# Induction of Clustered DNA Lesions by Ionizing Radiation – *Insights from Biophysical Modeling*

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Presented at

**2009 RRS Annual Meeting (**October 4-7, 2009**)**
**Savannah, GA**

**S1.** ***Complex DNA Damage: From Theory to Biological Consequences***
Chairs, Lynn Harrison and Aroumougame Asaithamby,
Ballroom D, Sunday October 4, 2009 from 10:15 am to 12:00 pm

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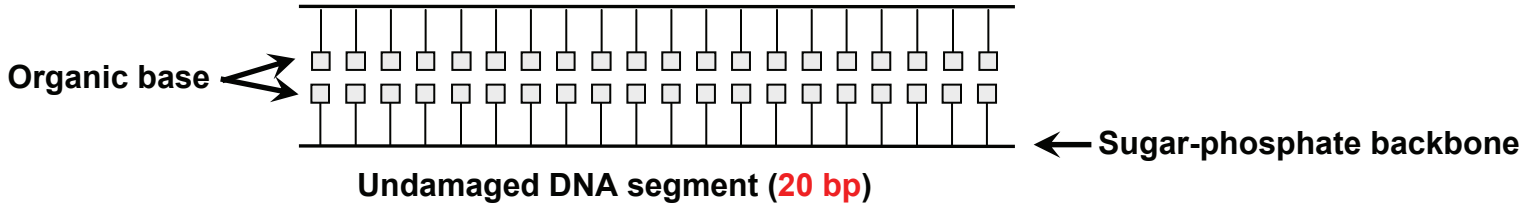
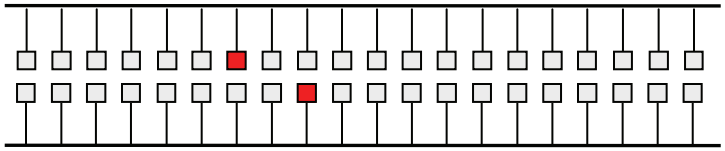
# Clustered DNA lesions

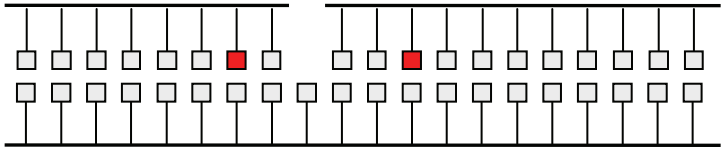
**Groups of several DNA lesions within one or two turns of the DNA are termed a *cluster* or *multiply damaged site* (MDS)***



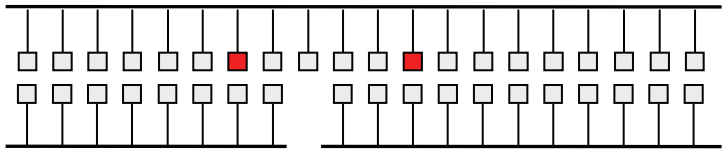

Undamaged DNA segment (20 bp)



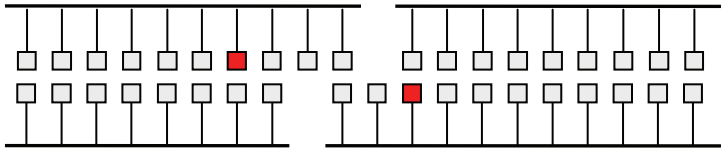
Base damage in opposed strands (2 lesions)



Single Strand Break (SSB)
(3 lesions)



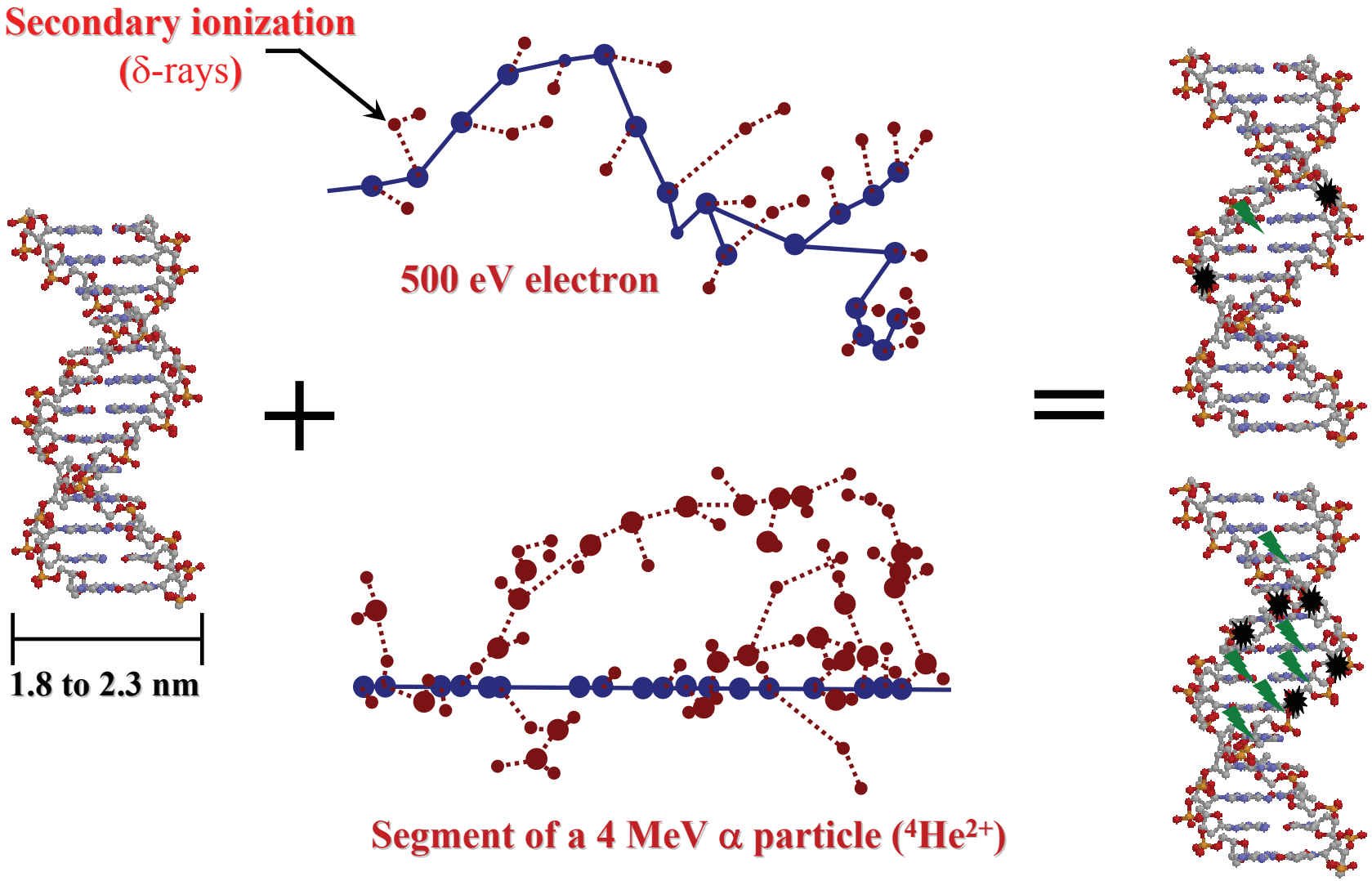
SSB with opposed base damage
(3 lesions)



