Mechanistic modelling of DNA Damage and Repair: Application to VHEE

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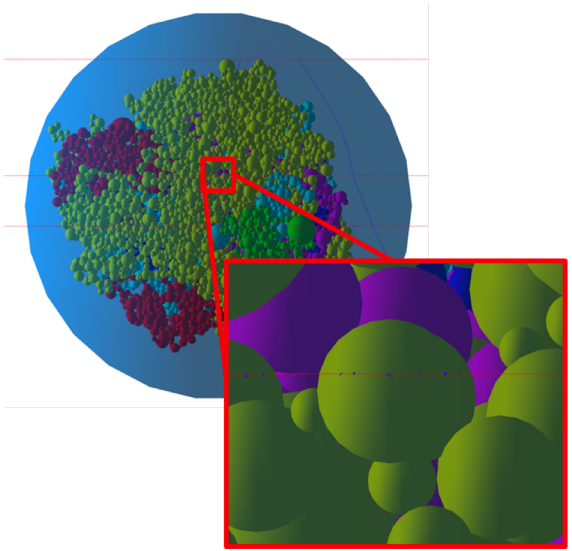
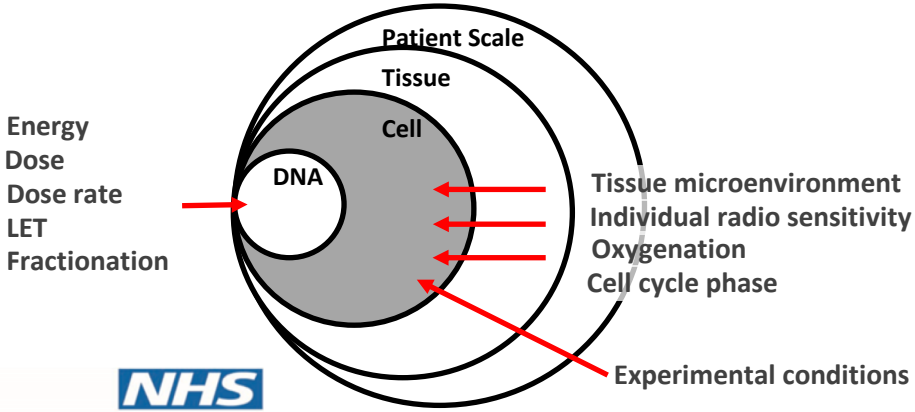


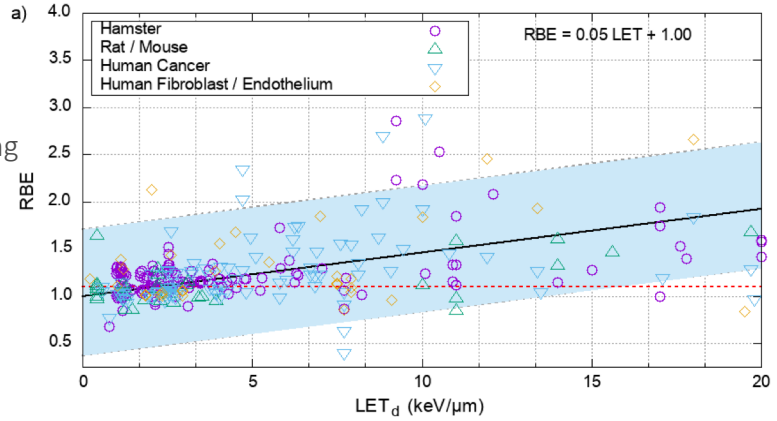
ANPP (Accelerator, Nuclear and Particle Physics) Division Seminar series,

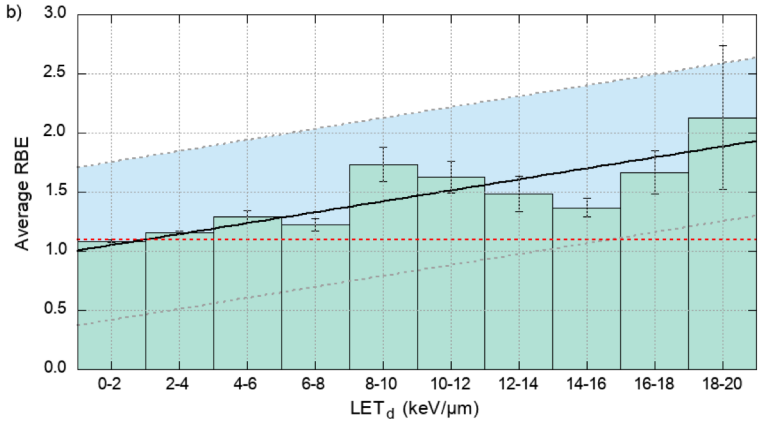
27th November 2020

Evidence for Proton RBE (survival)

- Proton RBE = 1.1
- No significant clinical evidence to suggest under- or over-dosing using constant RBE
  - Emerging evidence:
    Underwood *et al.,* Red Journal, 2018
- Significant amount of *in vitro* evidence to support variable RBE in proton therapy
  - Paganetti metareview publications, PIDE database (GSI)



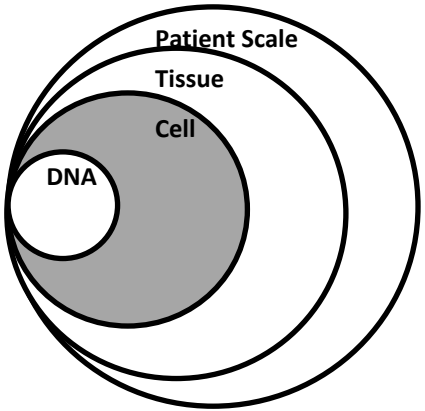




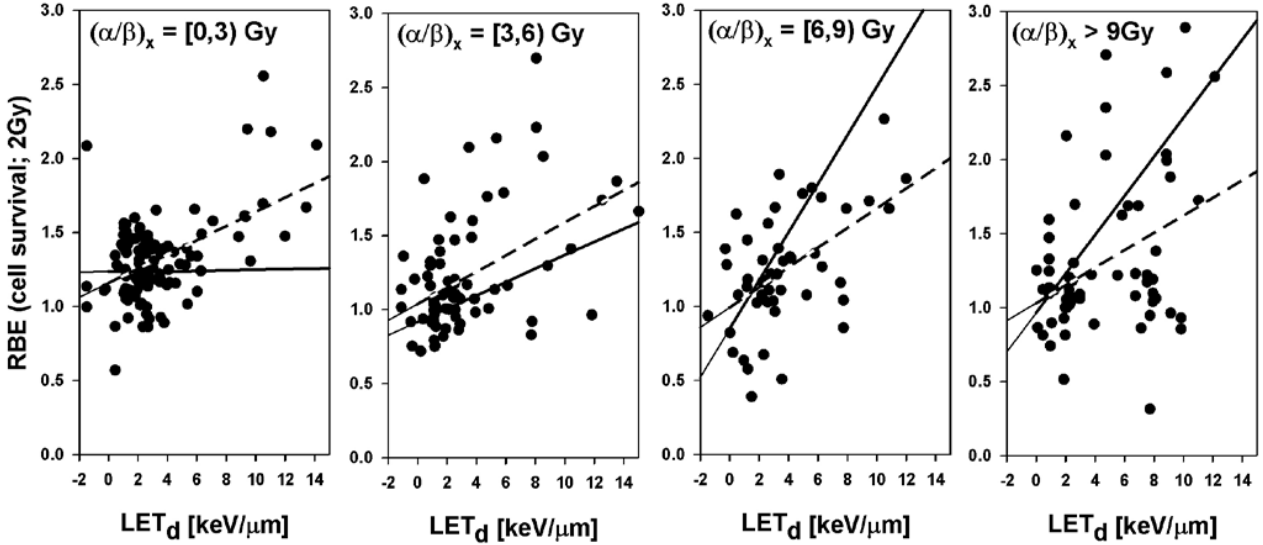
Data reproduced from Paganetti, Phys. Med. Biol., 2014

Why do we need a model?

We don't understand survival following radiation at a cell level



Proton RBE (survival) – in vitro



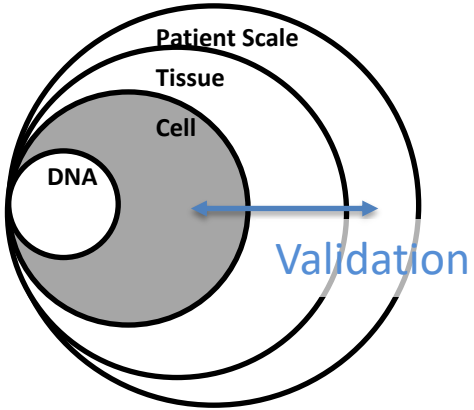
Paganetti, H. Relative Biological Effectiveness (RBE) Values for Proton Beam Therapy. Variations as a Function of Biological Endpoint, Dose, and Linear Energy Transfer. *Physics in Medicine and Biology* **2014**, *59*, R419–R472.

Cell scale approaches to Proton RBE

Phenomenological models

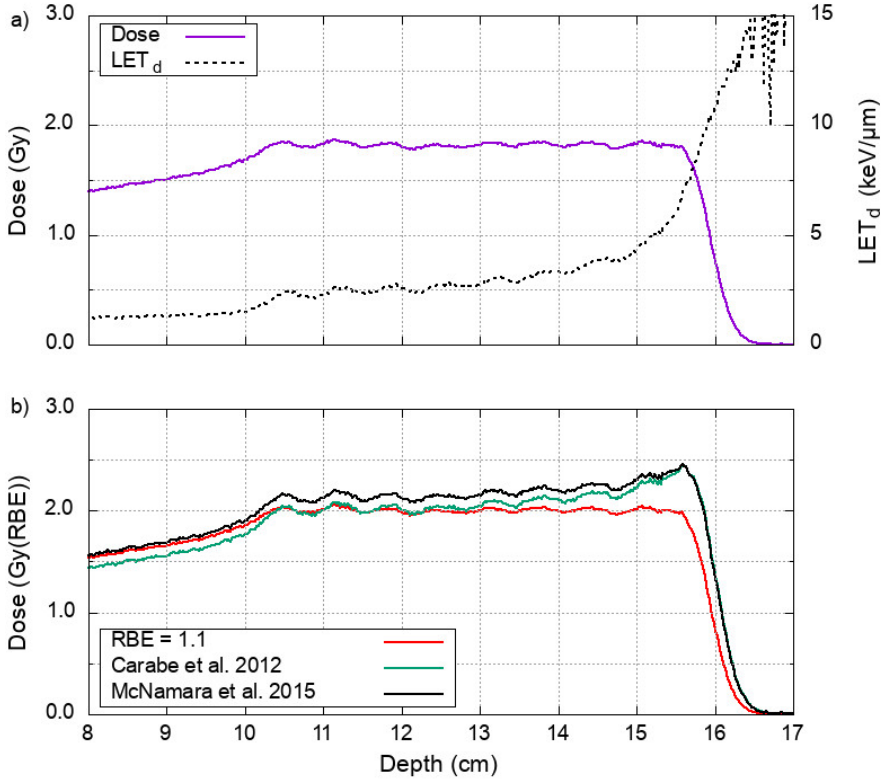
$$RBE = \frac{\sqrt{(\alpha/\beta)_x^2 + 4(\alpha/\beta)_x \boldsymbol{RBE_{max}} D_p + 4(\boldsymbol{RBE_{min}})^2 D_p^2} - (\alpha/\beta)_x}{2D_p}$$

RBE_{max} and RBE_{min} are fit to experimental data for cell survival



Many models proposed, but hard to validate using post-treatment imaging.

