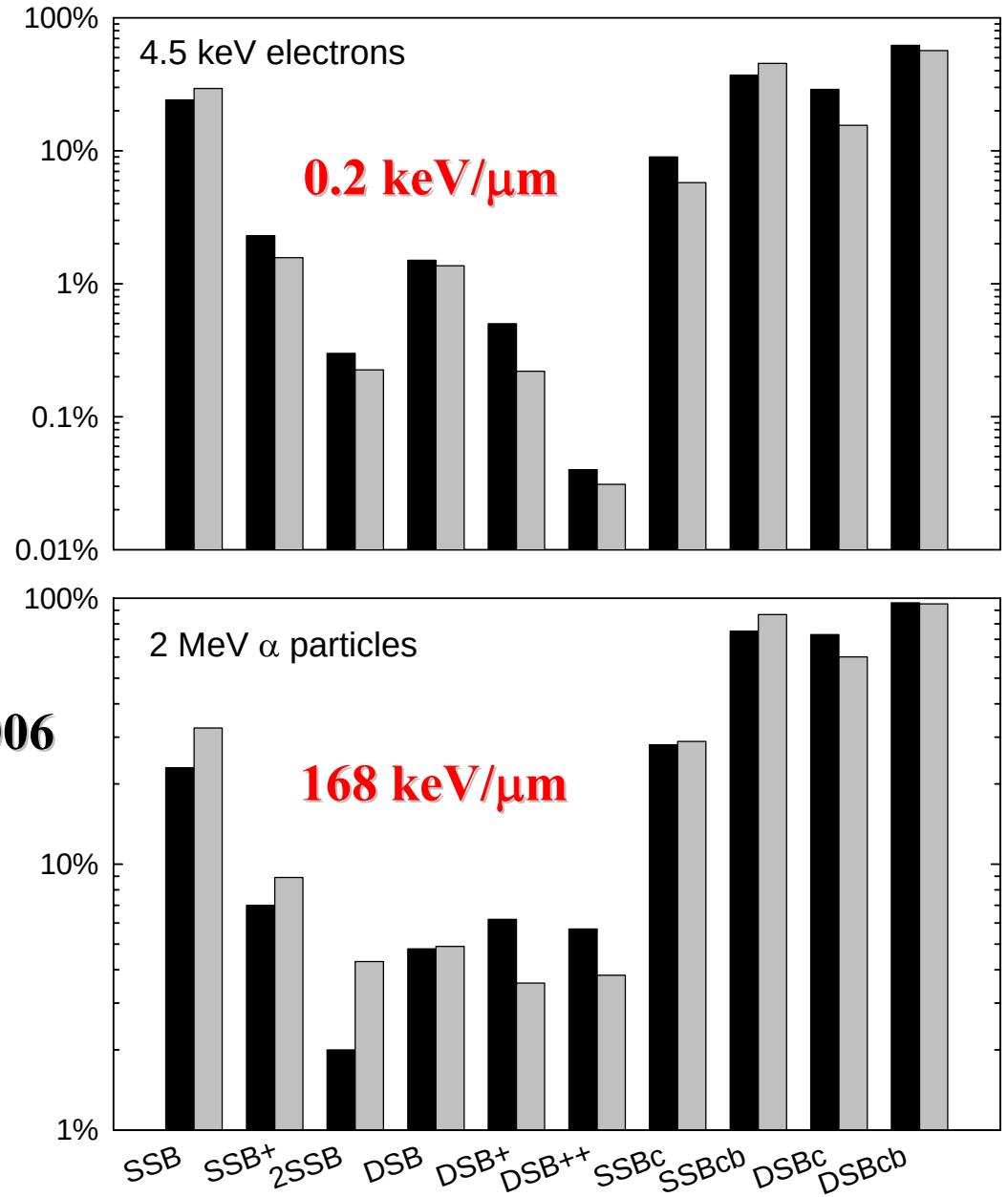


Filled symbols: Friedland *et al* 2003, 2005

Open symbols: Nikjoo *et al.* 1994, 1997, 1999, 2001, 2002

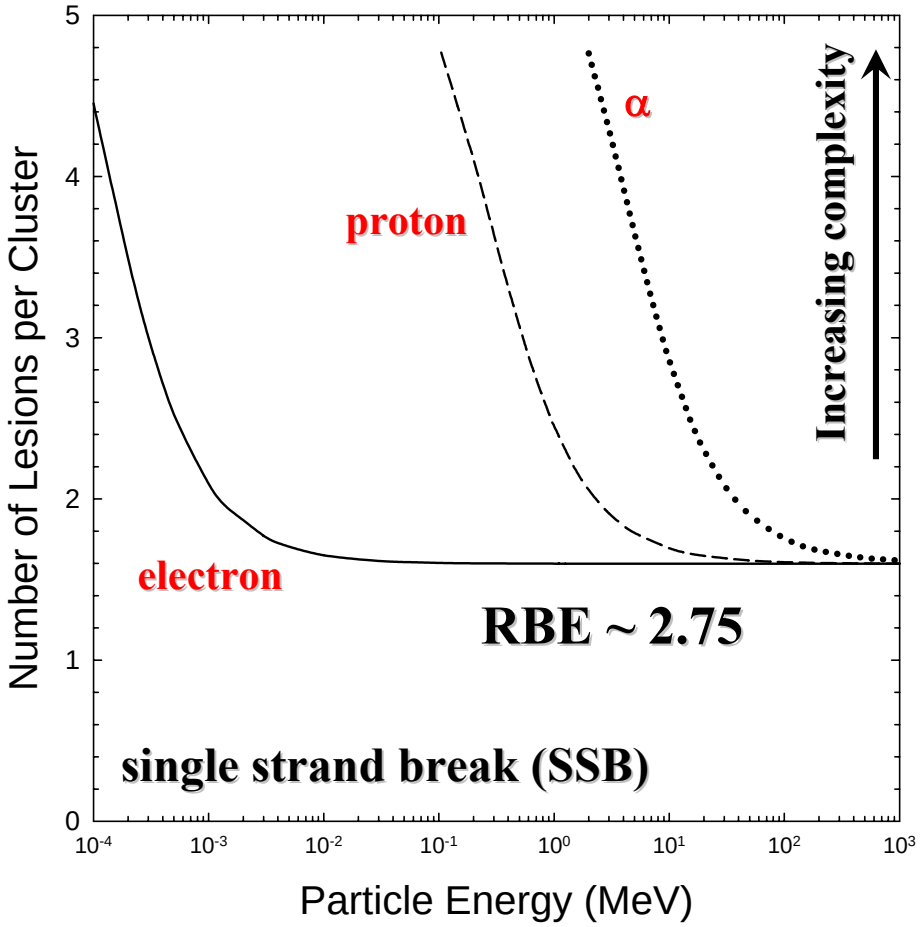
Local complexity (MCDS vs. track structure)

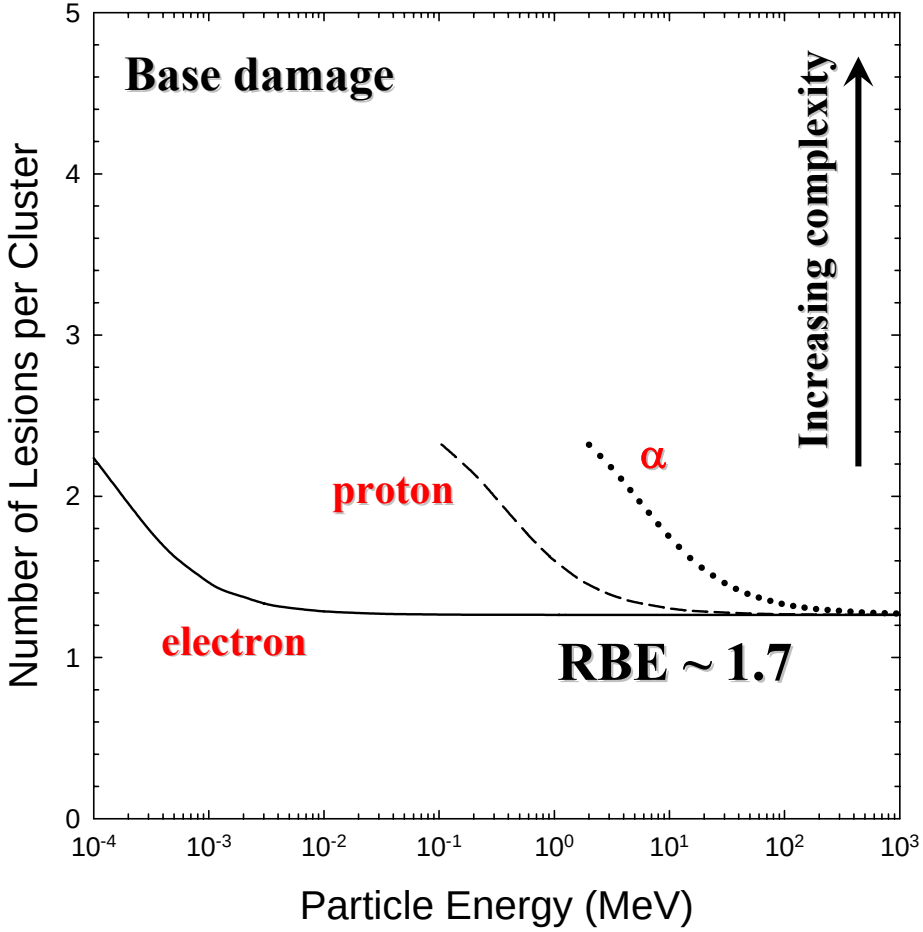
Simple and complex SSB and DSB yields in good agreement for low and high LET radiation