Double Strand Break (DSB)
(4 lesions)

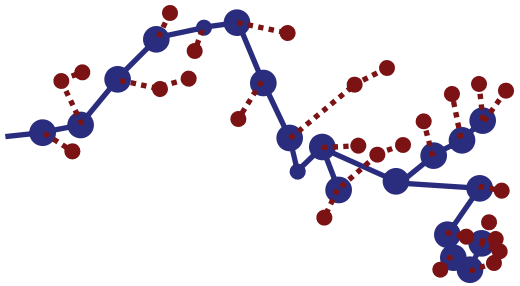
* Clustered lesions are also referred to as ***locally multiply damaged sites*** (LMDS)

# Mechanisms – Direct Effect



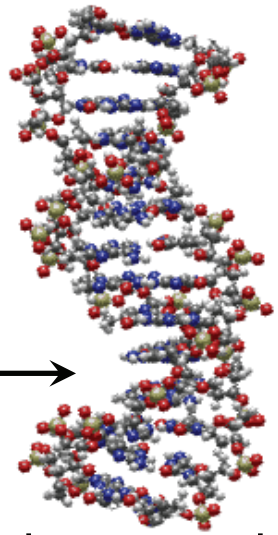
# Mechanisms – Indirect effect

**Ionization of excitation of a cellular constituent other than DNA produces chemically reactive species that diffuse and interact with the DNA**



$$H_2O \rightarrow H_2O^+ + e^-$$

$$H_2O^+ + H_2O \rightarrow \cdot OH + H_3O^+$$

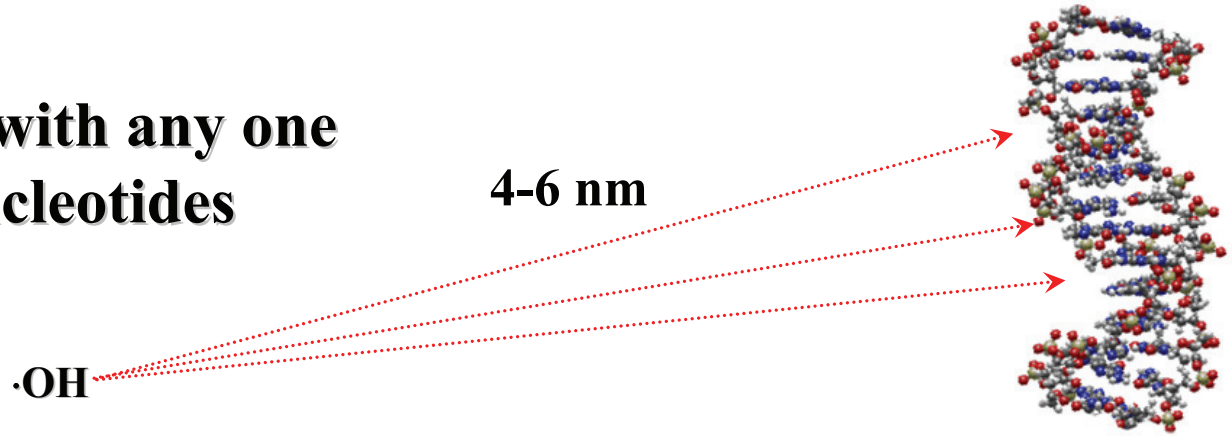


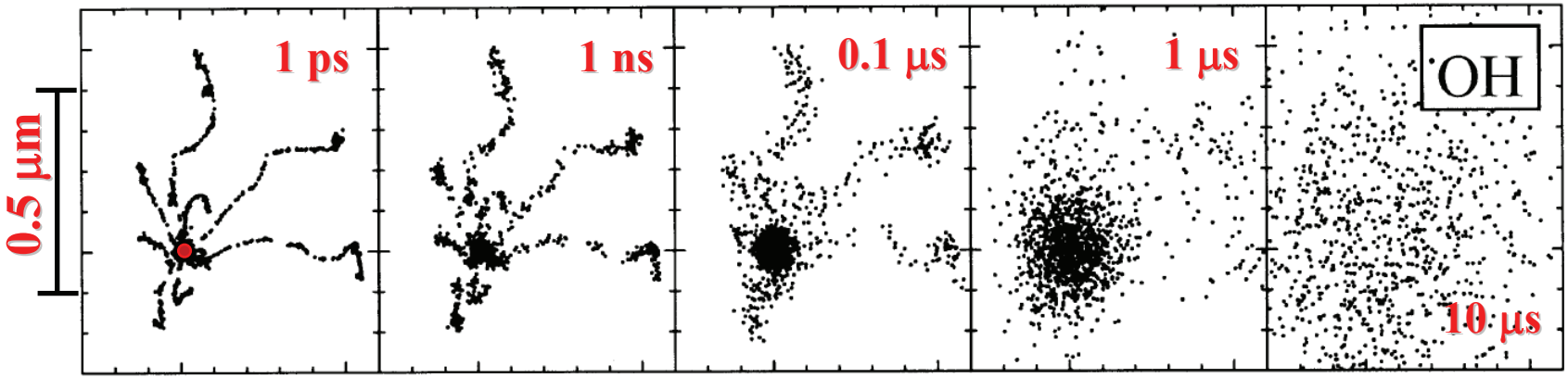

**Average diffusion distance of an •OH in a cellular milieu is about 4-6 nm (**Roots and Okada 1975**)**

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).

