Cell Activation of repair processes followed by cell signalling

Radiation exposure of cell DSBs

Activation of repair processes followed by cell signalling

What structural Changes?

Cell death Mutation Genetic diseases

EURODOS 2013 Barcelona
Some Questions

• In Radiation Physics
  Characterization of radiation field
  Accurate dosimetry

• In Radiation Protection
  Background levels for risk estimation (genetic & cancer)

• In Radiation therapy
  Clinical RBE values

• In Radiation Biology
  Mechanism of radiation induced DNA Damage Response & Repair
  What is the relevant type of damage induced by IR?
Hypothesis

Progress in radiation risk estimation and cancer therapy in the 21st century will be driven by the integration of genomic knowledge with that of DNA Damage, and DNA-repair mechanism.

Most radiation-induced mutations are large multi-gene deletions.

Spontaneous deletions induced in germ cells are more likely to be multi-system developmental abnormalities rather than single gene diseases.

EURODOS 2013 Barcelona

Sankaranarayanan & Nikjoo 2010
Energy density as a function of the mass for which energy density is determined. The horizontal line covers the region in which the absorbed dose can be established in a single measurement.  

Rossi 1968
## Microscopic consequences of 1cGy absorbed dose

<table>
<thead>
<tr>
<th>Radiation</th>
<th>Tissue</th>
<th>Cells</th>
<th>Chromatin fibre</th>
<th>DNA</th>
<th>Mean no. lethal lesions per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-ray</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
<td>~0.001</td>
</tr>
<tr>
<td>Dose uniformity</td>
<td>Uniform dose=1 cGy</td>
<td><del>Uniform dose</del>1 cGy</td>
<td>very non-uniform</td>
<td>very non-uniform</td>
<td></td>
</tr>
<tr>
<td>Mean no. tracks</td>
<td>~10^{11} gm^{-1}</td>
<td>~5000 cell^{-1}</td>
<td>~10^{-6} segment^{-1}</td>
<td>~10^{-6} segment^{-1}</td>
<td></td>
</tr>
<tr>
<td>226Ra 2-4 α particles</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
<td>~0.01</td>
</tr>
<tr>
<td>Dose uniformity</td>
<td>0 - 2 cGy</td>
<td>0 - ~30cGy</td>
<td>0 - ~10^{4} Gy</td>
<td>0-2x10^{6} Gy</td>
<td></td>
</tr>
<tr>
<td>Mean no. tracks</td>
<td>~10^{7} gm^{-1}</td>
<td>~0.26 cell^{-1}</td>
<td>~90% of cells unirradiated</td>
<td>~6x10^{-7} segment^{-1}</td>
<td>~10^{-9} segment^{-1}</td>
</tr>
</tbody>
</table>

Adopted from Goodhead 1987

EURODOS 2013 Barcelona
Why Microdosimetry

1. Dosimetry at a microscopic scale
   - Densely ionising radiation $\rightarrow$ dose inhomogeneity at cellular/subcellular levels
   - Dose calculations based on stopping power are inaccurate at a sub-mm precision.

2. Effective method to characterise the radiation quality of ion beams
   - Linear Energy Transfer (LET): average and macroscopic quantity $\rightarrow$ insufficient as a descriptor of biological effectiveness
   - Better alternative: a quantity related to energy depositions at a scale relevant to biological targets $\rightarrow$ Microdosimetry
Why Microdosimetry

• A better way of characterizing radiation track
• Microdosimetric quantities vary with the size of target volume
• Microdosimetry is a recommended method for characterising radiation quality when biological effectiveness is not well known
• Is there a volume that is most suitable to characterize clinical RBE-values?
Example 1

Energy deposition

Cell Survival

EURODOS 2013 Barcelona

Nikjoo (unpublished)
Absolute Frequency of deposition events of energy $>E$

$\text{Gy}^{-1}\text{cell}^{-1}$

Chromatin size target
25nm x 25 nm

- $1\text{keV e}^-$
- $10\text{keV p}$
- $24\text{keV p}$
- $4\text{MeV }\alpha\text{-particle}$

$\text{f(}>E\text{)}$

Energy(eV)

EURODOS 2013 Barcelona
Frequency of energy greater than $\varepsilon$ imparted to a target per Gy, $f(>\varepsilon)$

C 0.25 MeV/u
C 0.75 MeV/u
C 1 MeV/u
C 3 MeV/u

EURODOS 2013 Barcelona

Liamsuwan 2013 (thesis)
Frequency mean lineal energy \( (y_F) \) vs. LET for protons

**Lineal energy** \( (y) \)

= Ratio of energy imparted by a single track to the mean chord length of the target