Dark bar: Nikjoo *et al.* 2001
Light bar: Semenenko and Stewart 2006

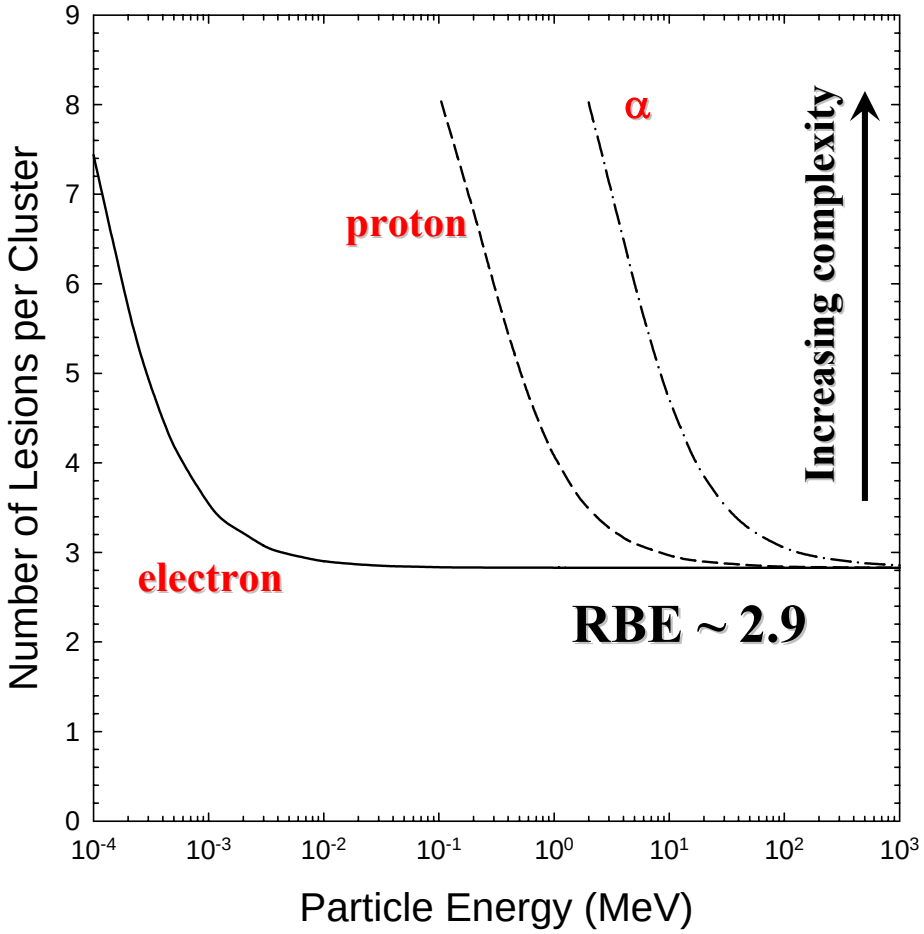
Average number lesions per cluster

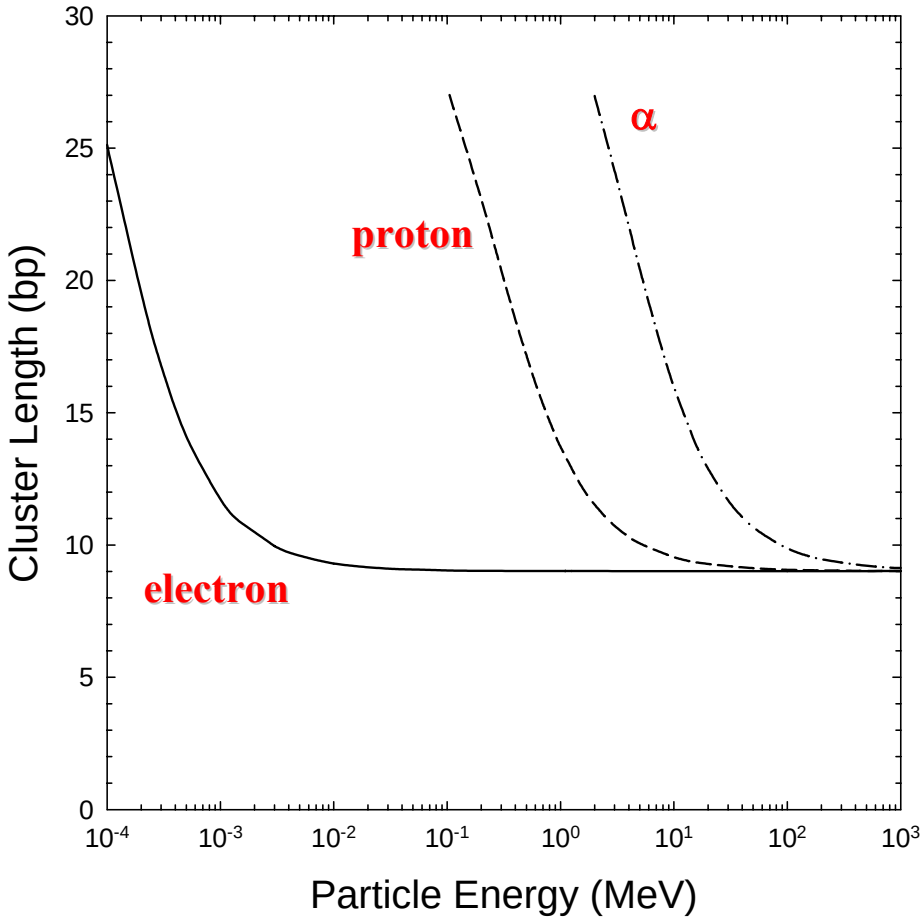




On average, SSB are more complex than clusters without any strand breaks (e.g., 1.6 vs. 1.3 lesion per cluster for large KE)

DSB complexity





Average DSB composed of 2.8 to 8 lesion distributed in a DNA segment less than 25 bp (8.5 nm) in length

MCDS summary

- Nucleotide-level damage maps are needed for cluster repair simulations
  - At present, can only be obtained through Monte Carlo simulations
- MCDS successfully reproduces cluster configurations predicted by track structure models
  - Electrons (> 80 eV), protons (> 105 keV), α (> 2 MeV)
  - Maximum kinetic energy ~ 1 GeV
- Three adjustable parameters
  - Lesion induction (σ_{Sb} = 216.67 Gy^{-1} Gbp^{-1} and $f \sim 3$)
  - Lesion clustering (n_{seg}, depends on LET and varies from 35-149 kbp)
- Computationally efficient
  - Damage configurations for 100,000 cells exposed to 1 Gy can be simulated on 2.8 Ghz Pentium in about 1.5 minutes

DSB repair

- Two main pathways for DSB repair
  - Non-homologous endjoining (NHEJ)
  - Homologous recombination (HR)
  - NHEJ is an error-prone repair mechanism whereas HR is potentially error free.
- In mammalian cells, over 99% of the initial DSB formed by radiation are successfully rejoined.
  - DSB rejoining most likely results in the deletion or insertion of small sections of DNA (point mutation), although larger-scale chromosome and chromatid aberrations can also occur

$$S(1\ \text{Gy}) \cong \exp\left(-40\,\frac{\text{DSB}}{\text{Gy cell}} \times 1\ \text{Gy}\right) = 4.25 \times 10^{-18}$$