A major challenge to gain clinical confidence.
(Limited evidence for RBE in vivo)



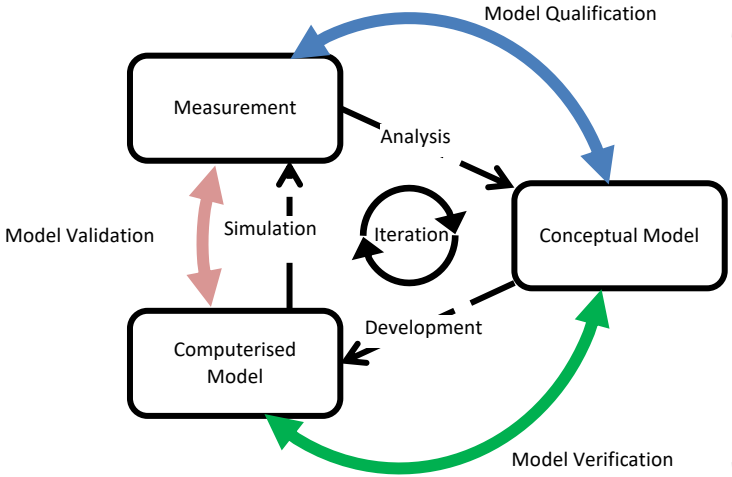
McNamara et al., Phys. Med. Biol., 2015
Carabe et al., Phys. Med. Biol., 2012

We need to understand what happens before cell death:

What are the mechanisms?

The University of Manchester

The Precise Group

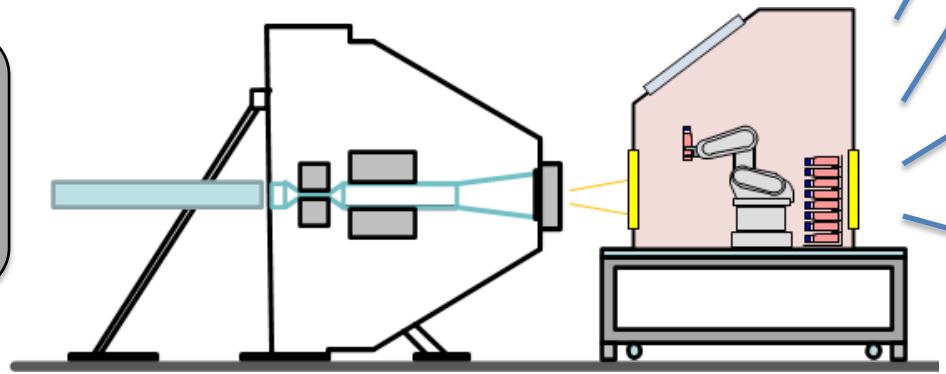


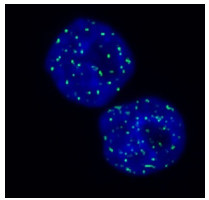
Clinical Translation

Multi-assay validation

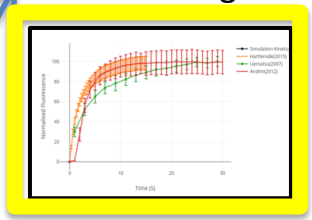
Reduction of errors
- Equipment designed for experimental need

Understanding the error chain
- What is the required experiment to reduce overall uncertainty?



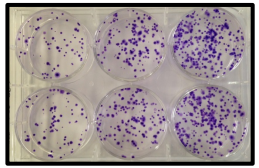


Foci counting



Protein Kinetics

Chromosome Aberrations

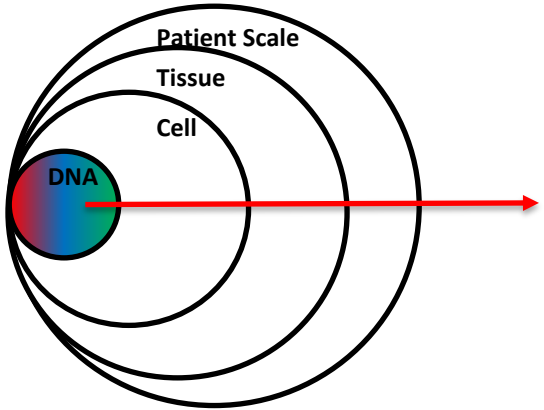


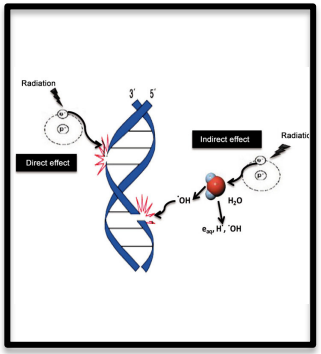
Cell survival

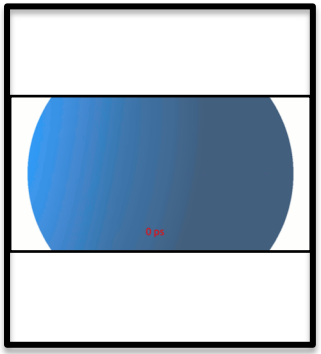
NHS The Christie NHS Foundation Trust

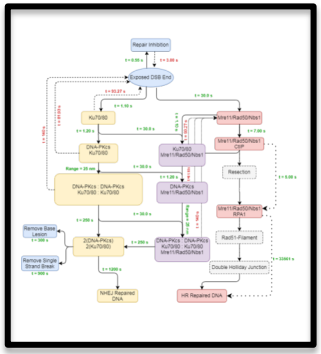
What happens at the DNA level?