**LET** = Ratio of average energy loss of a particle in its path length

---

Liamsuwan et al 2011

EURODOS 2013 Barcelona
Example 2

RBE v W values

Can α-ratio and $y_D$ be correlated?
### Weighting factors in Rad. Protection & Radiation Therapy

<table>
<thead>
<tr>
<th>Particle Type</th>
<th>Weighting Factor ($w_R$)</th>
<th>RBE&lt;sub&gt;clin&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{60}\text{Co }\gamma$/X-rays</td>
<td>1</td>
<td>1.25</td>
</tr>
<tr>
<td>$^{60}\text{Co }\gamma$/Protons</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>$^{60}\text{Co }\gamma$/Neutrons</td>
<td>2.5 -21</td>
<td>3.2</td>
</tr>
<tr>
<td>$^{60}\text{Co }\gamma$/C- ions</td>
<td>20</td>
<td>3.2 ?</td>
</tr>
</tbody>
</table>

Why are they different?

**EURODOS 2013 Barcelona**

Lindborg, Hultqvist, Carlsson, Nikjoo, 2013
<table>
<thead>
<tr>
<th>Radiation quality</th>
<th>α-ratio derived from the LQ relation</th>
<th>$\gamma_D$ Ratio $D=10\text{nm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray/$^{60}\text{Co }\gamma$</td>
<td>1.2</td>
<td>1.16</td>
</tr>
<tr>
<td>$p\ (175\text{ MeV})/^{60}\text{Co }\gamma$ in SOBP at 5cm</td>
<td>1.1</td>
<td>1.05</td>
</tr>
<tr>
<td>$^{12}\text{C SOBP}$/$^{60}\text{Co }\gamma$ centre</td>
<td>2.9</td>
<td>2.2</td>
</tr>
<tr>
<td>$^{12}\text{C SOBP}$/$^{60}\text{Co }\gamma$ distal end</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>$n/^{60}\text{Co }\gamma$</td>
<td>3.8</td>
<td>3.7</td>
</tr>
</tbody>
</table>
Conclusion

• All microdosimetry parameters and distributions can be obtained from absolute frequency of energy depositions in the target under consideration.

• We have calculated such data for electrons, and ions in target sizes 2nm – 100nm
Conclusions 2

1. Microdosimetry is a recommended method for characterising radiation quality when biological effectiveness is not well known (IAEA)
2. Size of the simulated target volume influence microdosimetric quantities.
3. For a simulated volume of about 10-15 nm, the $\alpha$-ratio increases approximately linearly with the $y_D$ ratio for all investigated radiation beams. (Lindborg et al 2013)
4. The correlation between $y$ and $\alpha$ provides the evidence for the use of $y$ to characterise therapy beam when weighting factors to be estimated
5. Low energy electrons play crucial role in characterizing radiation field

EURODOS 2013 Barcelona
I) 4D DESCRIPTION OF RADIATION TRACK \((x, y, z, t)\)
Monte Carlo track simulation at molecular level
With code KURBUC

<table>
<thead>
<tr>
<th>Kurbuc</th>
<th>Uehara &amp; Nikjoo</th>
<th>electron</th>
<th>1992</th>
<th>10eV - 10 MeV</th>
</tr>
</thead>
<tbody>
<tr>
<td>KurbucPits99</td>
<td>Wilson &amp; Nikjoo</td>
<td>ions</td>
<td>1999</td>
<td>≥ 0.3 MeV/u</td>
</tr>
<tr>
<td>Kurbuc_proton</td>
<td>Uehara &amp; Nikjoo</td>
<td>protons</td>
<td>2001</td>
<td>1keV – 1MeV</td>
</tr>
<tr>
<td>Kurbuc_alpha</td>
<td>Uehara &amp; Nikjoo</td>
<td>α-particles</td>
<td>2002</td>
<td>1keV/u – 2MeV/u</td>
</tr>
<tr>
<td>Kurbuc_Chem</td>
<td>Uehara &amp; Nikjoo</td>
<td>chemistry</td>
<td>2006</td>
<td>≥10^{-12} s</td>
</tr>
<tr>
<td>Kurbuc_Neutron</td>
<td>Nikjoo &amp; Uehara</td>
<td>neutrons</td>
<td>2007</td>
<td>thermal - 100 MeV</td>
</tr>
<tr>
<td>Kurbuc_liq</td>
<td>Emfietzoglou et al</td>
<td>electron</td>
<td>2008</td>
<td>10 eV – 10 keV</td>
</tr>
<tr>
<td>Kurbuc_Proton</td>
<td>Liamsuwan et al</td>
<td>protons</td>
<td>2010</td>
<td>1keV – 300 MeV</td>
</tr>
<tr>
<td>Kurbuc_Carbon</td>
<td>Liamsuwan &amp; Nikjoo</td>
<td>C-ions</td>
<td>2012</td>
<td>1keV/u – 10MeV/u</td>
</tr>
</tbody>
</table>
Physical and Radical Chemistry Simulation of Radiation Track at Molecular Level