For comparison, the human body is composed of less than 10^{14} cells. Also, experiments with mammalian cells indicate that $S(1\ \text{Gy}) \sim 0.1$ to 0.9

Base damage and SSB repair

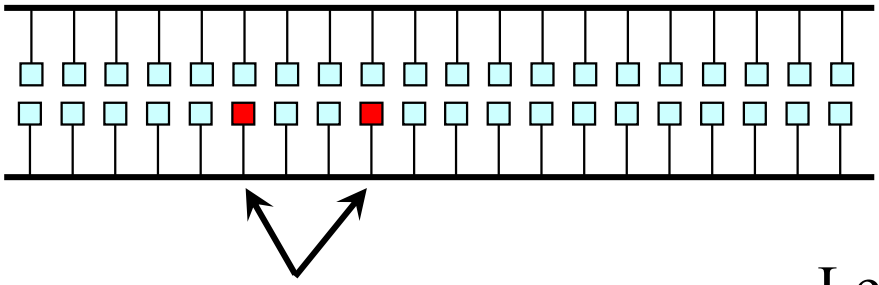
- Primary pathway for the repair of radiation damage other than the DSB is base excision repair (BER)
- BER is usually quite accurate and speedy compared to DSB repair
  - Accuracy and speed of repair tends to decrease as damage complexity increases
- Monte Carlo Excision Repair (MCER) model is the first and only model available to predict cluster repair outcomes for ionizing radiation
  - Correct repair, base substitution, conversion of a SSB into a DSB, number of repair cycles (presumably related to repair kinetics)

MCER Model

- Analog Monte Carlo simulation that captures selected important features of the excision repair process
  - (1) excision of a damaged base, (2) cleavage of the DNA backbone with the removal of the phosphodeoxyribose group (2) gap-filling synthesis by a DNA polymerase (4) sealing of the gap by a DNA ligase
- BER is a highly conserved repair pathway among all eukaryotes, i.e., from yeast to humans
- Significant homologies have also been demonstrated among bacterial and human proteins that participate in BER

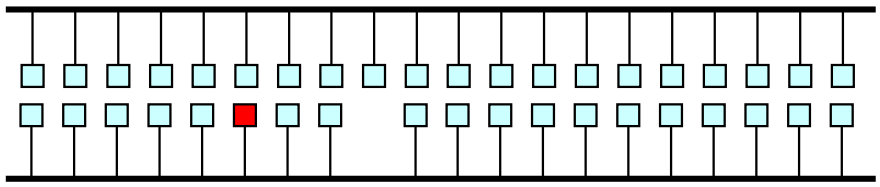
In the absence of information to the contrary, observations from prokaryotes and lower eukaryotes can be reasonably used to make inferences about mechanisms in humans

BER of base damage (Step 1)



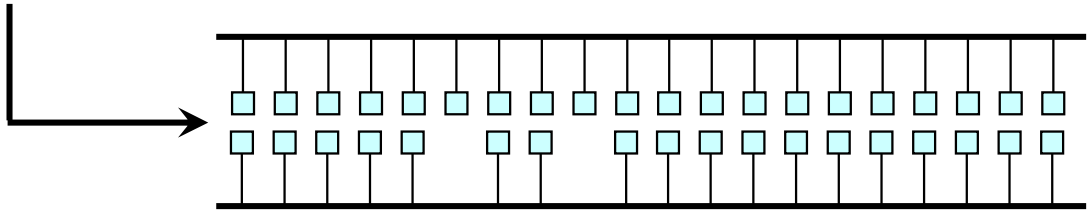
Equal chance of selecting either lesion

Lesion-specific glycosylase cleaves the bond between the sugar and damaged base



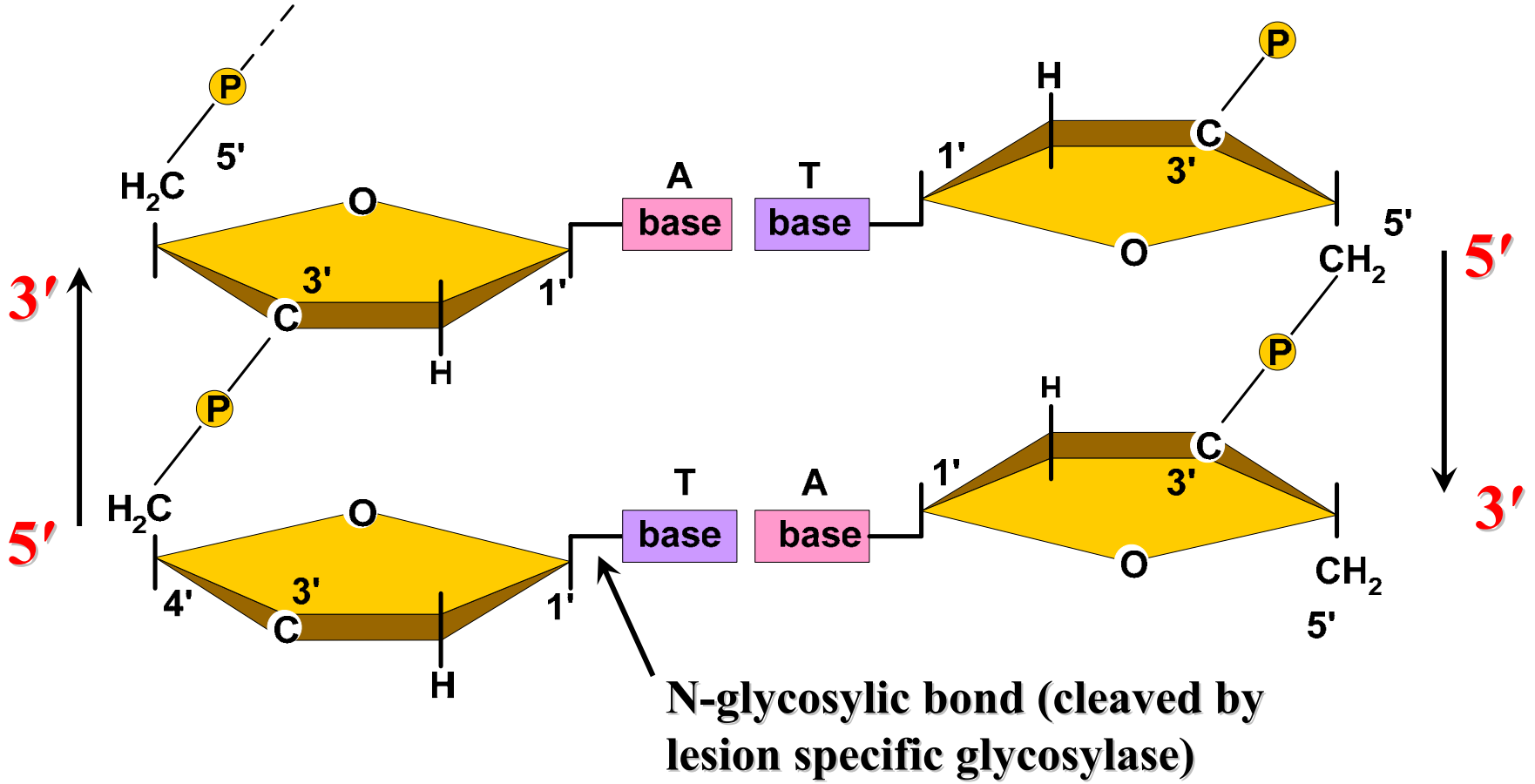
abasic site created
(also called AP site)

Possible (?) that several damaged bases are removed before next step in BER occurs. In MCER, we assume that initiate of BER at the location of one lesion proceeds to completion before BER is initiated at site of another lesion

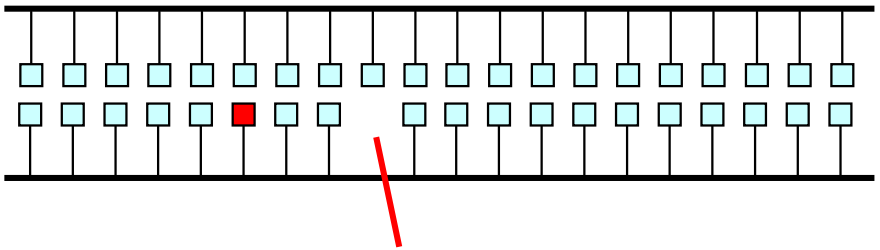


DNA helix and directionality

Sugar-phosphate linkages in one strand proceed from the 5′ to 3′ carbon. In the other strand, linkages proceed in 3′ to 5′ direction.