3 stages to mechanism of DNA response









Physics

Radiation-Chemistry

Repair

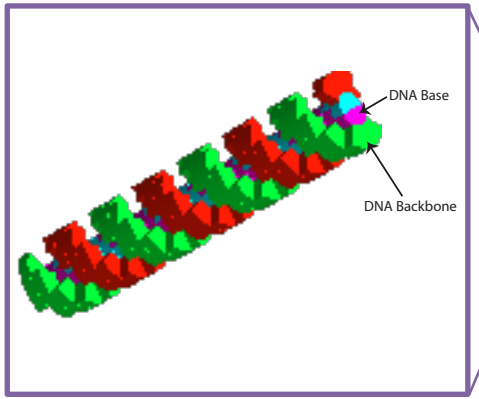
Ionisations

Strand breaks

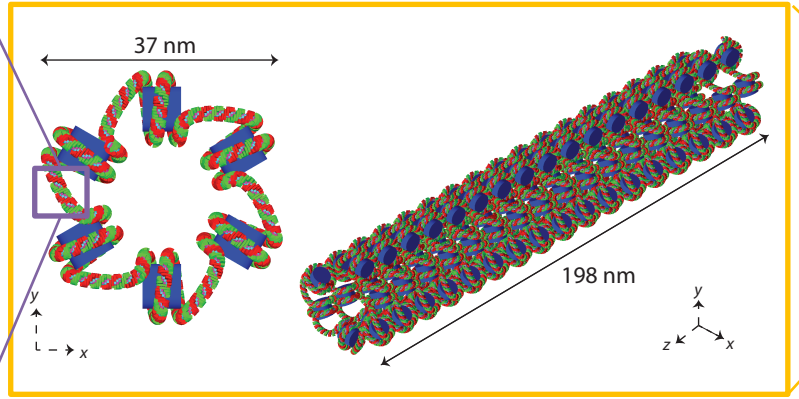
Repair fidelity

DNA Damage

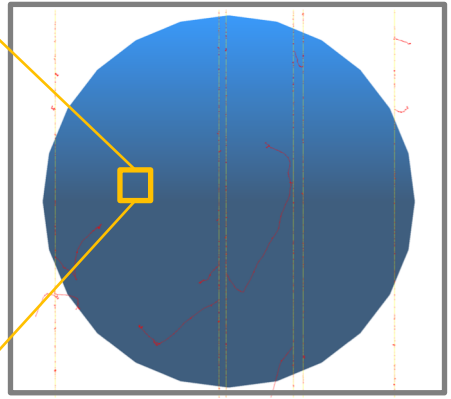
DNA Double Helix



Chromatin Fibre

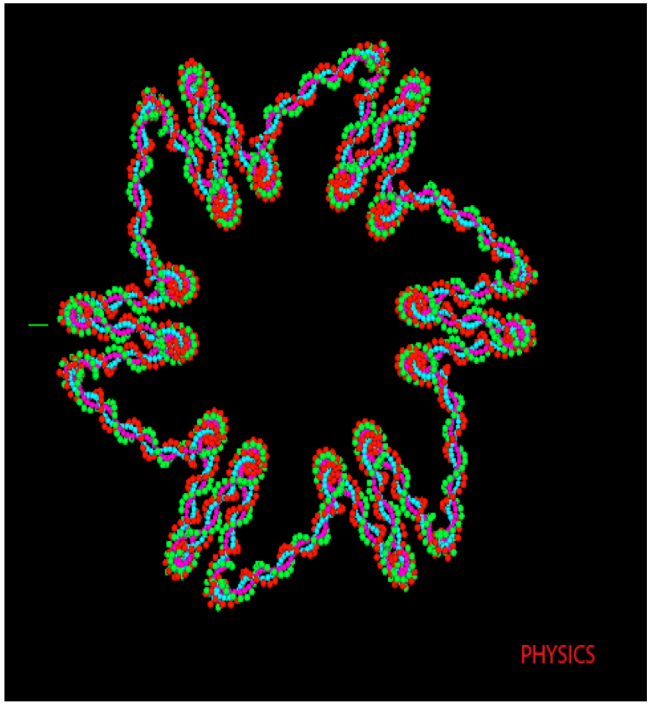


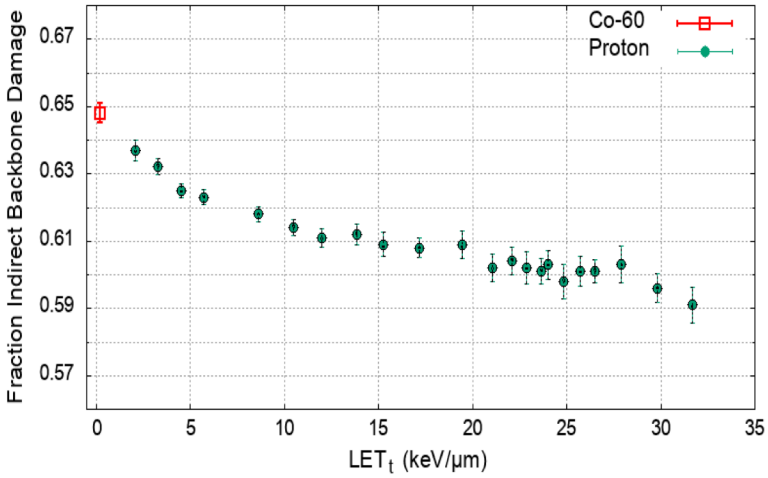
Cell Nucleus



- Geant4-DNA track structure simulation – protons and other ion species
- Energy deposition in DNA can cause a strand break – Mechanism fit to experimental data on plasmid irradiation
- OH radicals diffusing to DNA have a probability to cause a strand break – Mechanism fit to proportions proposed in literature
  - Damage mechanisms are experimentally measurable (experiments underway)
- Breaks on opposite strands separated by 10 bp or less cluster to form a DSB
- Model predicts DSB complexity and gives 4D map of position

Modelling Direct and Indirect DNA damage





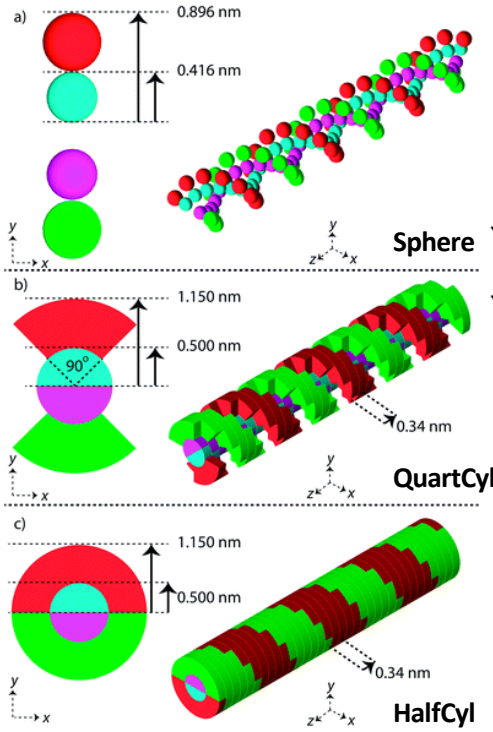
- For Co-60 irradiation 65% of the strand breaks are from indirect effects*

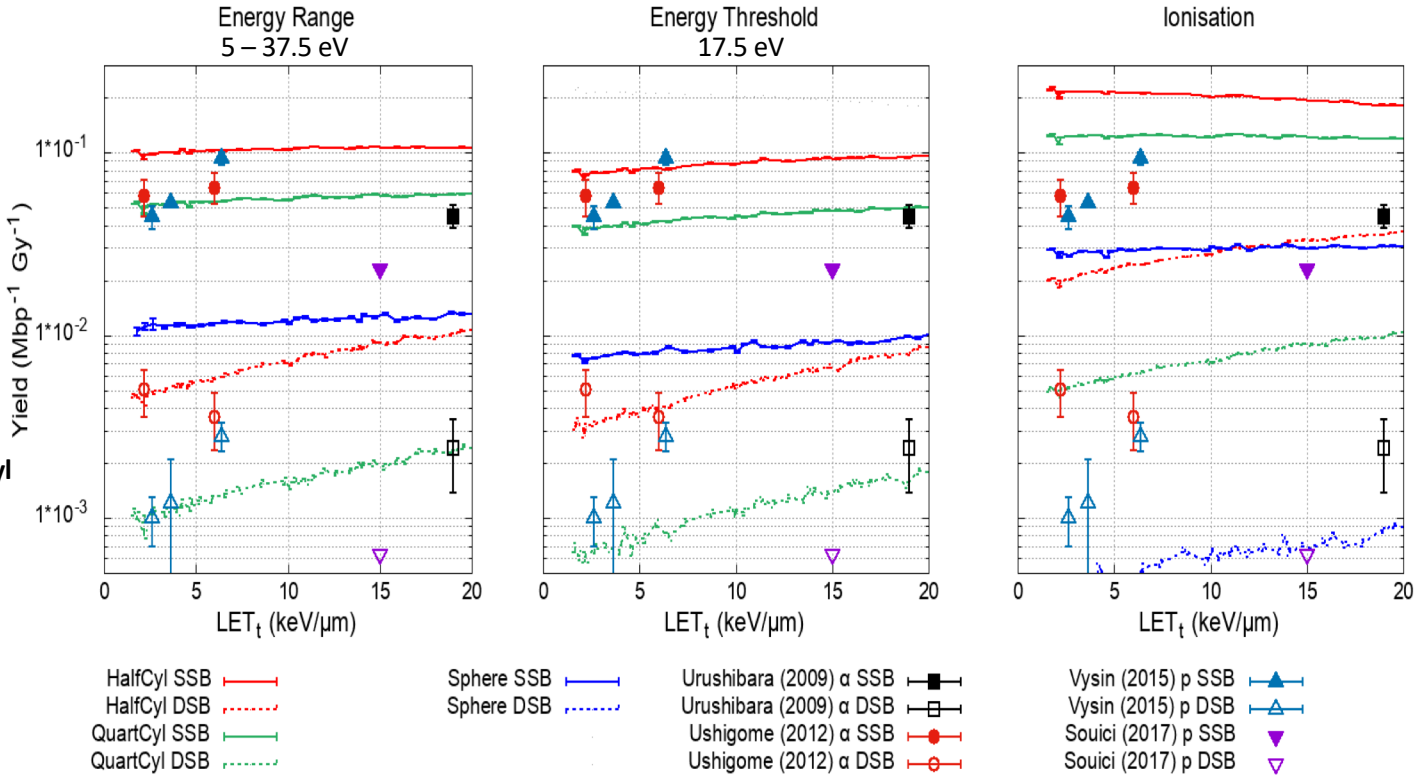
Modelling Assumption

- If an OH radical steps into a DNA backbone it reacts, set a probability that the reaction causes damage
- P=0.5 gives 65% indirect damage

**Ward, Radiat. Res., 1985*

Is the damage model accurate?
Plasmid data:





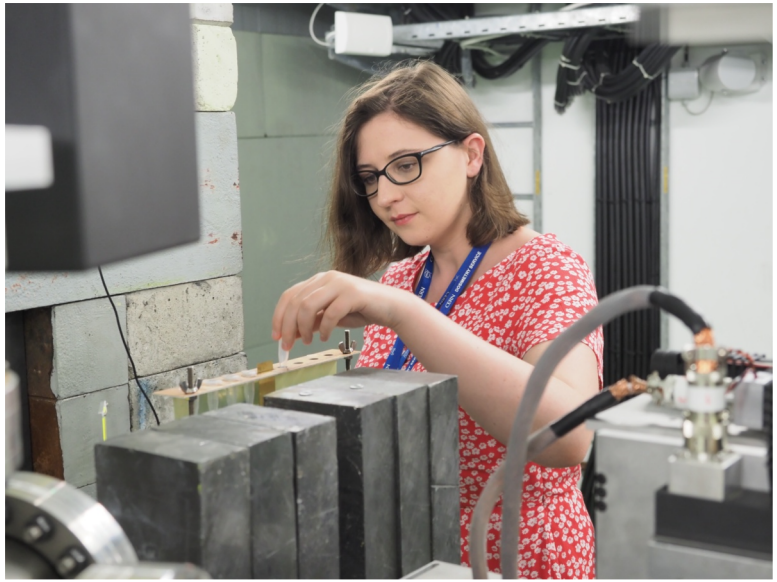
A quarter cylinder DNA model with direct strand breaks determined by an energy range probability most closely reproduces experimental data.

Henthorn, N.T., Warmenhoven, J.W., Sotiropoulos, M., Aitkenhead, A.H., Smith, E.A.K., Ingram, S.P., Kirkby, N.F., Chadwick, A.L., Burnet, N.G., Mackay, R.I., Kirkby, K.J. and Merchant, M.J.; Clinically relevant nanodosimetric simulation of DNA damage complexity from photons and protons; **RSC advances**; **2019**.

VHEE Plasmid irradiations

Kristina Small's PhD work!

- Successful series of plasmid experiments performed with 6-15 MeV electrons (Christie), 100-200 MeV (CLEAR) and ^{60}Co photons (DCF).
- Following tests in Dec 2018, beam exploitation planned at CLARA (20-50 MeV) in Apr 2020 – postponed due to COVID-19



Small et al, 2020, Under review, Scientific Reports

Plasmid Irradiation Experiments with VHEE

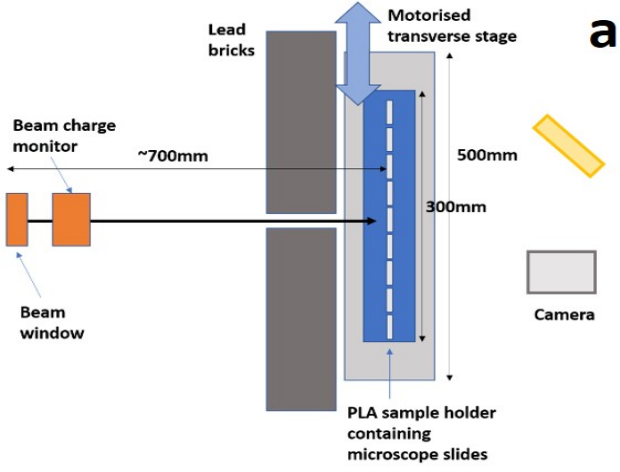
Experiment Aims

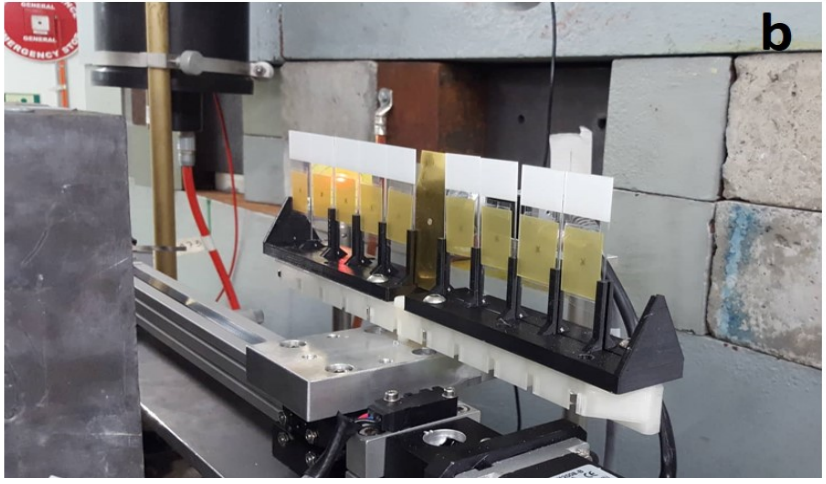
- Measure DSB yield following dry and wet plasmid irradiation – use to calculate VHEE RBE_{DSB}
- Variation of irradiation dose rate – evidence of FLASH effect at nanoscale?