# Radical diffusion – time and spatial scales

**Could interact with any one of about 100 nucleotides**



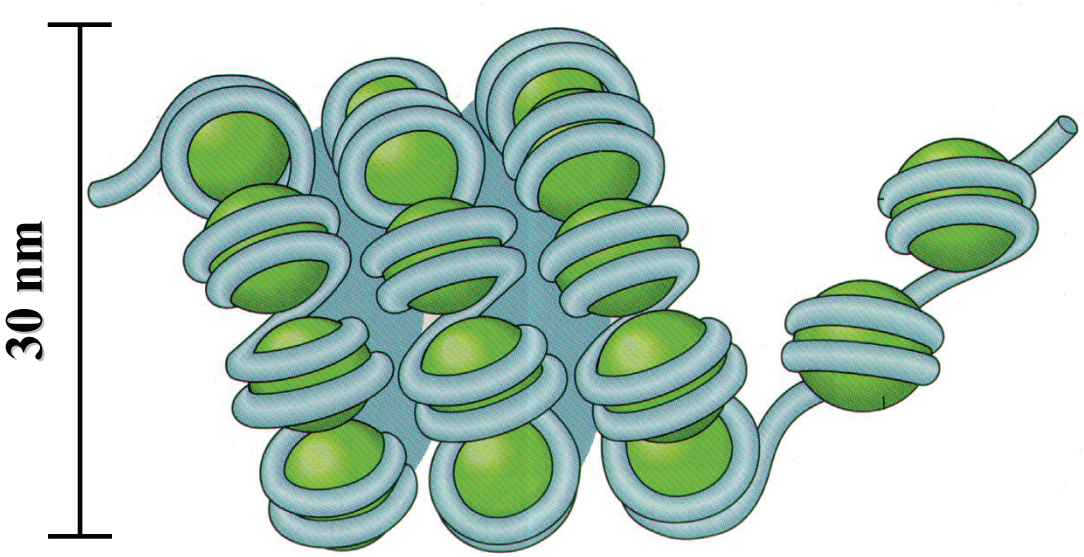

**Spatial distribution of ·OH radical in liquid water. Red dot indicates location of a 1 µm segment of a 24 MeV $^4He^{2+}$ ion (26 keV/µm) directed into the image**

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).

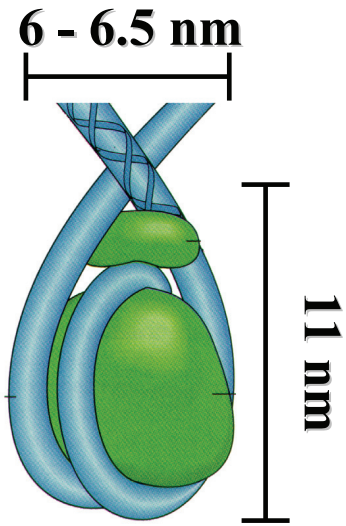
# Relevant time and spatial scales

## Induction of individual and clustered DNA lesions by ionizing radiation dominated by stochastic events and processes

- **Spatial scale < 10 nm (*100 to 1000 nucleotides*)**
- **Time scales < $10^{-3}$ to $10^{0}$ s**




**Local chemical environment most likely plays a role**
**(**histones, radical scavengers, …**)**




**Nucleosome**
(146 ± 1 bp)

# Clusters per $Gy^{-1}$ $Gbp^{-1}$ (yeast and humans)

Irradiation of human cells (~ 6 Gbp of DNA) by low LET radiation creates about **35 to 50 DSB $Gy^{-1}$ $cell^{-1}$**

$$35\frac{\text{DSB}}{\text{cell Gy}}\cdot\frac{1\text{ cell}}{6\text{ Gbp}}=5.8\frac{\text{DSB}}{\text{Gbp Gy}} \qquad 50\frac{\text{DSB}}{\text{cell Gy}}\cdot\frac{1\text{ cell}}{6\text{ Gbp}}=8.3\frac{\text{DSB}}{\text{Gbp Gy}}$$

In diploid yeast (24 Mbp of DNA), low LET radiation creates about **0.05 to 0.07 DSB $Gy^{-1}$ $cell^{-1}$**

$$0.07\frac{\text{DSB}}{\text{cell Gy}}\cdot\frac{1\text{ cell}}{24\text{ Mbp}}\frac{1000\text{ Mbp}}{1\text{ Gbp}}=2.9\frac{\text{DSB}}{\text{Gbp Gy}}$$

**250-fold difference in DNA content but only a factor 2-3 difference in DSB yield per unit genome length**

# Cell cycle and cell kinetics

| Duration of cell cycle (h) | | | | | DSB cell$^{-1}$ Gy$^{-1}$ |
|---|---|---|---|---|---|
| G1 | S | G2 | M | Total | |
| 4.00 | 6.00 | 0.50 | 0.50 | 11.00 | **40.91** |
| 12.00 | 8.00 | 1.00 | 0.50 | 21.50 | **37.67** |
| 36.00 | 6.00 | 0.50 | 0.50 | 43.00 | **32.79** |

25%

$G_1$ phase: 6 Gbp
$S$ phase: 9 Gbp
G2/M phase: 12 Gbp
5 DSB Gbp$^{-1}$ Gy$^{-1}$

**DSB Gy$^{-1}$ cell$^{-1}$ larger for rapidly dividing cells than for slowly dividing cells**

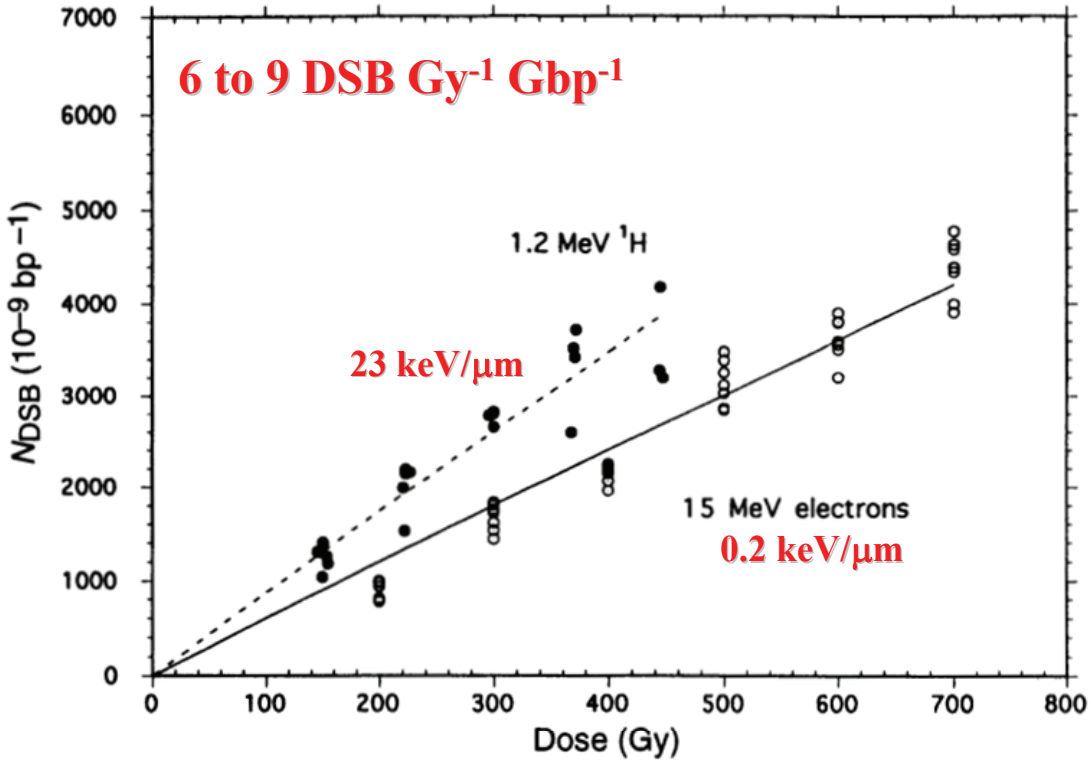
**Among mammalian cells ~ 25-40% difference in *clusters per cell* among proliferating cells – even if number of *clusters per Gbp* same**