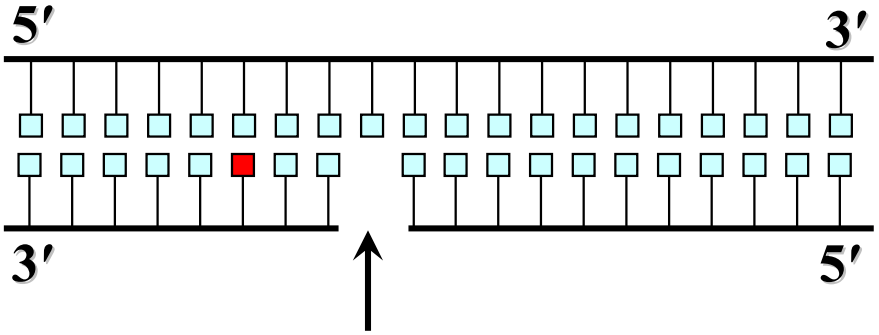


BER of a damaged base (step 2)

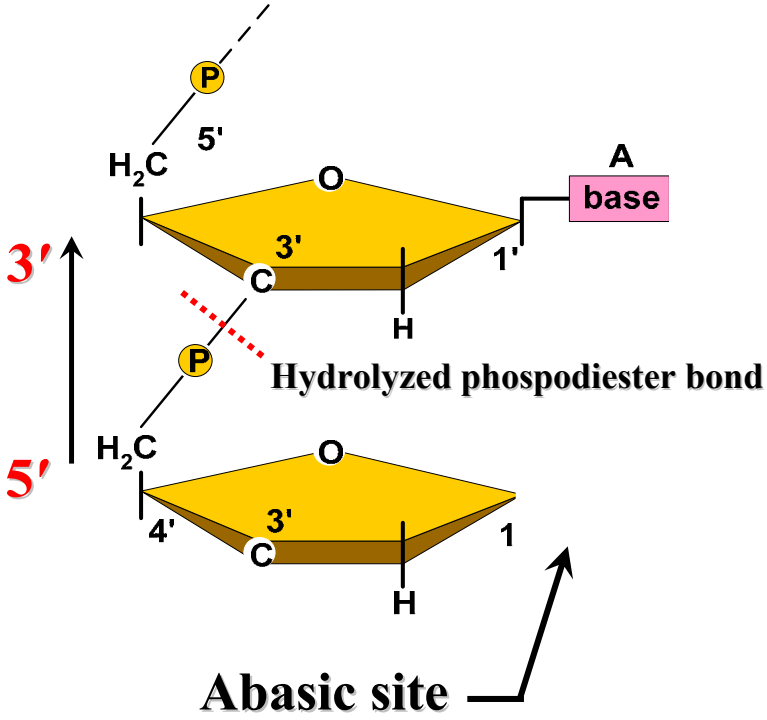


Sugar-phosphate backbone cleaved by nuclease

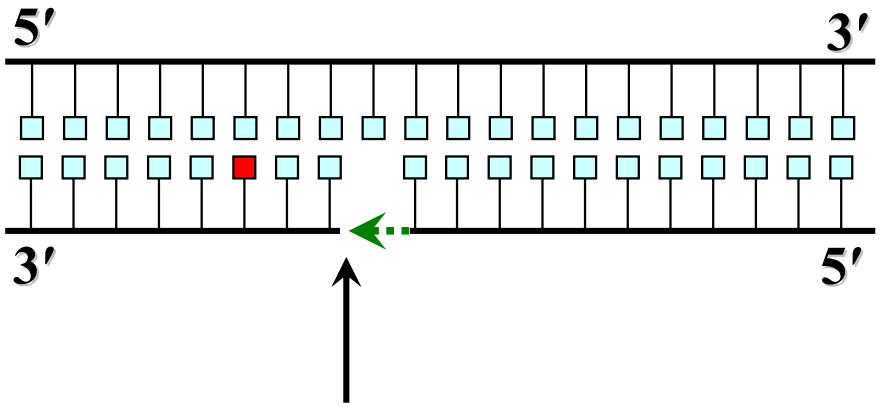
AP endonuclease (APE1) hydrolyzes the phospodiester bond 5′ to abasic site



Gap (nick) with a 3'-OH terminus suitable for extension by a DNA polymerase



BER of base damage (short patch BER)



Repair is complete when a DNA ligase seals the nick.

"repair cycle"

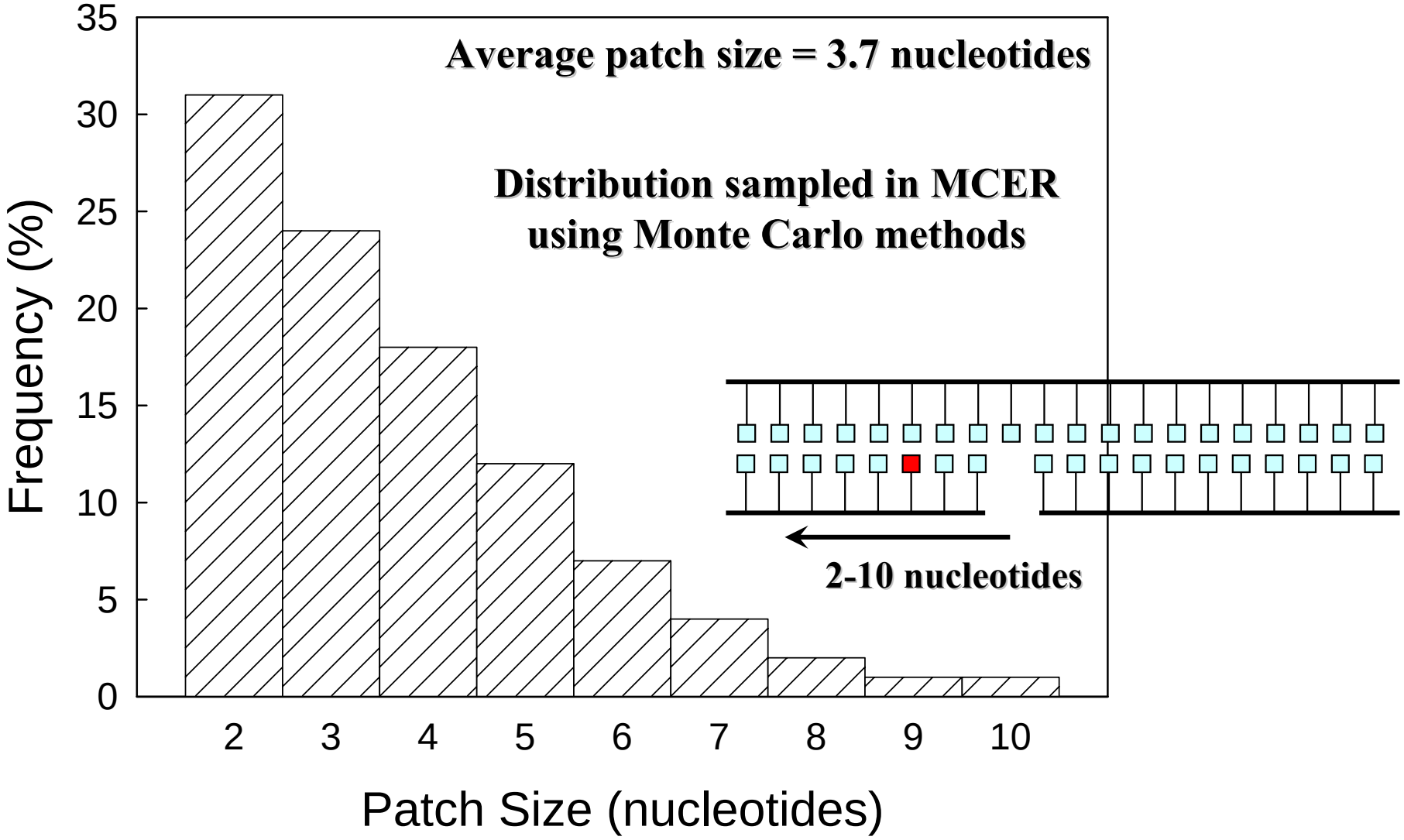
DNA polymerase β removes the 5′-deoxyribose phosphate residue. Opposing strand used as a template for nucleotide insertion