Experiment Plan

- Dry plasmid samples on glass microscope slides irradiated at 20-40 MeV, with doses of 0-6000 Gy delivered
- Wet plasmid samples in 1.5ml Eppendorf tubes irradiated at 20-40 MeV, with doses of 0-50 Gy
- Irradiated plasmids analysed through agarose gel electrophoresis at the Oglesby Cancer Research Centre (Manchester)

Dry Setup





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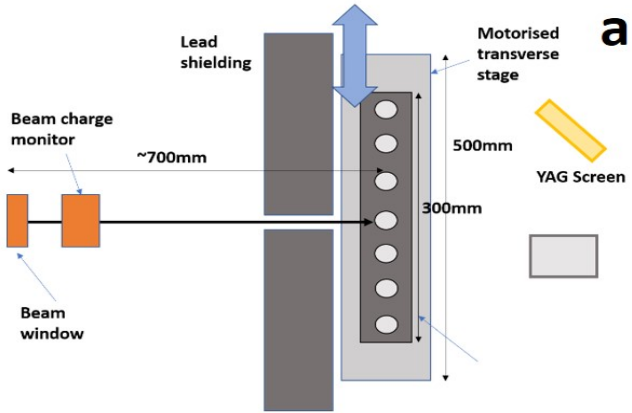
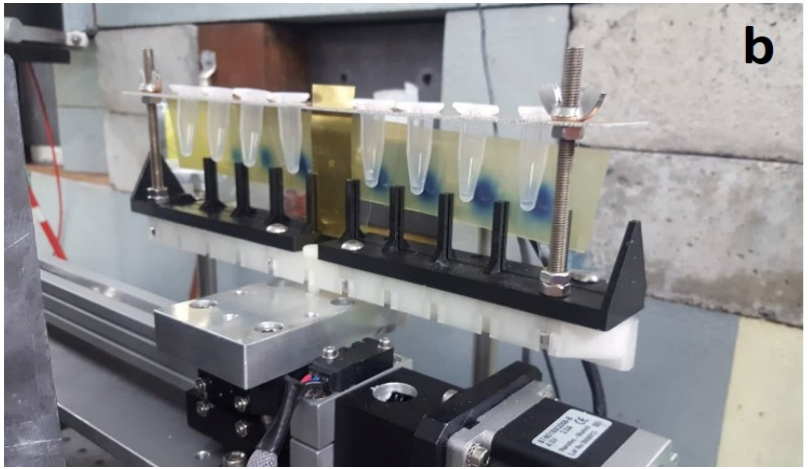
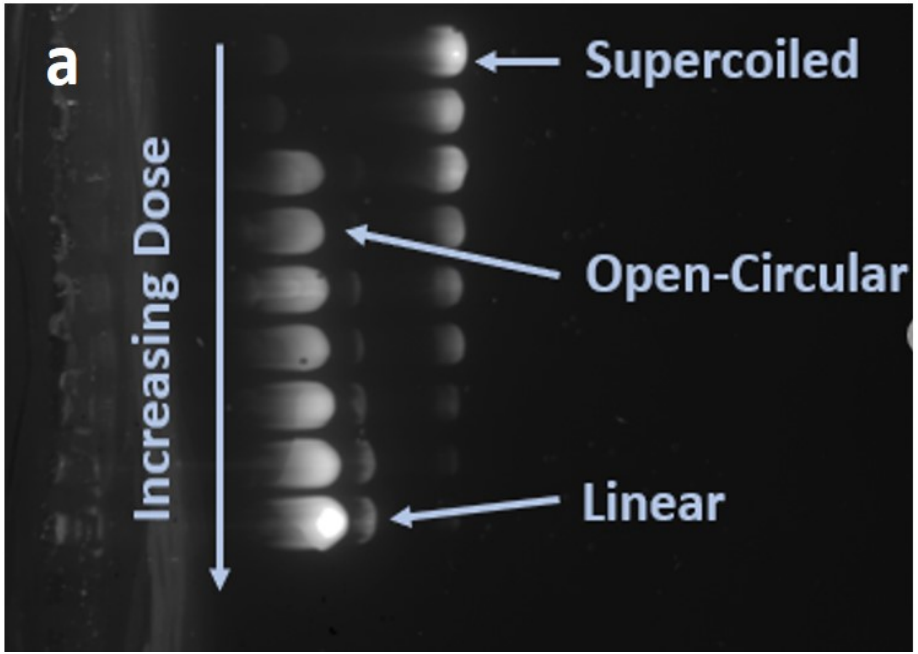
Wet Setup



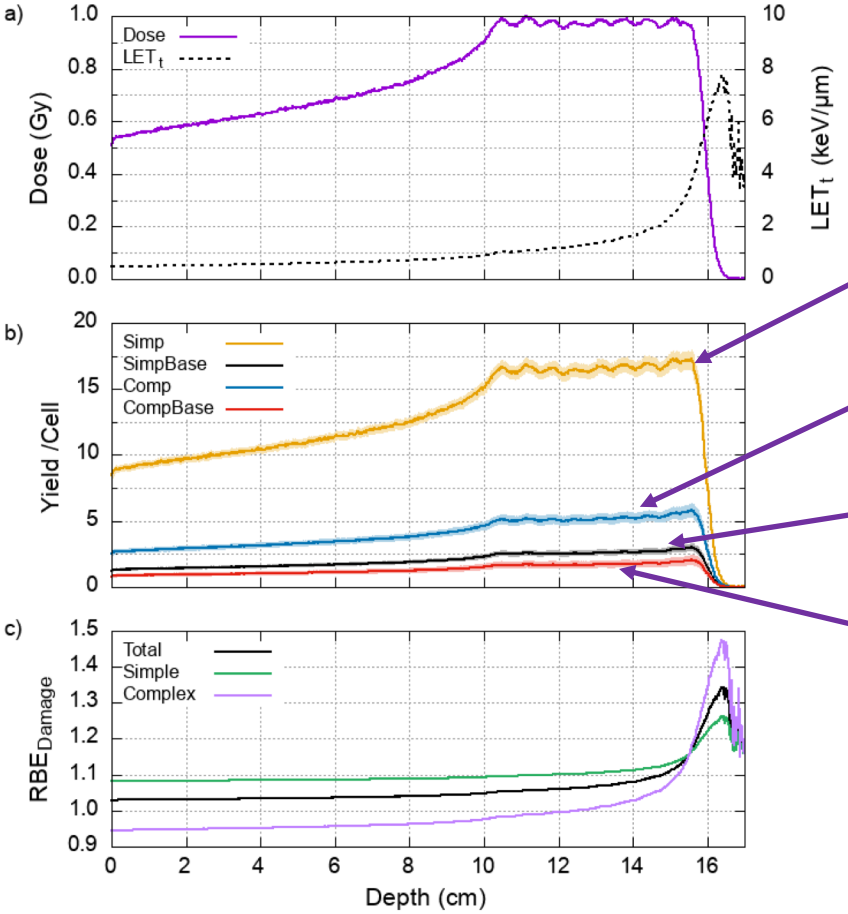


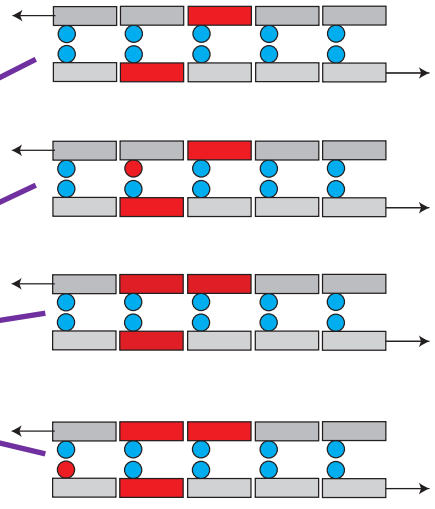
Plasmid Analysis – Agarose Gel Electrophoresis



- Unirradiated plasmid DNA exists in an undamaged, or supercoiled state.
- Open-circular plasmid results from a SSB, due to relaxation of the SC DNA.
- DSBs are detectable as linear forms of plasmid.