**Up to a factor of 2 difference in *clusters per cell* for dividing vs non-dividing cells**

# Effect of Dose

**Pulsed-field gel electrophoresis (PFGE) and DNA fragmentation analysis**

**human skin fibroblasts**




- **Number (*yield*) proportional to dose up to hundreds of Gy**
  - same types of clusters produced by low and high doses of radiation

Frankenberg D, Brede HJ, Schrewe UJ, Steinmetz C, Frankenberg-Schwager M, Kasten G, Pralle E. Induction of DNA double-strand breaks by $^{1}$H and $^{4}$He ions in primary human skin fibroblasts in the LET range of 8 to 124 keV/microm. *Radiat Res.* **151**(5), 540-549 (1999).

# Effect of Dose Rate

**To produce a dose rate effect, would need to modulate focused beams of radiation to targets with dimensions ~ 10 nm (**approximate diffusion distance of ·OH**) on time scales of μs to s (***time to complete initial chemical reactions***).**

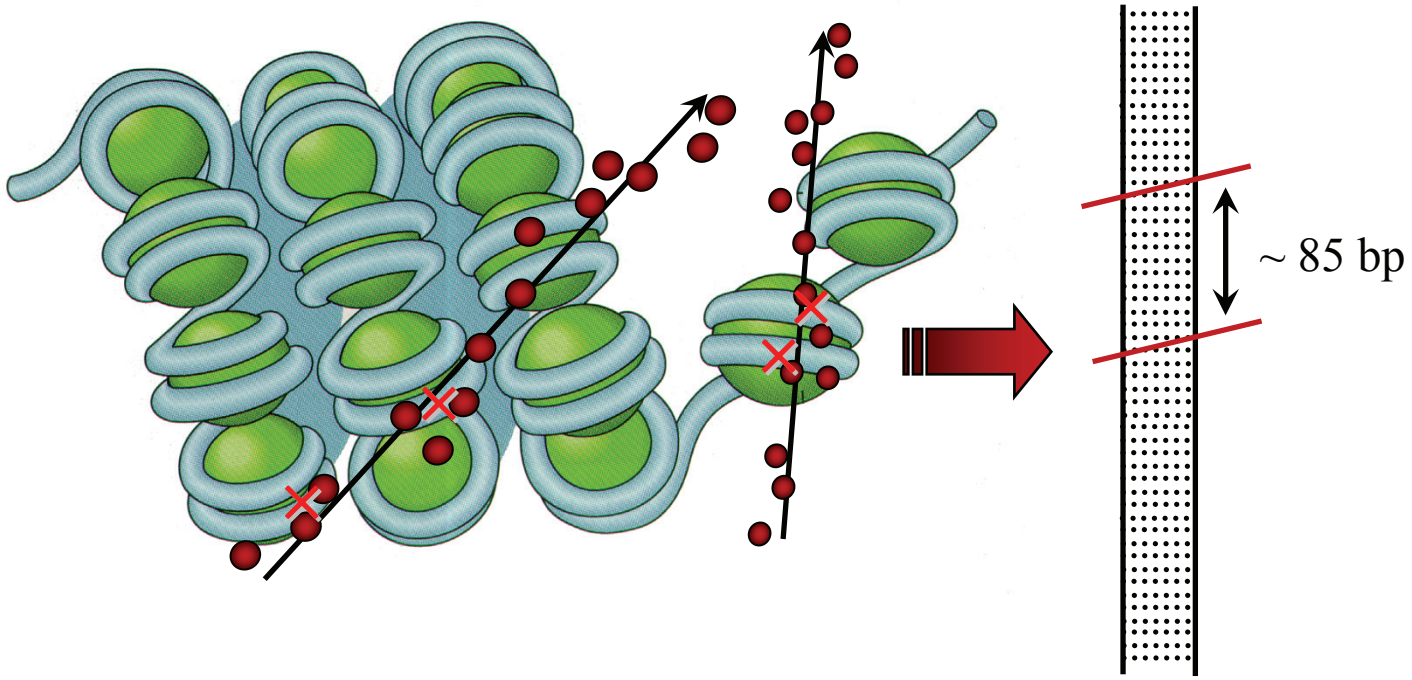
$$D = \frac{100\ \text{eV}}{(10\ \text{nm})^3 \cdot 1\ \text{g/cm}^3} \cdot \frac{1.602 \times 10^{-19}\ \text{J}}{1\ \text{eV}} \cdot \frac{10^3\ \text{g}}{1\ \text{kg}} \cdot \left(\frac{10^7\ \text{nm}}{1\ \text{cm}}\right)^3 \cdot \frac{1\ \text{Gy}}{1\ \text{J/kg}}$$

$$= 16{,}020\ \text{Gy}$$

$$\dot{D} = \frac{16{,}020\ \text{Gy}}{1\ \text{s}} \cdot \frac{3600\ \text{s}}{1\ \text{h}} = 57.7 \times 10^6\ \text{Gy/h}$$

- **Types and yield of individual and clustered DNA lesions independent of dose rate**
  - repair during and after irradiation reduces number of clusters observed in experiments