A-T or G-C
Error rate ~ 10^{-4}

Replacement of a single lesion is referred to as *short patch BER*

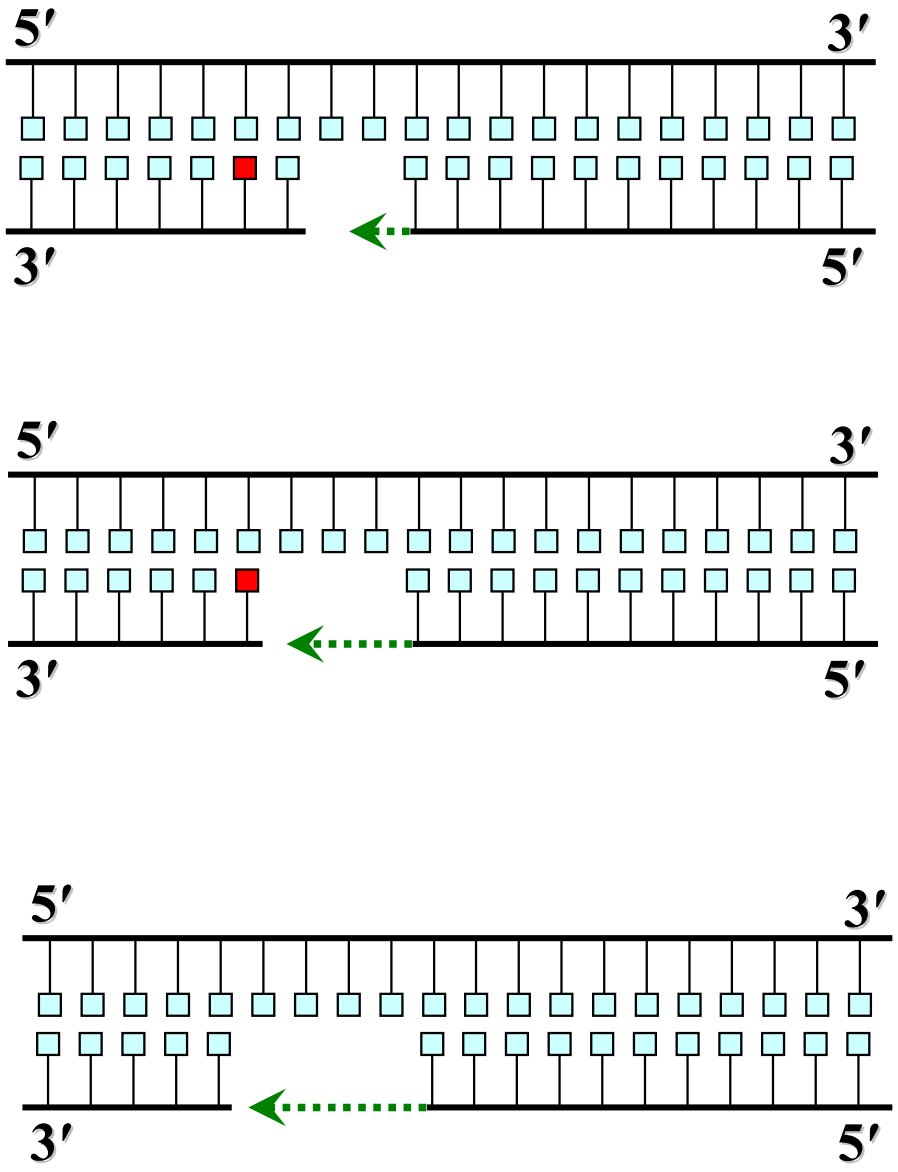
If the abasic site (sugar residue) is oxidized or reduced prior to initiation of repair, long patch BER is initiated instead of short-patch BER. In long patch BER, DNA polymerases δ/ε remove and replace several nucleotides in the 5′ to 3′ direction. The size of this "repair patch" is …

Patch size distribution (long patch BER)



V.A. Semenenko, R.D. Stewart, E.J. Ackerman. Monte Carlo Simulation of Base and Nucleotide Excision Repair of Clustered DNA Damage Sites. I. Model Properties and Predicted Trends. *Radiat. Res.* **164**, 180-193 (2005).

BER of base damage (long patch BER)



DNA polymerase δ/ε remove and resynthesize nucleotides in the 5′ to 3′ direction. Opposing strand used as template to insert new nucleotide

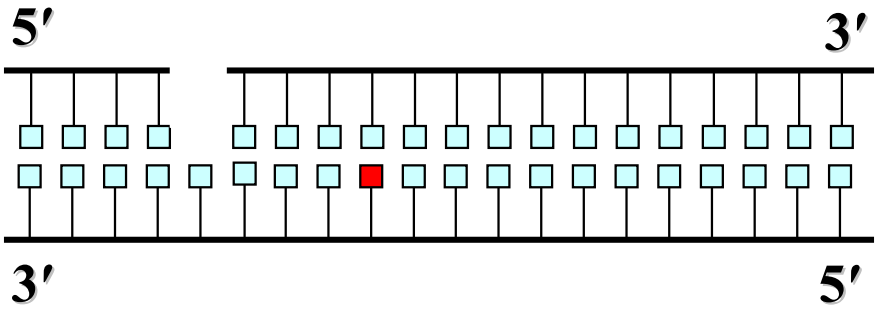
A-T or G-C

Error rate ~ 10^{-6}

If patch extends past other lesions (5′→3′ direction), a single round ("cycle") of BER may remove multiple lesions

Repair is complete when a DNA ligase seals the nick.

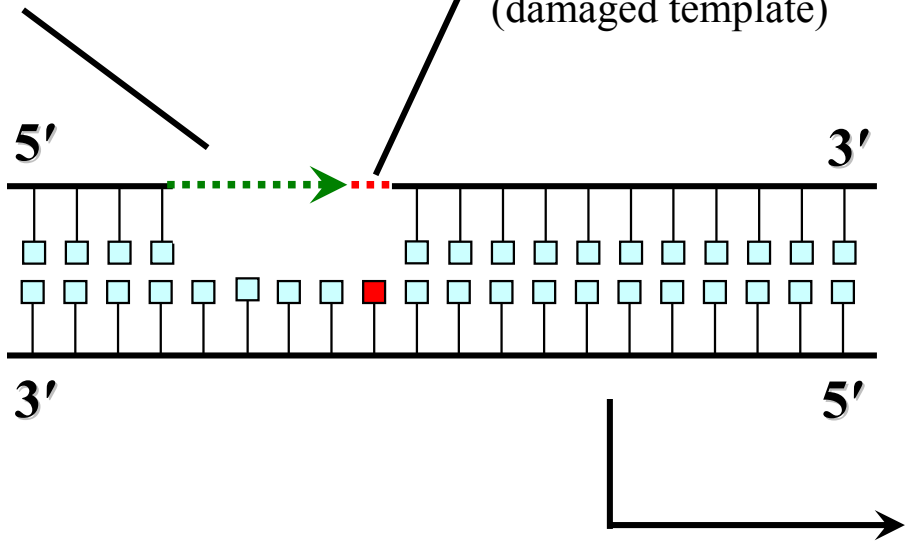
Base substitution (point mutation)



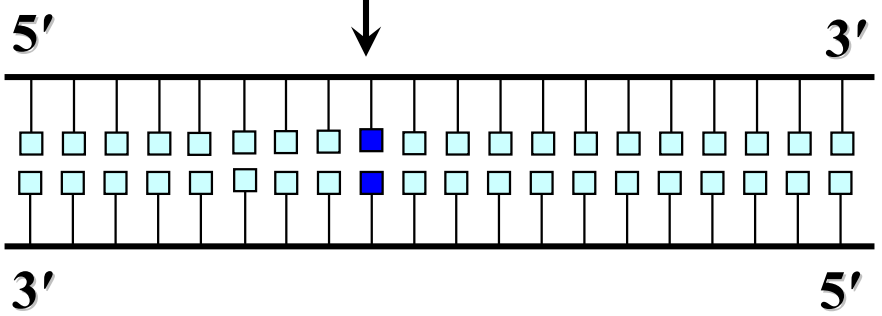
Equal chance of selecting either lesion

Error rate 10^{-6}
(undamaged template)