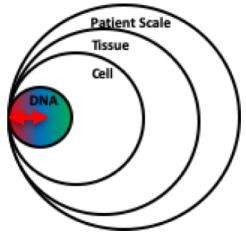
Protons: RBE of Damage Complexity





"Simple"

"Complex"



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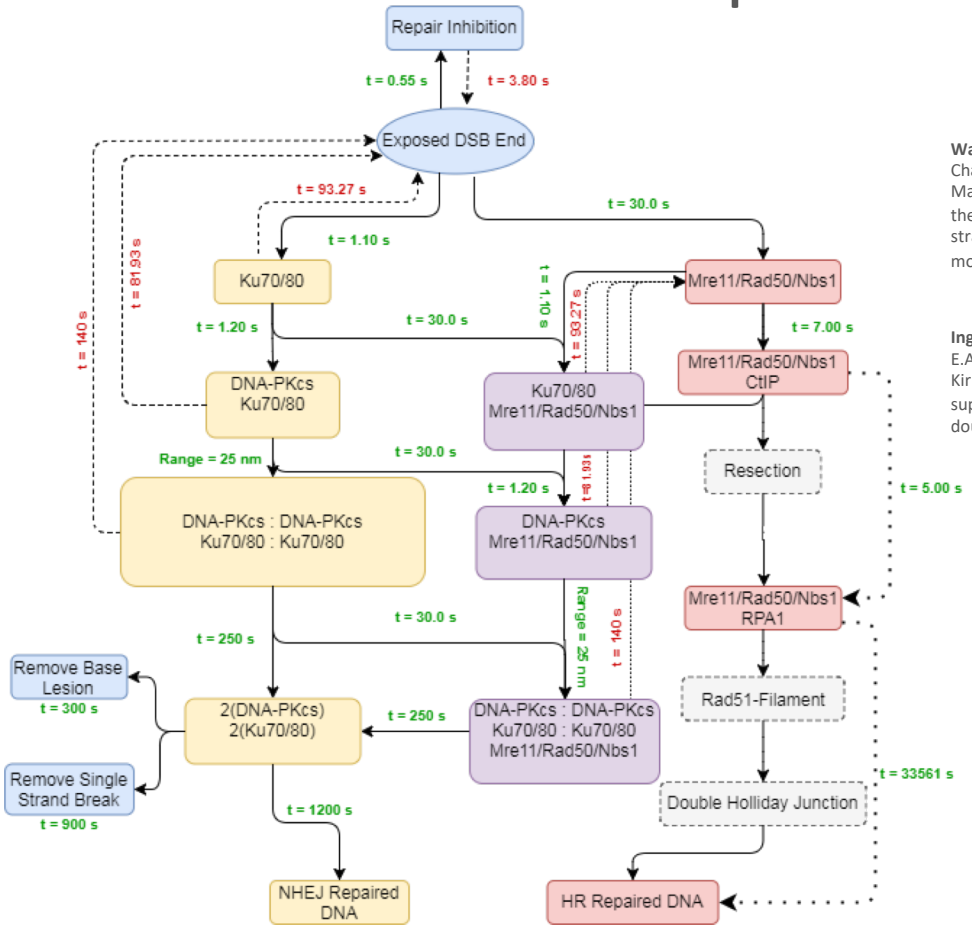
DNA Repair

DaMaRiS – The DNA Mechanistic Repair Simulator



Warmenhoven, J.W., Henthorn, N.T., Ingram, S.P., Chadwick, A.L., Sotiropoulos, M., Korabel, N., Fedotov, S., Mackay, R.I., Kirkby, K.J. and Merchant, M.J.; Insights into the non-homologous end joining pathway and double strand break end mobility provided by mechanistic *in silico* modelling; **DNA repair**; **2020**.

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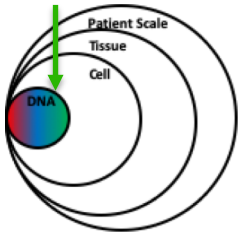
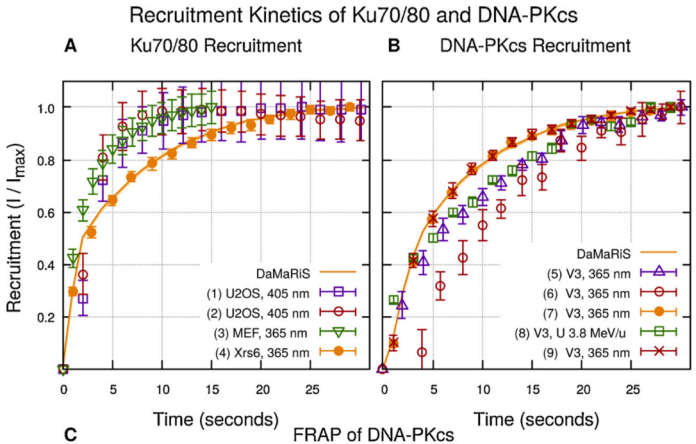
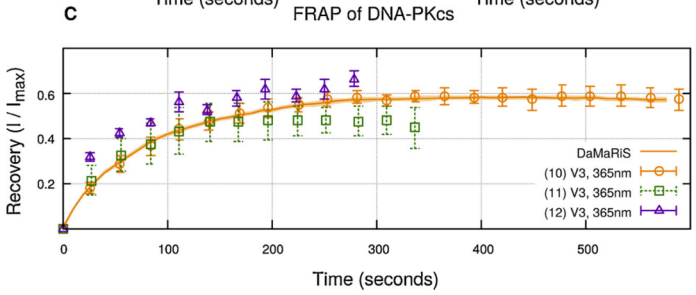
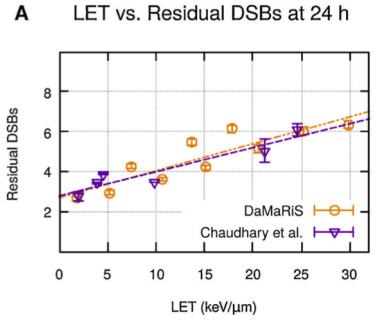


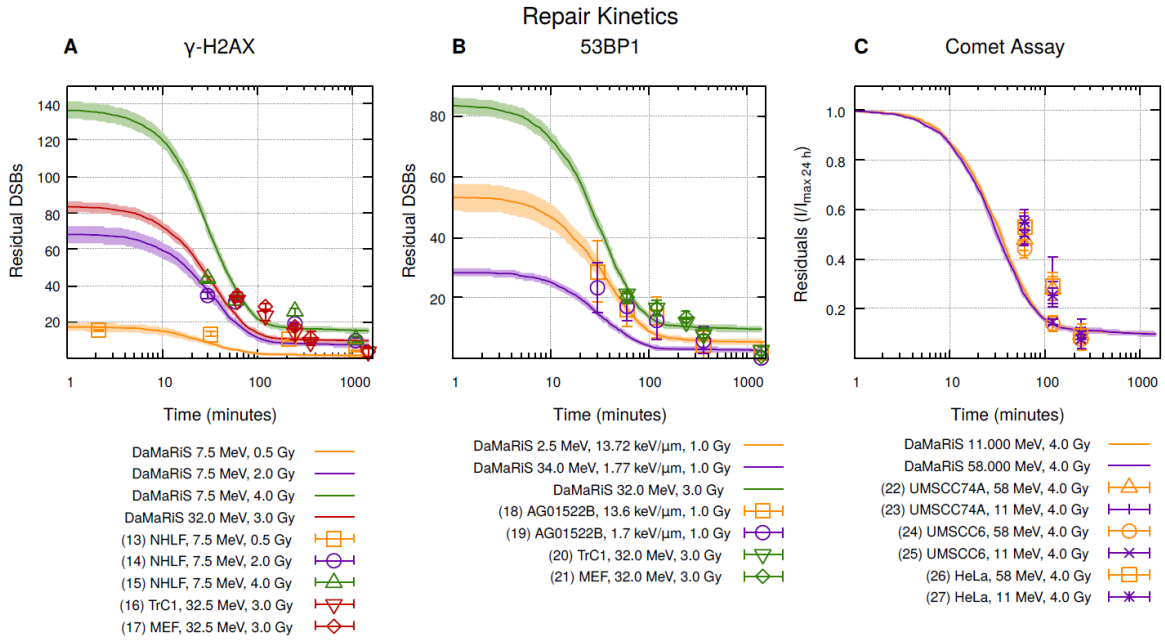
DNA Repair:
Fitting Protein Kinetics

The Precise Group









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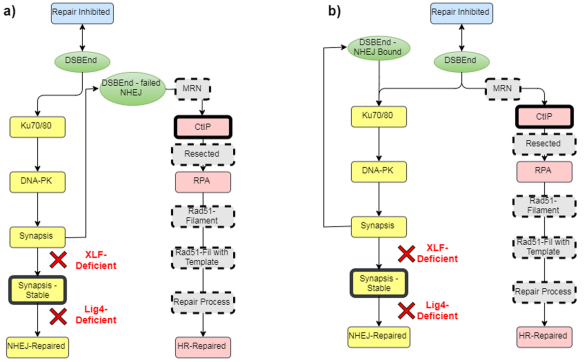
DNA Repair: Pathway interactions