1 MeV alpha track

$10^{-15}$ s
$N=1016$

$10^{-12}$ s
$N=1491$

$10^{-7}$ s
$N=365$

EURODOS 2013 Barcelona

Uehara & Nikjoo 2006
Radiation track structure

Primary ion

Electrons

δ-ray track

10 MeV/u C⁶⁺

1 μm

EURODOS 2013 Barcelona

MCTS simulation with sensitivity at a single cell level

<table>
<thead>
<tr>
<th>Radiation</th>
<th>Depth at</th>
<th>Dose Gy cm²</th>
<th>Fluence for 1 cGy /cm²</th>
<th>Track # per cell Nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{60}$Co γ rays</td>
<td>5 mm</td>
<td>5.8 x10^{-12}</td>
<td>1.72 x 10^9</td>
<td>6900</td>
</tr>
<tr>
<td>200 MeV proton</td>
<td>Bragg peak</td>
<td>4.7 x10^{-9}</td>
<td>2.13 x 10^6</td>
<td>8.5</td>
</tr>
<tr>
<td>10 MeV/u C ions</td>
<td>Bragg peak</td>
<td>1.3 x10^{-6}</td>
<td>7.69 x10^3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

EURODOS 2013 Barcelona

## Number of events in a 200 MeV full slowing down proton track

<table>
<thead>
<tr>
<th>Event Type</th>
<th>Energy (eV)</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>elastic scattering</td>
<td>200,908</td>
</tr>
<tr>
<td>p</td>
<td>ionization</td>
<td>1,943,795</td>
</tr>
<tr>
<td>p</td>
<td>excitation</td>
<td>1,117,797</td>
</tr>
<tr>
<td>p</td>
<td>e-capture</td>
<td>1,294</td>
</tr>
<tr>
<td>H</td>
<td>elastic scattering</td>
<td>2,259</td>
</tr>
<tr>
<td>H</td>
<td>ionization</td>
<td>1,084</td>
</tr>
<tr>
<td>H</td>
<td>excitation</td>
<td>676</td>
</tr>
<tr>
<td>H</td>
<td>electron loss</td>
<td>1,293</td>
</tr>
</tbody>
</table>

### Secondary electrons

<table>
<thead>
<tr>
<th>Event Type</th>
<th>Energy (eV)</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>ionization (12.62 eV)</td>
<td>12.62 eV</td>
<td>1,977,465</td>
</tr>
<tr>
<td>ionization (14.75 eV)</td>
<td>14.75 eV</td>
<td>1,494,653</td>
</tr>
<tr>
<td>ionization (18.51 eV)</td>
<td>18.51 eV</td>
<td>877,941</td>
</tr>
<tr>
<td>ionization (32.40 eV)</td>
<td>32.40 eV</td>
<td>20,812</td>
</tr>
<tr>
<td>ionization (539.7 eV)</td>
<td>539.7 eV</td>
<td>7,135</td>
</tr>
<tr>
<td>excitation (A₁B₁)</td>
<td></td>
<td>198,989</td>
</tr>
<tr>
<td>excitation (B₁A₁)</td>
<td></td>
<td>648,345</td>
</tr>
<tr>
<td>excitation (Rydberg A+B)</td>
<td></td>
<td>246,620</td>
</tr>
<tr>
<td>excitation (Rydberg C+D)</td>
<td></td>
<td>347,007</td>
</tr>
<tr>
<td>excitation (diffuse band)</td>
<td></td>
<td>1,204,030</td>
</tr>
<tr>
<td>excitation (H* Lyman a)</td>
<td></td>
<td>343,570</td>
</tr>
<tr>
<td>excitation (H* Balmer a)</td>
<td></td>
<td>67,931</td>
</tr>
<tr>
<td>excitation (OH*)</td>
<td></td>
<td>836,387</td>
</tr>
<tr>
<td>sub-excitation electrons</td>
<td></td>
<td>634,450</td>
</tr>
</tbody>
</table>

EURODOS 2013 Barcelona

Liamsuwan et al 2011
Liamsuwan & Nikjoo 2013
### Some physical characteristics of radiation tracks