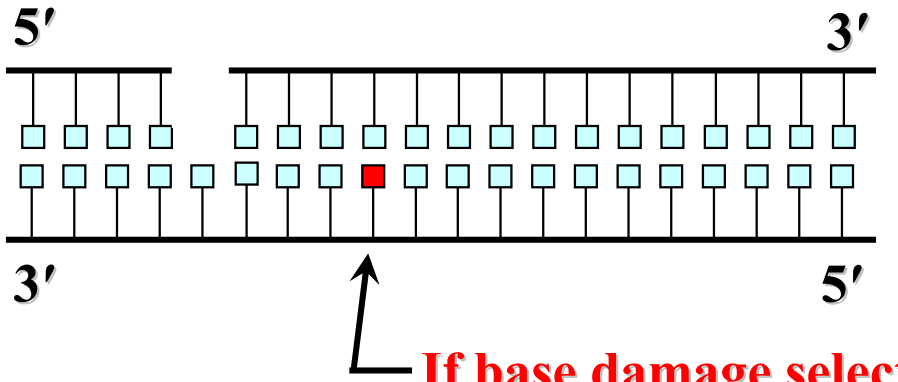
Error rate 3/4 = Randomly insert A, G, T or C
(damaged template)



base substitution

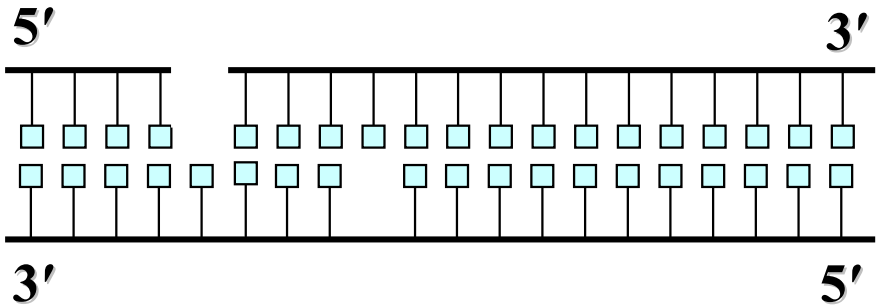


Conversion of SSB into DSB



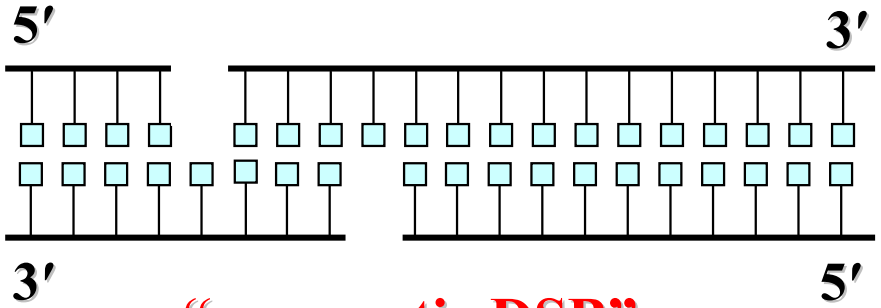
Equal chance of selecting either lesion

If base damage selected…



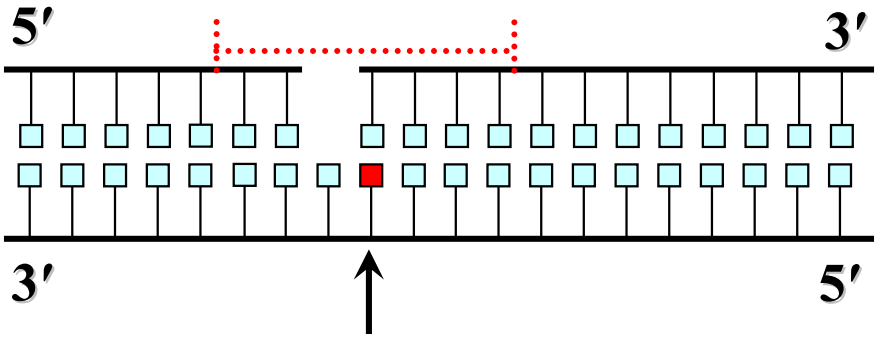
Glycosylase generates an abasic site

AP endonuclease generates a DSB



“enzymatic DSB”

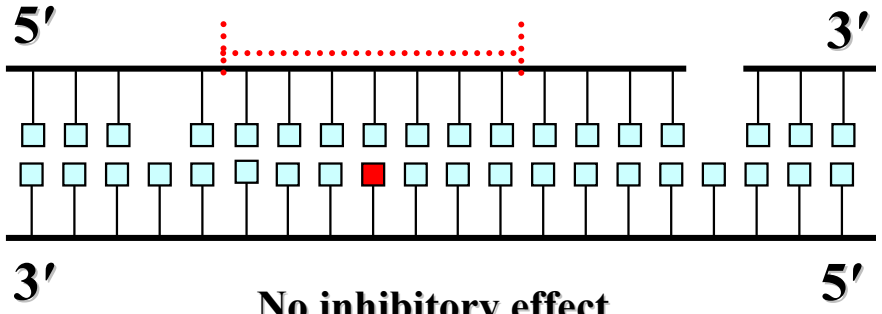
Inhibitory effect of strand breaks



Strand break inhibits BER
(*prevents DSB formation*)

If a strand break occurs within 3-8 bp on opposite strand, BER of the damaged base is strongly inhibited

N_{inh} parameter in MCER
(3 bp in E. coli and 8 bp in humans)



No inhibitory effect
(*DSB formed*)

If strand break is more than 3-8 bp away from damaged base, no inhibitory effect

For additional details...

- Monte Carlo Damage Simulation (MCDS)
  - V.A. Semenenko and **R.D. Stewart**. A fast Monte Carlo algorithm to simulate the spectrum of DNA damages formed by ionizing radiation. *Radiat Res.* **161**(4), 451-457 (2004).
  - V.A. Semenenko and **R.D. Stewart**. Fast Monte Carlo simulation of DNA damage formed by electrons and light ions. *Phys. Med. Biol.* **51**(7), 1693-1706 (2006).
- Monte Carlo Excision Repair (MCER)
  - V.A. Semenenko, **R.D. Stewart**, E.J. Ackerman. Monte Carlo Simulation of Base and Nucleotide Excision Repair of Clustered DNA Damage Sites. I. Model Properties and Predicted Trends. *Radiat. Res.* **164**, 180-193 (2005).
  - V.A. Semenenko and **R.D. Stewart**. Monte Carlo Simulation of Base and Nucleotide Excision Repair of Clustered DNA Damage Sites. II. Comparisons of Model Predictions to Measured Data. *Radiat. Res.* **164**, 194-201 (2005).

http://rh.healthsciences.purdue.edu/mcds/
http://rh.healthsciences.purdue.edu/mcer/

Repair outcomes for ionizing radiation

Number of cycles to completely remove a cluster

Repair with mutation

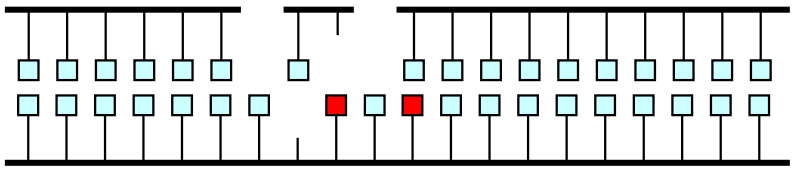
Half-time for cluster repair $\propto$ **Repair Cycles**