The University of Manchester

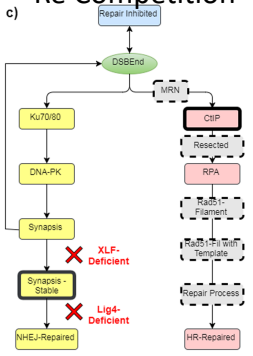
The Precise Group

NHEJ First

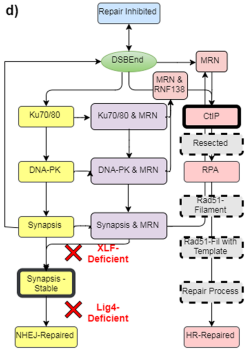


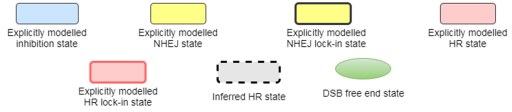
Competition

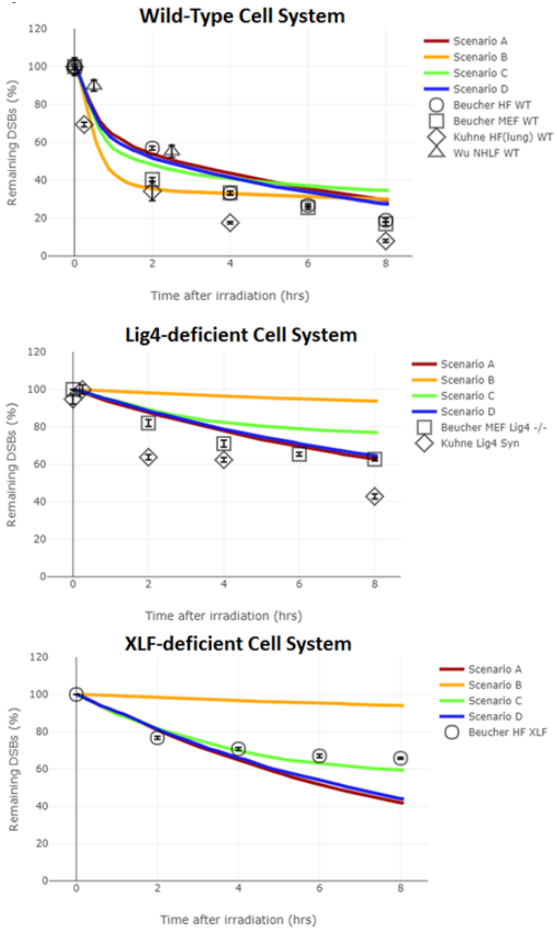
Re Competition

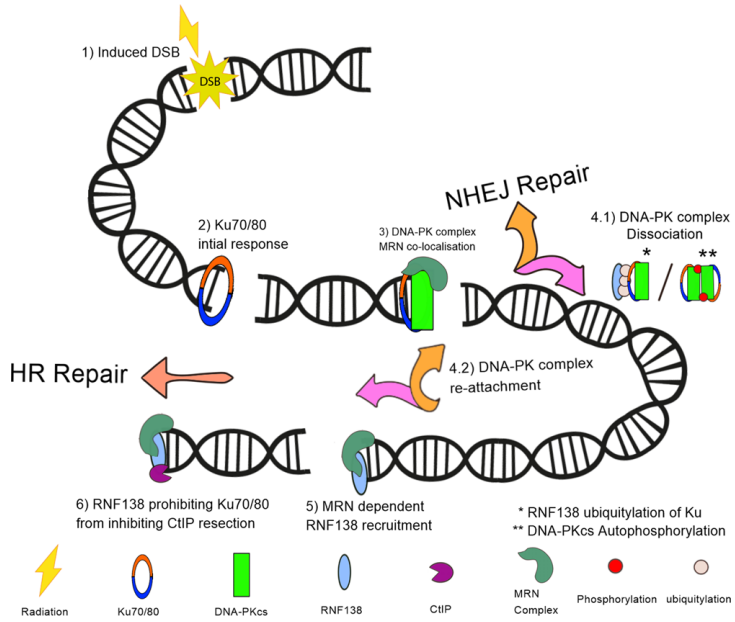


Entwined









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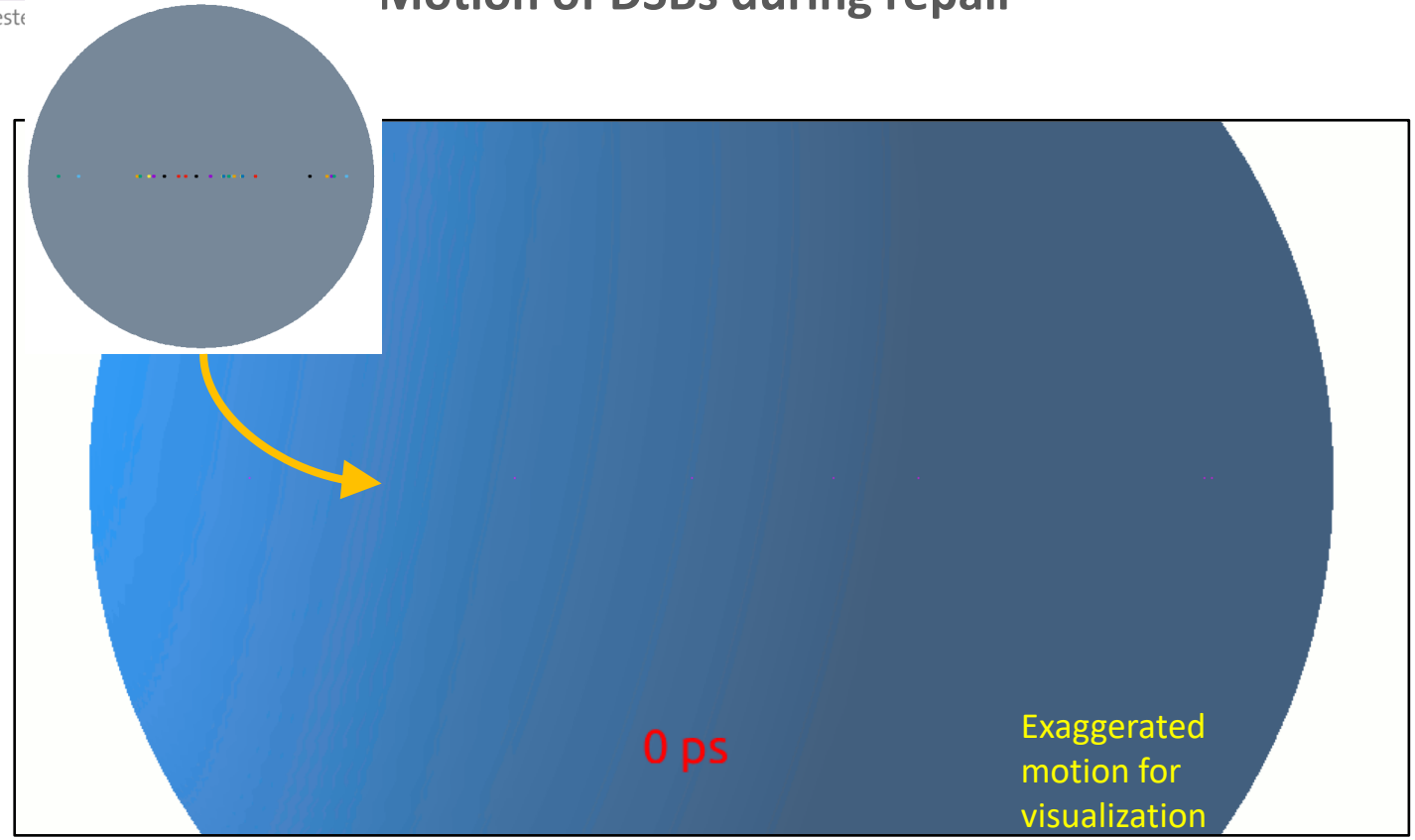
DNA Repair:
Motion of DSBs during repair



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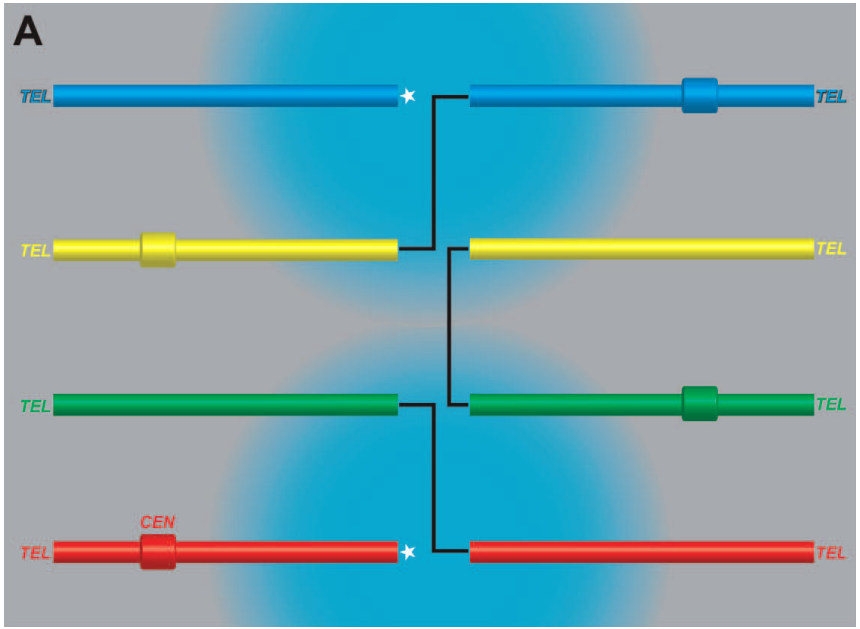
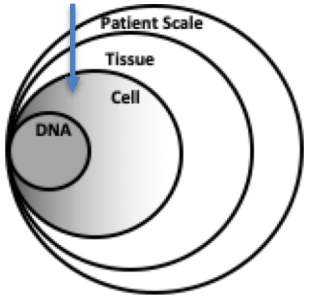
A critical parameter of repair fidelity:

DSB motion



Loucas, B.; Cornforth, M. The LET Dependence of Unrepaired Chromosome Damage in Human Cells: A Break Too Far? Radiat Res 2013



Moving to the Cell Scale:
Chromosome Aberrations

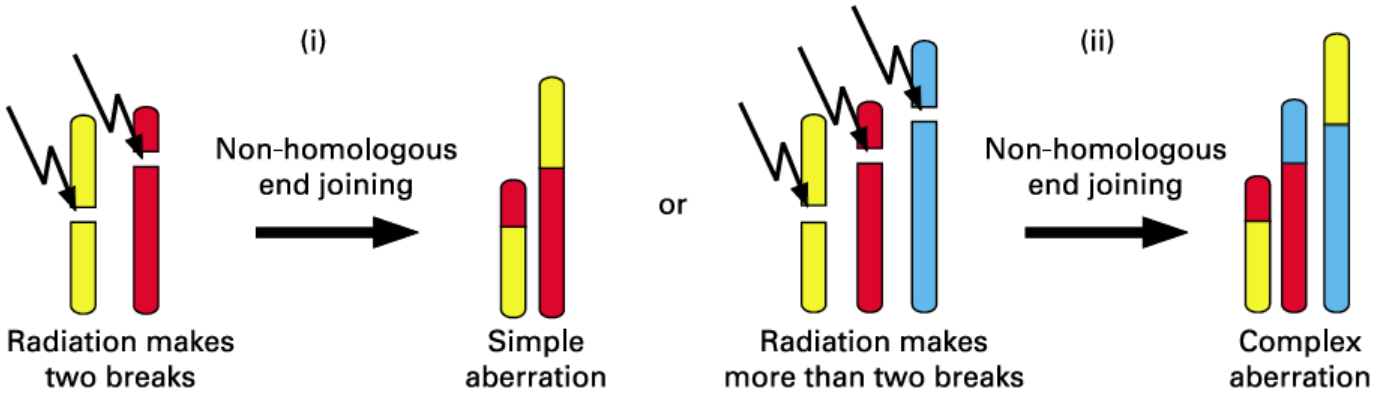
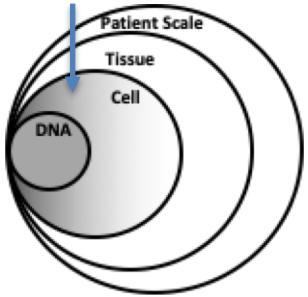
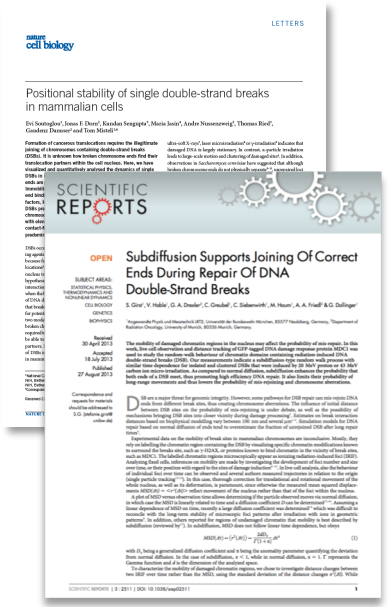
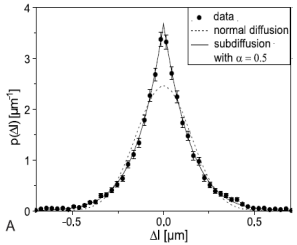


Figure from: Sachs, RK; Levy, D; Hahnfeldt, P; Hlatky, L Quantitative Analysis of Radiation-Induced Chromosome Aberrations.*Cytogenetic and genome …* **2004**



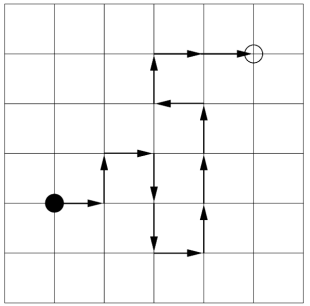
Anomalous diffusion of DSB Ends





somes. Similarly to single DSBs, the broken DNA ends were positionally stable (Fig. 2c). No coalescence was observed at times up to 24 h after breakage, even between arrays separated by less than 400 nm (data not shown). We conclude that DSBs are positionally immobile within the mammalian cell nucleus.