<table>
<thead>
<tr>
<th>Particle type</th>
<th>Energy (keV)</th>
<th>primary interactions (%)</th>
<th>secondary electron interactions (%)</th>
<th>dE/dx (keV/µm)</th>
<th>Energy in track by primary %</th>
<th>Energy in track by delta electrons %</th>
</tr>
</thead>
<tbody>
<tr>
<td>e^-</td>
<td>1keV</td>
<td>25</td>
<td>75</td>
<td>12</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>e^-</td>
<td>10keV</td>
<td>23</td>
<td>77</td>
<td>2.3</td>
<td>37</td>
<td>63</td>
</tr>
<tr>
<td>e^-</td>
<td>100keV</td>
<td>22</td>
<td>78</td>
<td>0.42</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>Proton</td>
<td>1MeV</td>
<td>21</td>
<td>79</td>
<td>27</td>
<td>30%</td>
<td>70%</td>
</tr>
<tr>
<td>Proton</td>
<td>20MeV</td>
<td>20</td>
<td>80</td>
<td>2.5</td>
<td>34%</td>
<td>66%</td>
</tr>
<tr>
<td>Proton</td>
<td>200MeV</td>
<td>20</td>
<td>80</td>
<td>0.4</td>
<td>30%</td>
<td>70%</td>
</tr>
<tr>
<td>Carbon</td>
<td>20MeV/u</td>
<td>20</td>
<td>80</td>
<td>93</td>
<td>20%</td>
<td>80%</td>
</tr>
</tbody>
</table>

Nikjoo and Lindborg 2010
Nikjoo & Goodhead 1989
EURODOS 2013 Barcelona
Conclusions 3

• Radiation track description in space and time is possible for electrons and ions in homogenous biological media

• How good is the technology?
Electron energy loss in condensed matter

- Inelastic interactions with atomic electrons leading to electronic excitations (including ionizations) is the most important energy-loss mechanism for electrons at all energies of practical interest.

- Electronic excitations depend not only upon the atomic composition of the system but also upon its state of aggregation (gas vs. solid or liquid).

Example: single-collision energy-loss spectrum of vapor vs. liquid water

The discrete excitation peaks of the vapor are suppressed in the liquid

The liquid spectrum is dominated by a broad peak at ~20 eV

Differences vanish at high energy transfer

EURODOS 2013 Barcelona
Liquid vs. Ice data

The IXS data are in better agreement with the ice data.

EURODOS 2013 Barcelona

Emfietzoglou et al Nikjoo, 2012
Electron stopping power in liquid water

**Graph:**
- **X-axis:** Electron energy (eV)
- **Y-axis:** Stopping power (eV/nm)
- **Legend:**
  - ICRU '70
  - Kutcher & Green '76
  - Sugiyama '85
  - LaVerne '86
  - Ritchie '86
  - Ashley '88
  - Luo (ice) '91
  - Kaplan '91
  - Zaider (ice) '94
  - Watt '96
  - Dingfelder '98
  - Akkerman '99
  - Pimblott '02
  - Tan '04
  - Gumus '05
  - Akar '05
  - Tung '06
  - Bethe (l=75eV)
  - Ours

**Conference:** EURODOS 2013 Barcelona

**Authors:** Emfietzoglou et al. 2012
Conclusions 4

Physics of electron energy loss in liquid water

- For electrons >200 eV optical-data model calculations for the IMFP are not very sensitive to the choice of the physics model used
  - The differences between models are less than ~10%

- For electrons <200 eV the IMFP is very sensitive to the choice of physics model
Conclusions 4
Is Trajectory Simulation Correct?

An explicit trajectory picture of the transport of low energy (< 1 keV) electrons in liquids and amorphous solids is certainly not valid according to the Heisenberg uncertainty principle. In fact, it does not correspond to physical reality. But, a trajectory treatment provide a good approximation of multiple quantum scattering down to electron energies in the order of $\sim 10$ eV, due to the incoherence introduced by a randomlike structure of the medium and to the presence of multiple inelastic scattering.