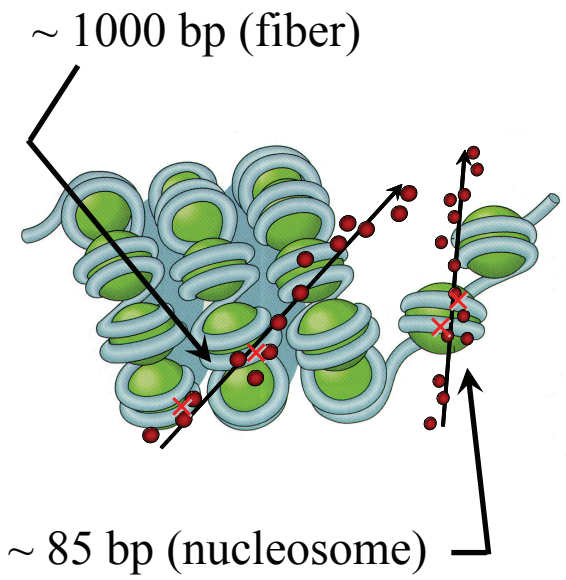
# Regionally multiply damage sites (RMDS)

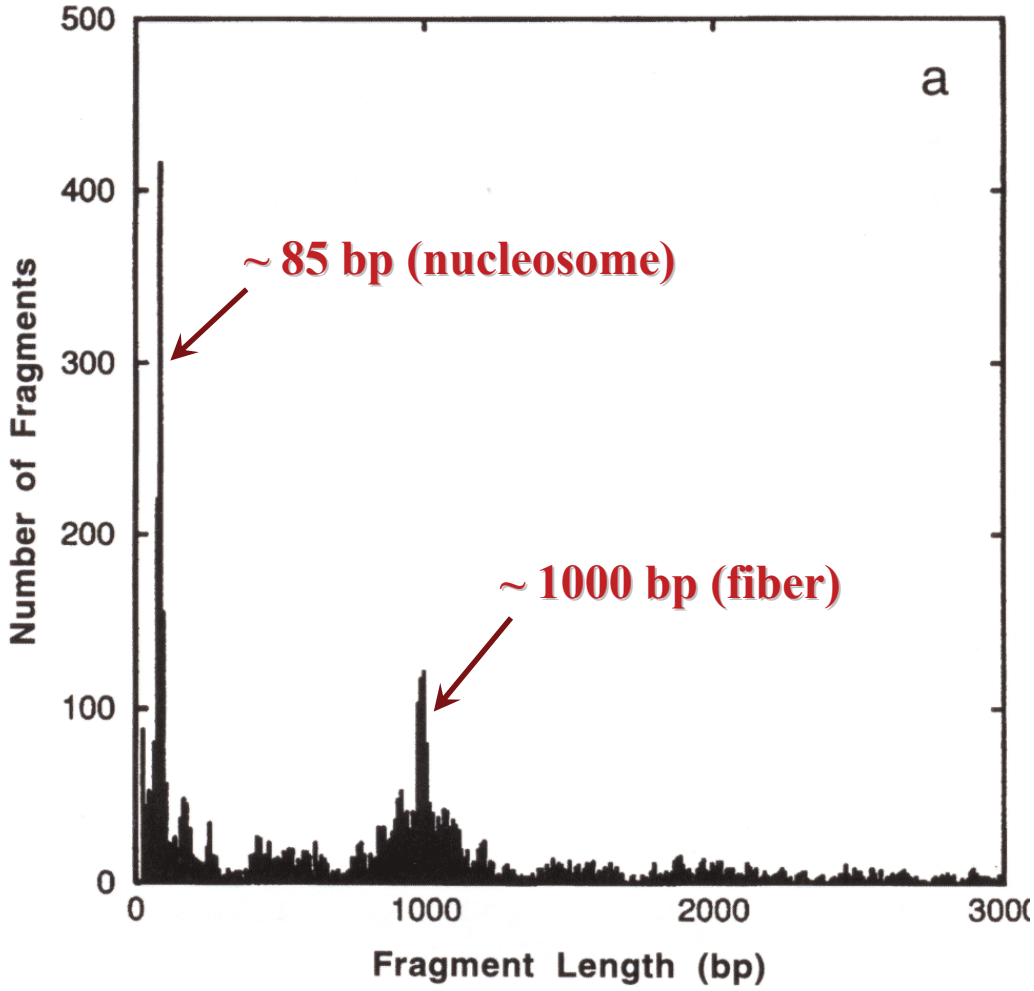



**DSB separated by about 85 bp (one turn around nucleosome) and about 1,000 bp (chromatin fiber) sometimes formed, especially for high LET radiations – termed *regionally multiply damaged sites* (or RMDS).**

Holley WR, Chatterjee A. Clusters of DNA induced by ionizing radiation: formation of short DNA fragments. I. Theoretical modeling. *Radiat Res.* **145**(2):188-99 (1996). Rydberg B. Clusters of DNA damage induced by ionizing radiation: formation of short DNA fragments. II. Experimental detection. *Radiat Res.* **145**(2):200-9 (1996).

# Dectection vs (*true*) Induction



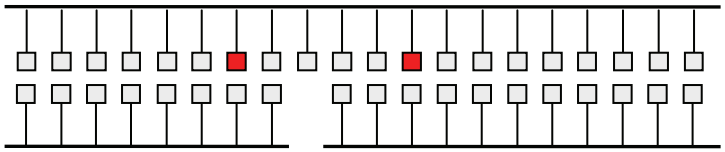




**Small fragments not always counted ("detected") in published PFGE studies**

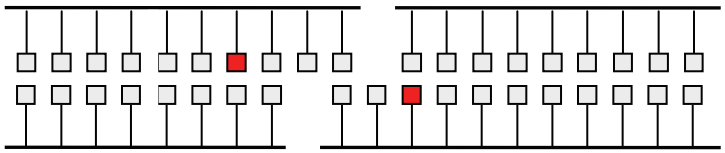
Holley WR, Chatterjee A. Clusters of DNA induced by ionizing radiation: formation of short DNA fragments. I. Theoretical modeling. *Radiat Res.* **145**(2):188-99 (1996). Rydberg B. Clusters of DNA damage induced by ionizing radiation: formation of short DNA fragments. II. Experimental detection. *Radiat Res.* **145**(2):200-9 (1996).