○ 'Standard' diffusion



○ Anomalous diffusion

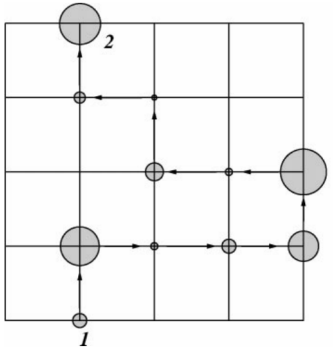
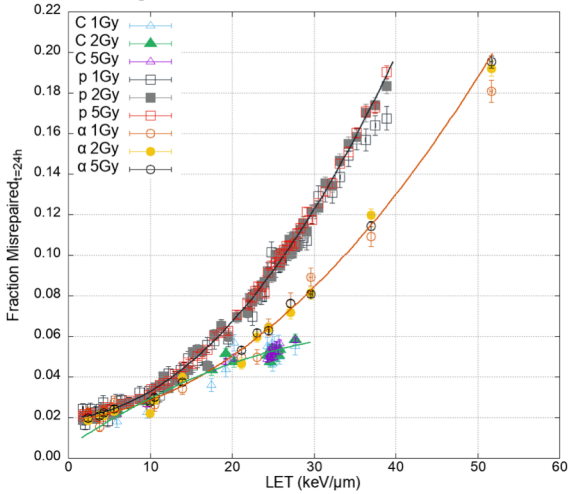


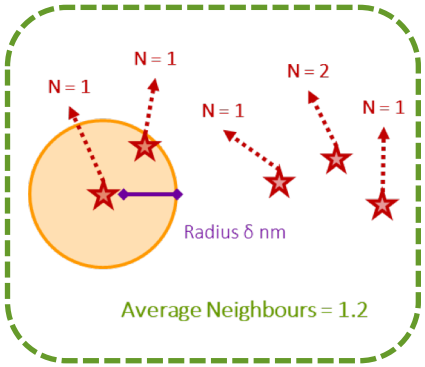
Figure 4. & 5.Metzler, R. and Klafter, J (2000) The Random Walk's Guide to Anomalous Diffusion: A Fractional Dynamics Approach, Physics Reports

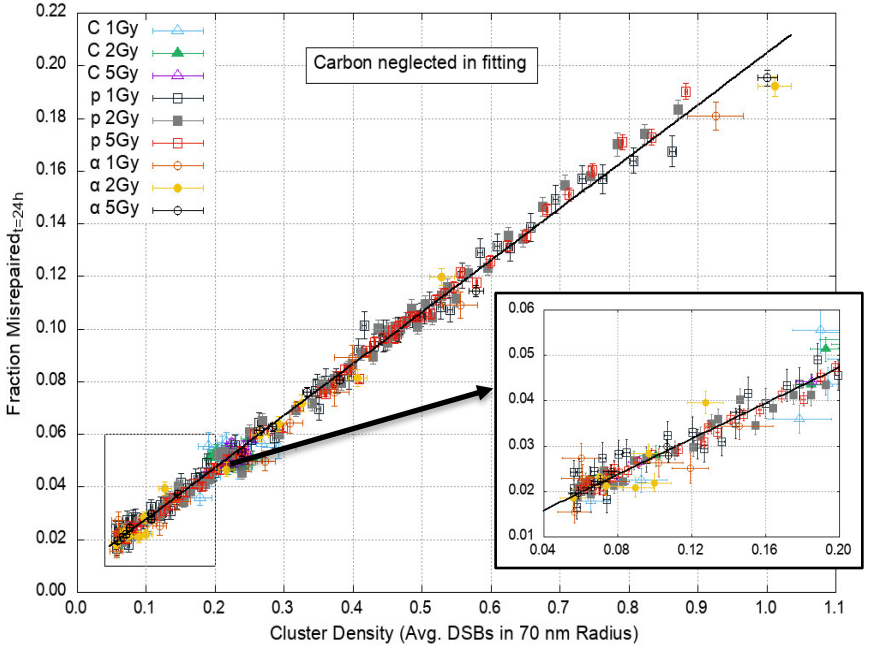
EPSRC BioProton, working with:
Dr Nickolay Korabel,
Prof Sergei Fedotov
School of Mathematics, University of Manchester.

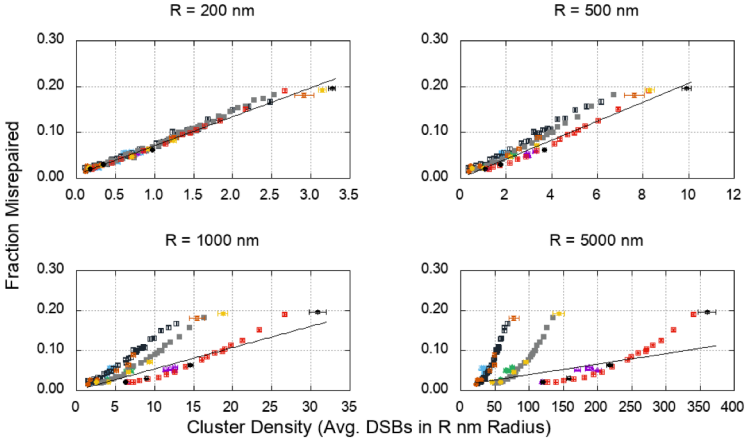
DNA Damage & Repair:

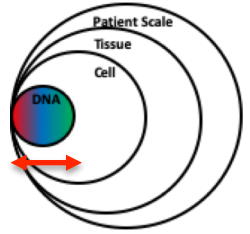
DSB cluster size – a better predictor than LET?





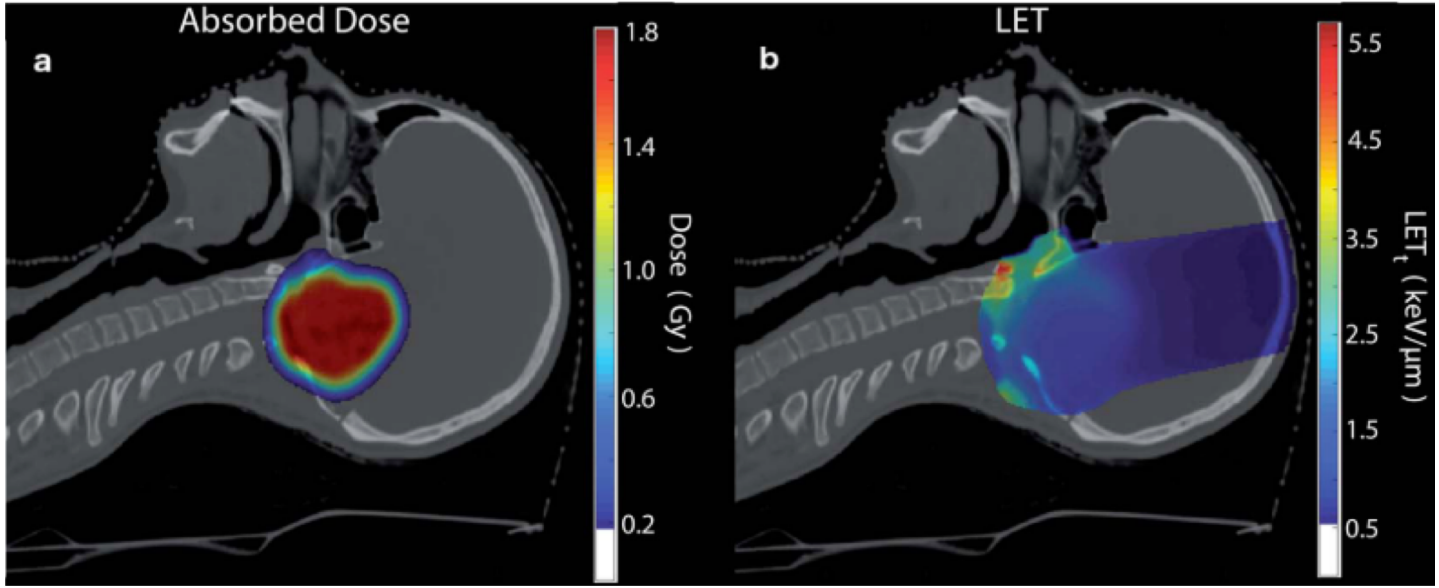






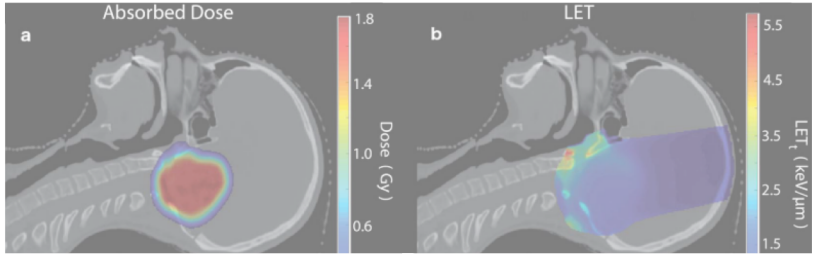
Henthorn NT, Warmenhoven JW, Sotiropoulos M, Mackay RI, Kirkby NF, Kirkby KJ, Merchant MJ; In silico non-homologous end joining following ion induced DNA double strand breaks predicts that repair fidelity depends on break density; **Scientific reports; 2018**.