Correct Repair ← **MCER** → **Conversion to DSB**

MCDS

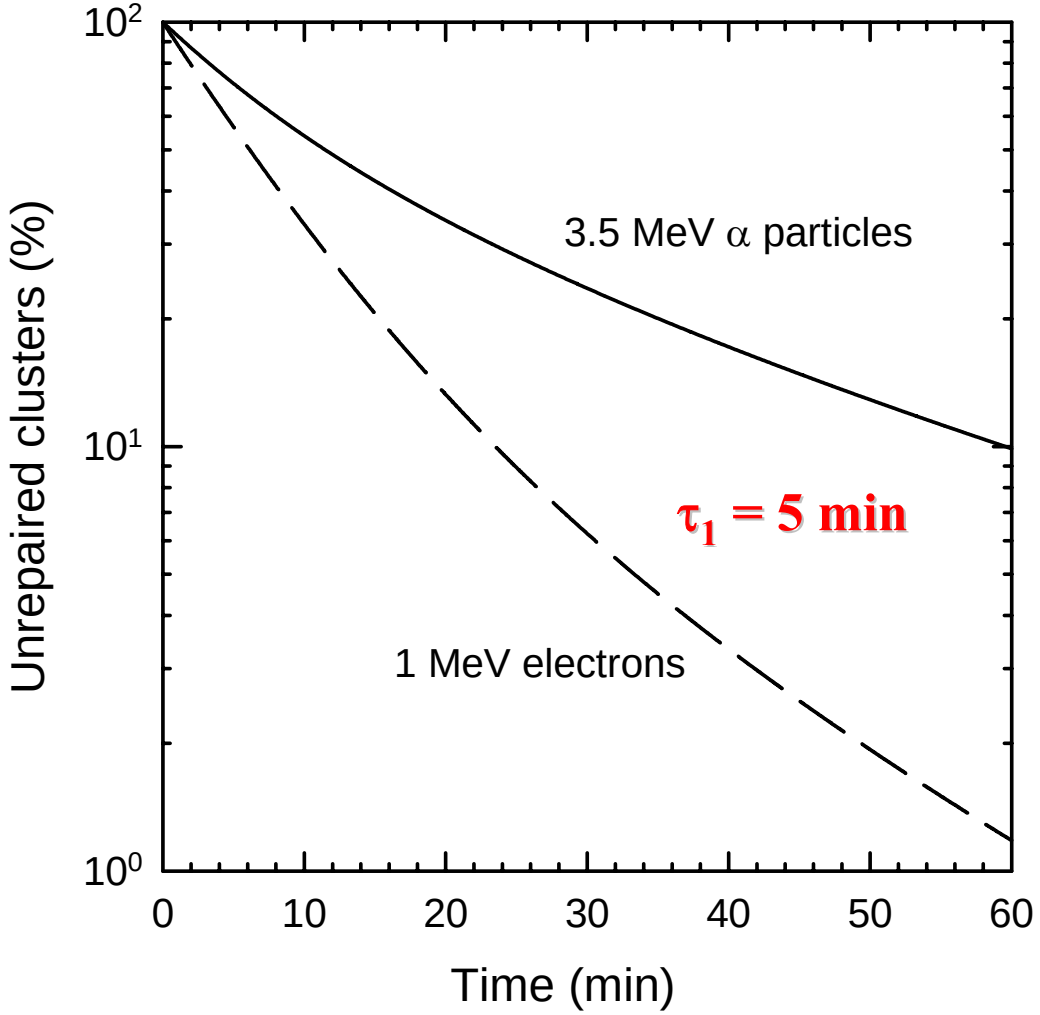
Combined MCDS/MCER simulation takes ~ 4-6 h on a 2.8 GHz Pentium

Generate ~ 10^9 cluster configuration
(10^6 cells exposed to 1 Gy)



Complex SSB (6 lesions)

Cluster repair kinetics (working hypothesis)



Multi-exponential cluster repair kinetics:

$$U(t) = D\sum_{i=1}^{100}\sigma_i e^{-\lambda_i t}$$

σ_i = number of *i*th type cluster Gy^{-1} cell^{-1} (MCDS)

$\lambda_i = \ln(2)/(\kappa_i \times \tau_1)$

↓

Repair cycle *i*th type of cluster (**MCER**)

τ_1 = time to repair a 1-cycle cluster

Over two-fold reduction in rate of repair predicted for e^- compared to α particle

Point mutations in HPRT gene (low LET)

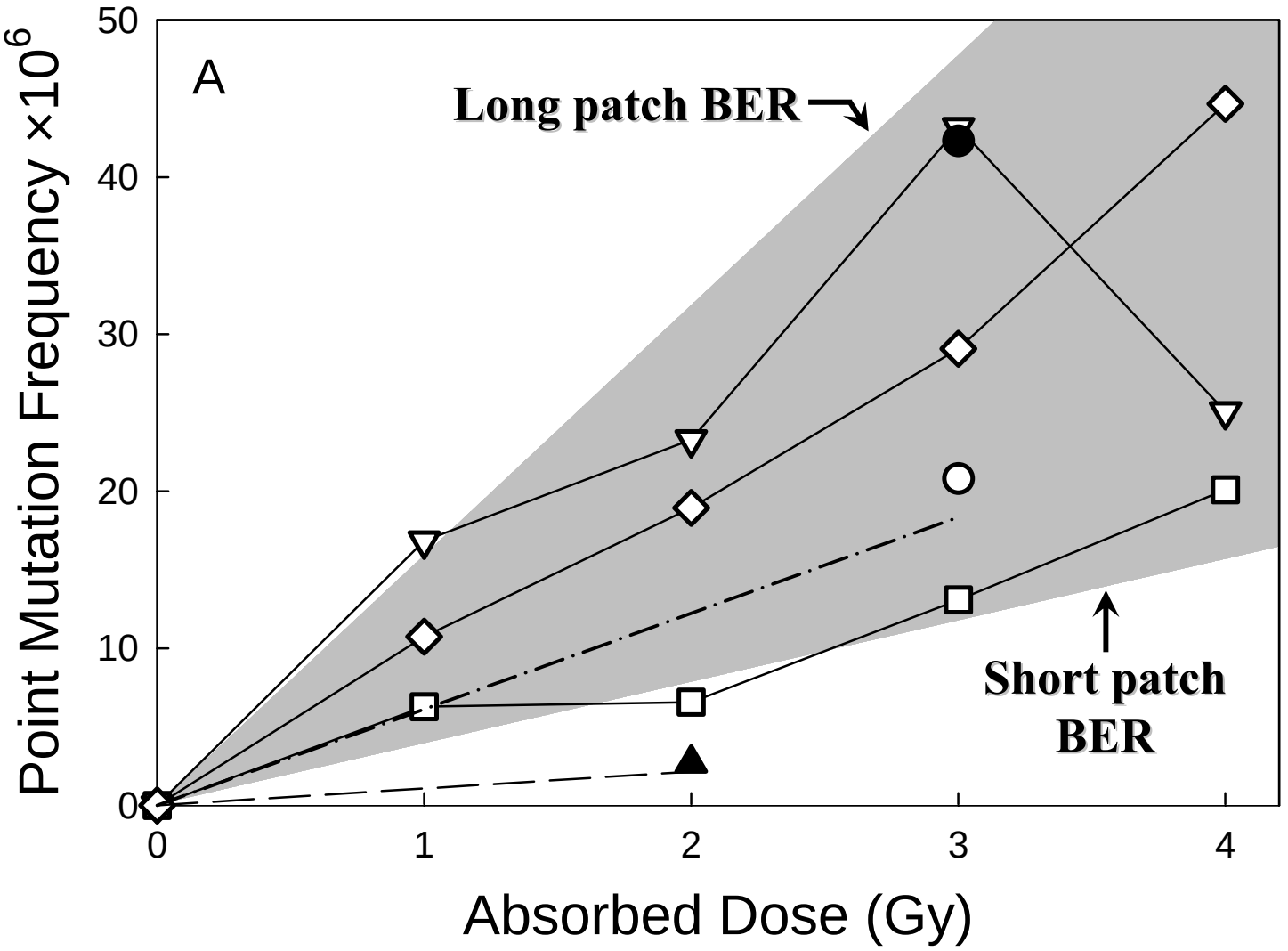


Figure 2 in V.A. Semenenko and R.D. Stewart. Monte Carlo Simulation of Base and Nucleotide Excision Repair of Clustered DNA Damage Sites. II. Comparisons of Model Predictions to Measured Data. *Radiat. Res.* **164**, 194-201 (2005).