# Effect of Radiation Quality

- **Same number of individual lesions produced, per unit dose per unit genome length, by all types of radiation**
  - About 217 strand breaks $Gy^{-1}$ $Gbp^{-1}$ and 650 AP sites or damaged bases $Gy^{-1}$ $Gbp^{-1}$ (*3 bases damaged per strand break*)
- **Cluster complexity tends to increase with increasing linear energy transfer (LET)**
  - Clusters cannot be a DSB and a SSB (*mutually exclusive types of cluster*)
  - DSB formed by *at least* 2 lesions and SSB formed by *at least* 1 lesion
  - Ratio of SSB per DSB *decreases* with *increasing* LET

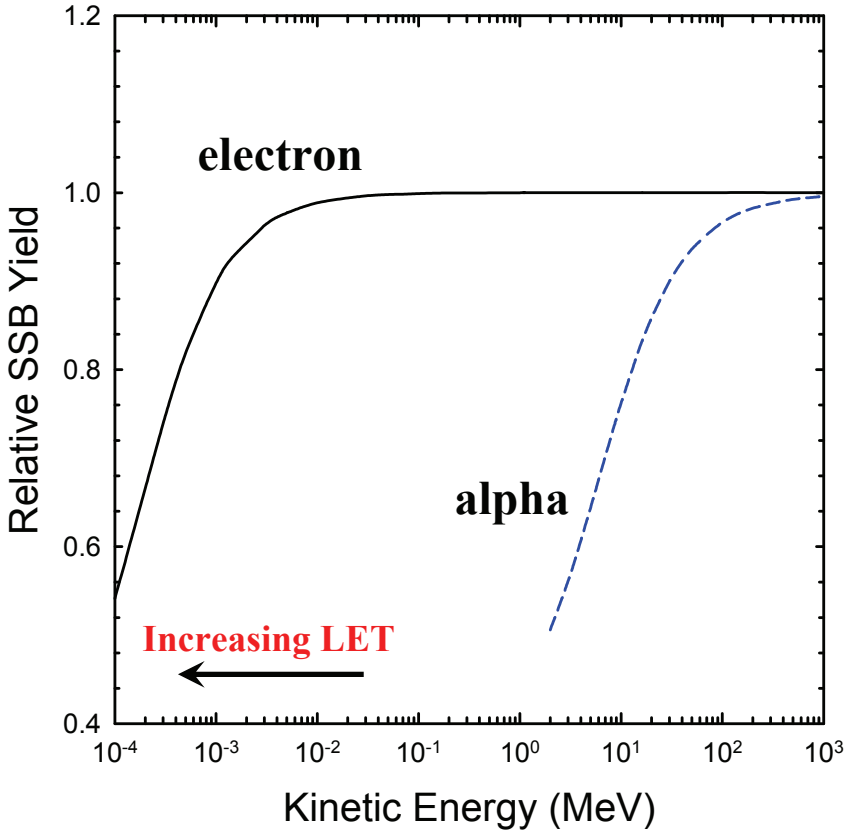


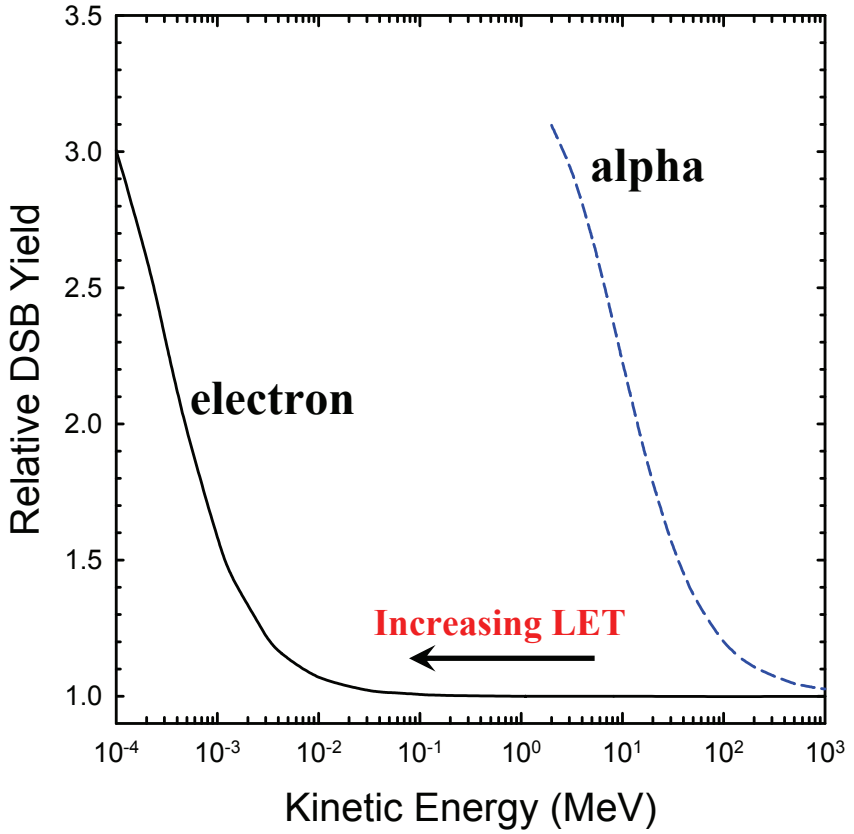
SSB composed of 3 lesions



DSB composed of 4 lesions

# Relative SSB and DSB induction




**Ratio of SSB per DSB decreases with increasing LET**

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)

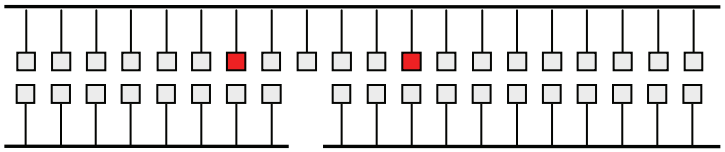
# Cluster complexity and repair

## Expect cluster repair to depend on factors such as

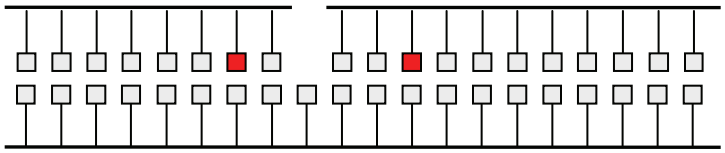
**(1) Number of lesions forming the cluster**