EURODOS 2013 Barcelona

Liljequist and Nikjoo 2013
Conclusions 5

a. We now have the technology to describe physical and chemical interactions of electrons and ions at molecular level. (track segment and full-slowing down)

b. Low energy electrons <100eV are responsible for most of energy depositions by a track

c. Although we know a lot about physics of radiation track but at energies below 100eV our understanding is incomplete and uncertain, especially in condensed matter

d. Microdosimetry considerations proves LET is not a good parameter for characterising radiation track
II) DNA DAMAGE
DNA Damage in human genome

Intersecting domains:
70, 44, 18, 13, 20

Nikjoo & Girard 2012
Goorely, Terrissol, Nikjoo 2008

30 nm fibre loops from a ‘factory’

HPRT gene ~ 40 kbp

<1 loop> ~ 86.5 kbp ~ 5x10^6 atoms

EURODOS 2013 Barcelona

1 factory ~ 50x10^6 atoms

Umrania, Nikjoo, Goodfellow 1995

Nikjoo, Girard, 2012
Cell Response to Radiation: I- track interaction with the genome

Track of 70 keV electron

Ionization & excitation

Water of hydration

EURODOS 2013 Barcelona

Watanabe & Nikjoo 2002
Cell Response to Radiation: Yield of SSB & DSB

EURODOS 2013 Barcelona

Charlton, Humm, Nikjoo, 1989
Nikjoo et al., 2001
Low-LET
Complex DSB ~20%

High-LET
Complex DSB ~70%

EURODOS 2013 Barcelona
SSB, DSB, & BD in cell nucleus from a single track of 0.5 MeV/u proton

TOTAL ENERGY = 178.  TRACK: 1,  DOMAIN: 10,  FACTORY: 115,  LOOP: 6
00276  43609  43642  46495
....1................................1- [x = SSB]
.....                                      [1 & - not an SSB]
.....--
1.....1...x...1x................................-

TOTAL ENERGY = 331.  TRACK: 1,  DOMAIN: 3,  FACTORY: 124,  LOOP: 9
00394  00480  00529  00575  00604  01426  01513  01610  79795
..x.x..  .  ..     ..... 1................................ 1..1
....1  1 1 ..... 1 x..  ...................................x  ......
...x..  .  .  ..... 1 ...  ...................................
...-x..  .  .  1.x..  .1-  ................................-

TOTAL ENERGY = 179.  TRACK: 1,  DOMAIN: 3,  FACTORY: 125,  LOOP: 9
00802  01662  01959  03089  04140  04172  05255  05283  06381
  .  . ................................................. 1 -...1..........-..  .  .  .
  .  . ................................................. 1...-  1-......-..  -..  1
  .  . ................................................. 1...-  1-......-..  -..  .
x  1 1..........................-1............ -.................. 1 .-

TOTAL ENERGY = 94.  TRACK: 1,  DOMAIN: 90,  FACTORY: 28,  LOOP: 11
01661  01756  02445  02592  02672
... .  .  .........
... .  .  x.....
... .  x  ..-...
1  .  .  ....x -
DSB-Repair Kinetics

NHEJ
- Ku70/80
- DNA-PKcs
- Artemis
- Ligase IV

HR
- MRN, RPA, BRCA1, BRCA2, RAD52, RAD51, RAD54, Helicase, Ligase

SSA
- MRN, RPA, ERCC1/XPF, RAD52, Ligase

MMEJ
- MRN, PARP-1
- FEN1, Polymerase, Ligase

EURODOS 2013 Barcelona
### Biochemical repair of initial damage

<table>
<thead>
<tr>
<th>Step Description</th>
<th>Elapsed Time (min)</th>
<th>Elapsed Time (Average DSB&lt;sub&gt;c&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ku70/80 finds the DSB ends and translocates inward</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>DNA-PKcs is recruited to the damage site</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>First autophosphorylation of DNA-PKcs</td>
<td>4.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Second autophosphorylation of DNA-PKcs</td>
<td>7.8</td>
<td>7.6</td>
</tr>
<tr>
<td>End processing by Artemis</td>
<td>187.3</td>
<td>184.1</td>
</tr>
<tr>
<td>Gap filling by Polymerase λ - μ</td>
<td>339.5</td>
<td>300.9</td>
</tr>
<tr>
<td>Ligation of complex DSB</td>
<td>391.9</td>
<td>348.7</td>
</tr>
<tr>
<td>Repair time (min) for complex damage</td>
<td>391.9</td>
<td>348.7</td>
</tr>
</tbody>
</table>
Radiation Biophysics Group (RBG)

Hooshang Nikjoo, Professor, Group Leader

Peter Girard: Radioprobing/Molecular computational studies
Reza Taleei: DNA Damage-Repair
Thiansin Liamsuwan: Physics and track simulation
Lennart Lindborg: Microdosimetry
Alfredo Metere: Genome Technology/IT
Shirin Rahmanian: DNA damage response & repair

Scientific Collaborators

Dimitris Emfietzoglou: Medical Physics, Ioannina Medical School
K. Sankaranarayanan: Toxicogenetics Department, Leiden
Shuzo Uehara: School of Health Sciences, Kyushu

EURODOS 2013 Barcelona