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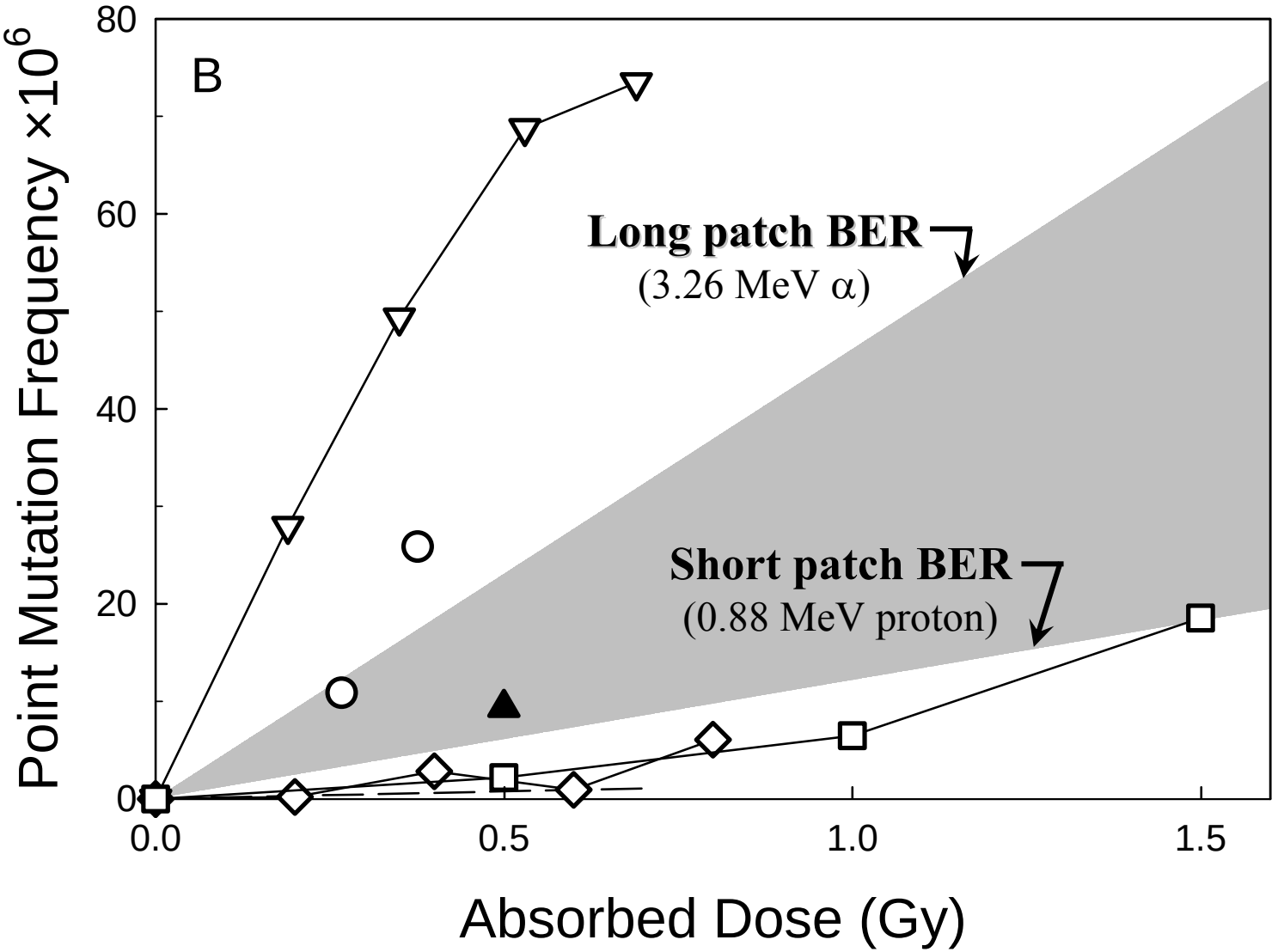


Figure 2 in V.A. Semenenko and R.D. Stewart. Monte Carlo Simulation of Base and Nucleotide Excision Repair of Clustered DNA Damage Sites. II. Comparisons of Model Predictions to Measured Data. *Radiat. Res.* **164**, 194-201 (2005).

Repair outcomes (selected radiations)

Radiation Type	L_∞ (keV/μm)	Yield (Gy^{-1} $cell^{-1}$)				Correct Repair (%)		Enzymatic DSB	
		DSB	SSB	Base Damage	Total	SSB	Base Damage	number	% SSB
1 MeV electrons	0.19	49.8	1136	2567	3753	96.2	99.4	29.4	2.59
250 MeV protons	0.39	50.1	1135	2562	3747	96.1	99.4	29.5	2.60
100 keV electrons	0.41	50.1	1135	2559	3744	96.1	99.4	29.6	2.61
10 keV electrons	2.3	53.3	1123	2483	3659	95.8	99.3	31.3	2.79
1 MeV protons	26	95.6	944	1558	2598	91.2	98.3	51.6	5.47
6.29 MeV α particles	75	127	776	981	1884	86.1	97.1	62.5	8.06
5.49 MeV α particles	83	131	750	908	1789	85.2	96.9	63.8	8.50
3.5 MeV α particles	113	144	664	703	1511	82.2	96.1	65.9	9.93

Experiments with mammalian cells suggest 0.3-0.8 enzymatic DSB per prompt DSB compared to 0.4-0.6 from MCDS/MCER simulations

MCER summary

- Combined MCDS and MCER simulations correctly predict trends in repair outcomes with LET and have right order of magnitude
  - Conversion of SSB into DSB
  - Point mutations in HPRT gene
- Excision repair model captures some important mechanistic aspects with a minimum number of parameters
  - Patch size distributions (size and directionality)
  - Inhibitory effects of strand breaks
- Supports the hypothesis that clustered damage is repaired less accurately and efficiently than singly lesions

Acknowledgements

U.S. Department of Energy, Office of Science, Grant DE-FG02-03ER63541 and DE-FG02-03ER63665.

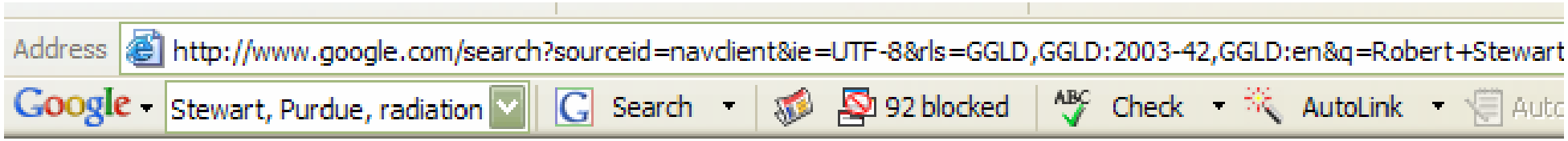
Low Dose Radiation Research Program
http://lowdose.tricity.wsu.edu/

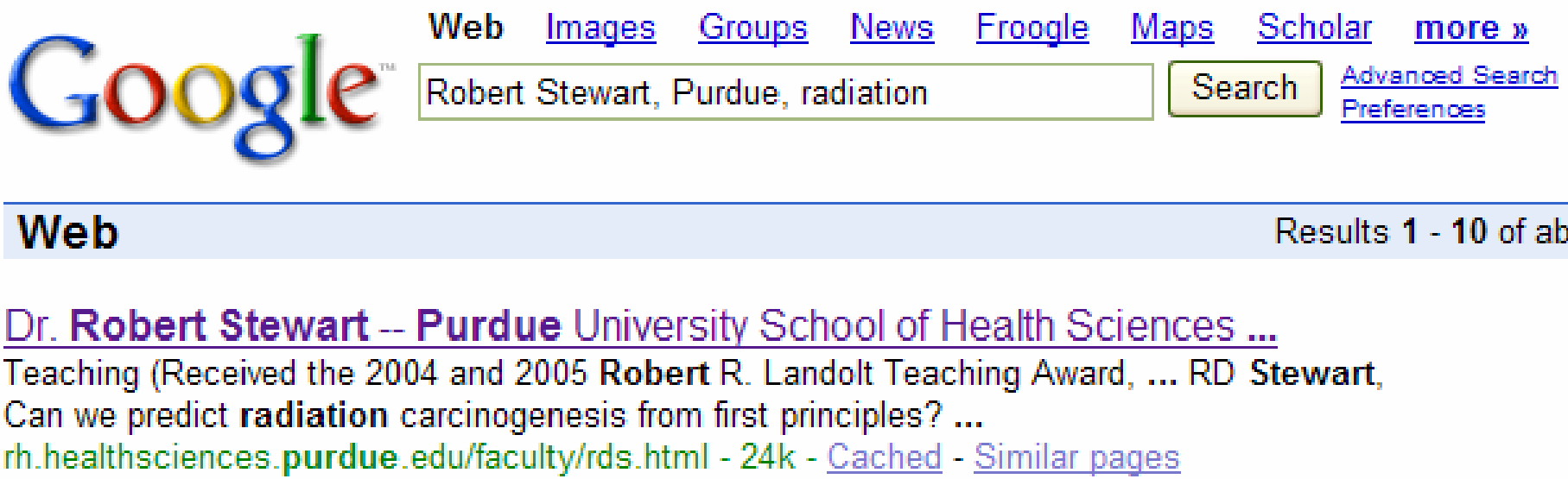
Vladimir Semenenko, Ph.D.
(now at Medical College of Wisconsin)

Software and presentation (*online*)

Google search with key words

"Robert Stewart, Purdue, radiation"





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