**(2) Types of lesions forming the cluster (**AP site, base damage, strand break**)**

**(3) Distribution of lesions on same and opposed DNA strand**

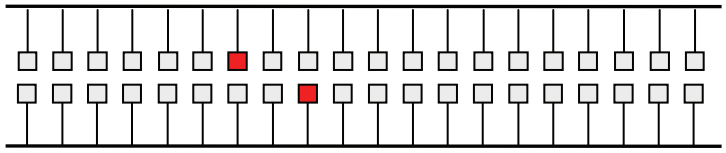


**Cluster composed of 3 lesions**
**(1 strand break and 2 base damages)**

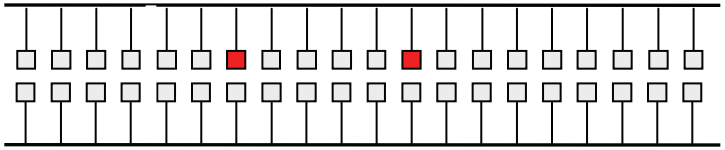


**Cluster composed of 3 lesions**
**(1 strand break and 2 base damages)**

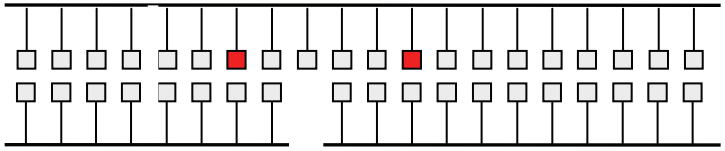
# Number of potential cluster types



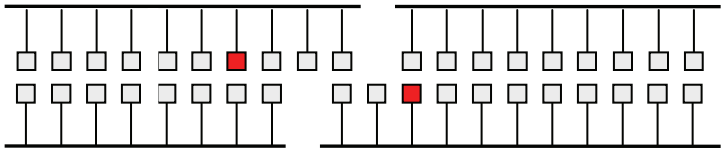
Base damage in opposed strands (2 lesions)



Base loss and damage in same strand
(3 lesions)



Base loss, base damage and a strand break
(4 lesions)



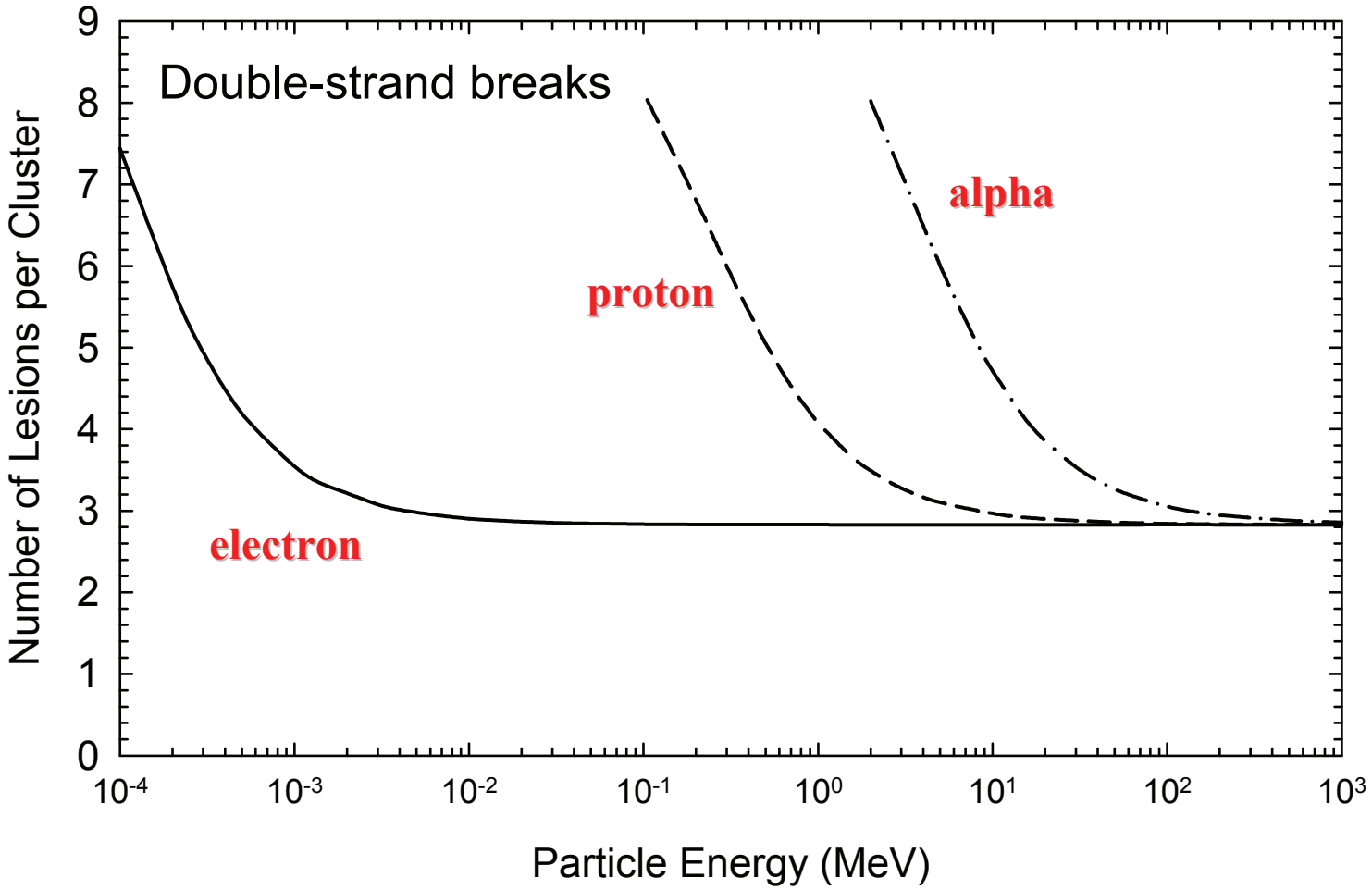
Base loss, base damage and strand breaks
(5 lesions)

**Number of possible ways a 10 bp segment of DNA can be damaged is on the order of $4^{20} = 10^{12}$ possible types of clustered damage**

→ (20 nucleotides and 4 nucleotide "states")

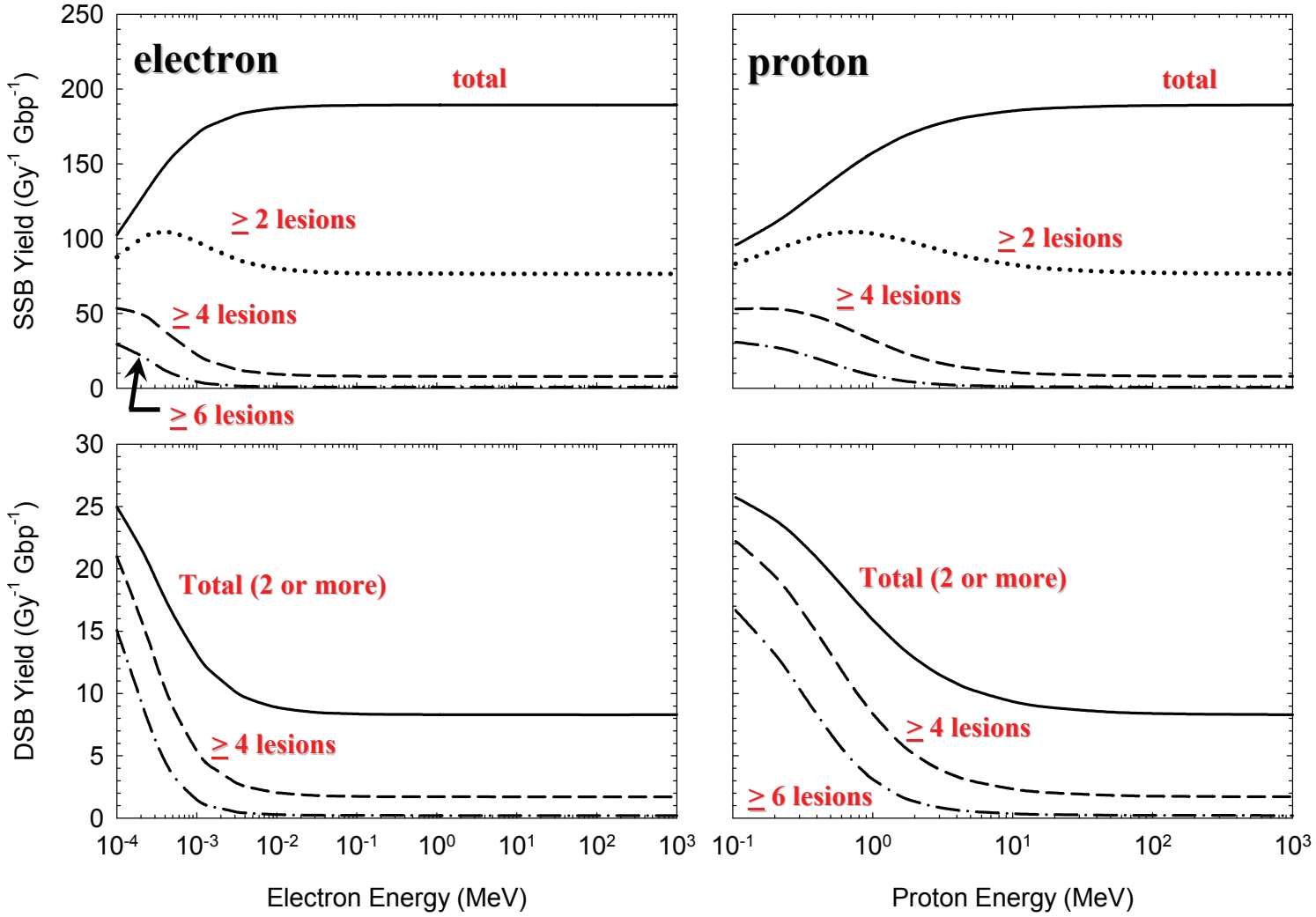
**Monte Carlo simulations are the only available method to sample and quantify the properties of a large number of cluster configurations**

# DSB complexity




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)

# SSB and DSB yield and complexity




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)

# Cluster Complexity and BER Outcome

| Radiation Type | LET (keV/μm) | Induction ($Gy^{-1}$ $Gbp^{-1}$) | | Correct Repair (%) | | SSB→DSB (%) |
|---|---|---|---|---|---|---|
| | | SSB | Base Damage | SSB | Base Damage | |
| 1 MeV $e^-$ | 0.19 | 189.3 | 427.8 | 96.2 | 99.4 | 2.6 |
| 250 MeV $p$ | 0.39 | 189.2 | 427.0 | 96.1 | 99.4 | 2.6 |
| 100 keV $e^-$ | 0.41 | 189.2 | 426.5 | 96.1 | 99.4 | 2.6 |
| 10 keV $e^-$ | 2.30 | 187.2 | 413.8 | 95.8 | 99.3 | 2.8 |
| 1 MeV $p$ | 26.00 | 157.3 | 259.7 | 91.2 | 98.3 | 5.5 |
| 6.29 MeV α | 75.00 | 129.3 | 163.5 | 86.1 | 97.1 | 8.1 |
| 5.49 MeV α | 83.00 | 125.0 | 151.3 | 85.2 | 96.9 | 8.5 |
| 3.5 MeV α | 113.00 | 110.7 | 117.2 | 82.2 | 96.1 | 9.9 |

**MC model for damage induction** → **MC model for base excision repair (BER)** → **Outcome**

R.D. Stewart and V.A. Semenenko, Induction and Repair of DNA Damage Formed by Energetic Electrons and Light Ions, In Handbook of Cancer Models With Applications to Cancer Screening, Cancer treatment and Risk Assessment, W.Y. Tan and A. Yakovlev, Editors. World Scientific Publishing Company, (August 2008). ISBN-13: 978-9812779472.

# Software and Literature Citations

## Monte Carlo Damage Simulation (MCDS)

- Y Hsiao and R.D. Stewart, Monte Carlo Simulation of DNA Damage Induction by X-rays and Selected Radioisotopes. *Phys. Med. Biol.* **53**, 233-244 (2008)
- 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).
- **http://rh.healthsciences.purdue.edu/mcds/**

## 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).
- R.D. Stewart and V.A. Semenenko, Induction and Repair of DNA Damage Formed by Energetic Electrons and Light Ions, In Handbook of Cancer Models With Applications to Cancer Screening, Cancer treatment and Risk Assessment, W.Y. Tan and A. Yakovlev, Editors. World Scientific Publishing Company, (August 2008). ISBN-13: 978-9812779472.
- **http://rh.healthsciences.purdue.edu/mcer/**

**http://rh.healthsciences.purdue.edu/faculty/rds.html**

# Future Direction

- **Predict the yield of clusters that can be detected using new experimental techniques**
  - Fpg, Endo III and Endo IV clusters (**S103 Alex Georgakalis**)

- **Simulate chemical repair and oxygen fixation in a cellular environment (“*oxygen effects*”)**

**(*1*) DNA + ionizing radiation → DNA lesion (*biochemical repair required*)**

**(*2*) DNA + ionizing radiation → DNA· (*various*)**

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**(*3*) DNA· + $O_2$ → DNA-$O_2$· (*“oxygen fixation” – biochemical repair required*)**

**(*4*) DNA· + RSH → DNA (*“chemical repair” – restoration of the DNA**)**

**(*5*) DNA· → DNA lesion (*biochemical repair required*)**

# Acknowledgements


- Vladimir A. Semenenko, Ph.D.
  - Medical College of Wisconsin, Department of Radiation Oncology, Milwaukee, WI
- Alexandros Georgakilas, Ph.D.
  - East Carolina University, Biology Department, Greenville, NC
- Yayun Hsaio, Ph.D.
  - Chung Shang Medical University (Taiwan)
- Anshuman Panda and Victor Yu
  - Ph.D. Students in Medical Physics, Purdue University, School of Health Sciences, West Lafayette, IN