Protons: Model Translation:
From DNA scale to Patient Scale



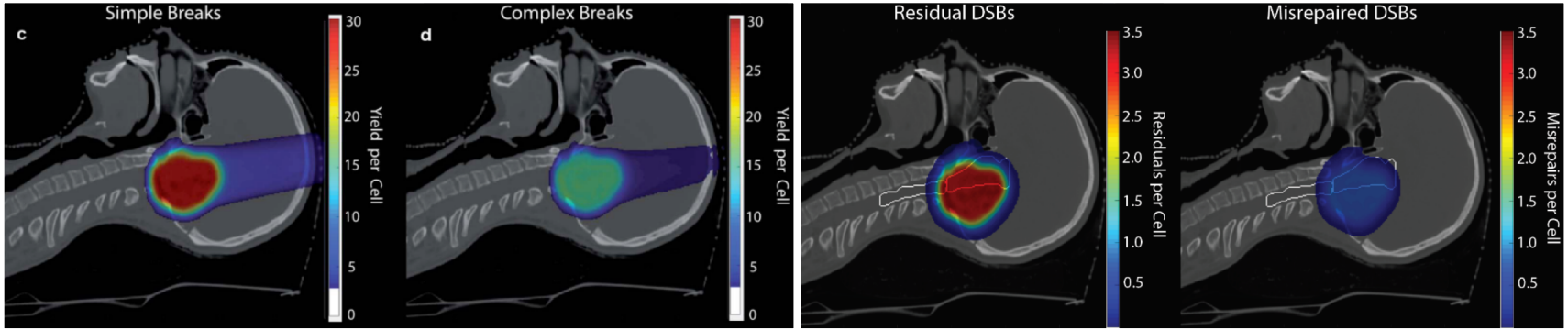
E. Smith et al., Nat. Sci. Rep 2019

Model Translation:
From DNA scale to Patient Scale



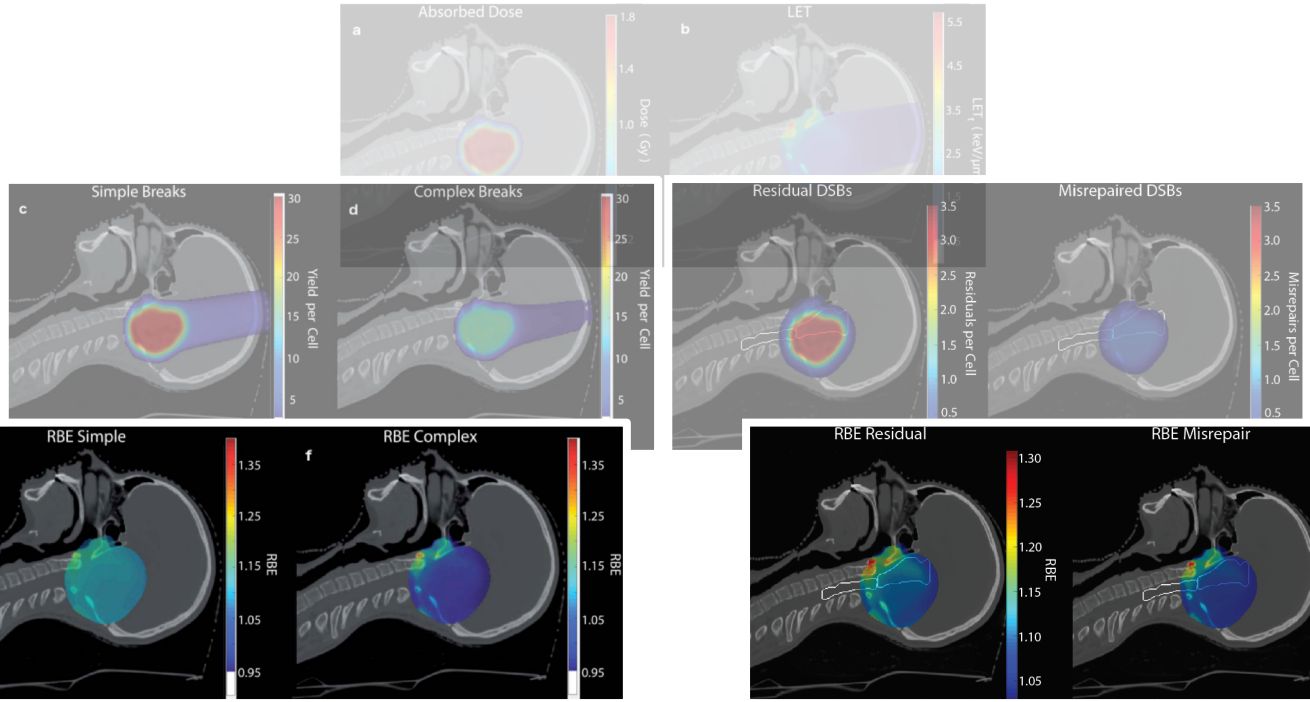
N. Henthorn et al., RSC Advances, 2019

E. Smith et al., Nat. Sci. Rep 2019



Model Translation:
From DNA scale to Patient Scale

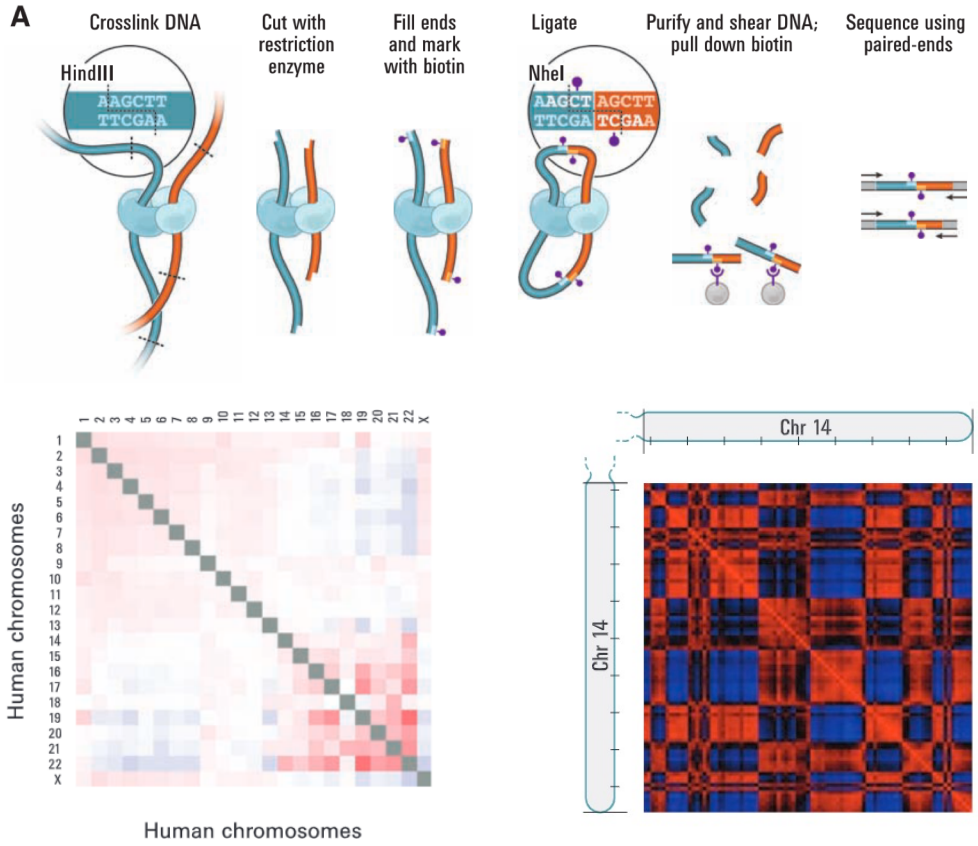
N. Henthorn et al., RSC Advances, 2019



E. Smith et al., Nat. Sci. Rep 2019

Note: – unvalidated research model – Not used clinically!

Using genomic data to model cell nucleus:
Hi-C



A biological technique to measure gene-gene contacts

- Cells are crosslinked to join nearby chromatin segments
- Crosslinked segments are marked and analysed to give contact frequency
- Repeats over many cells gives an average contact frequency map

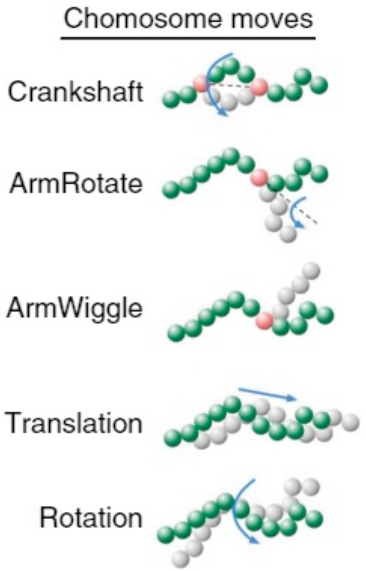
Technique identifies “Topologically Associated Domains”, highlighting frequent gene-gene interactions

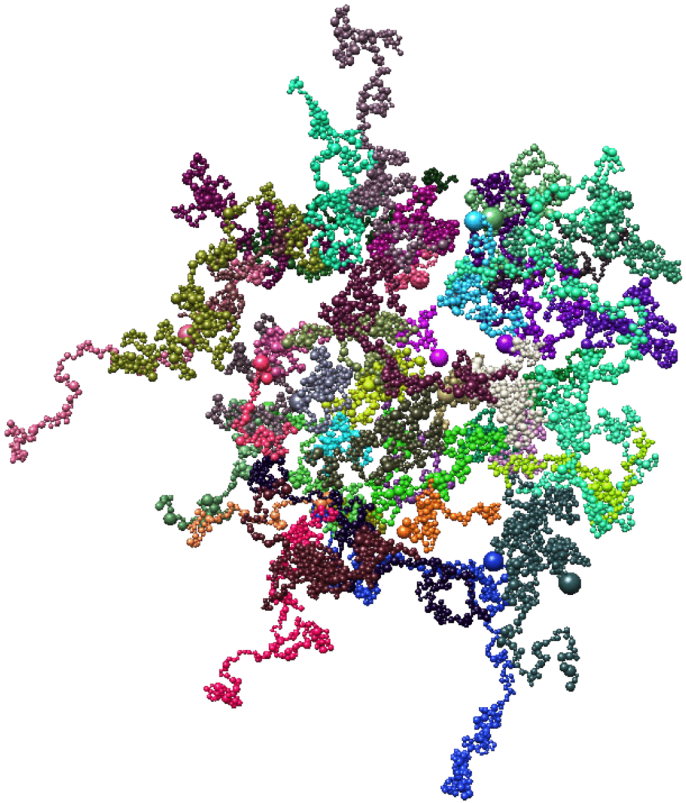
Data can be used to reconstruct a 3D geometry

Resolution depends on restriction enzymes, Typically ~10kbp

Liberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., Sandstrom, R., Bernstein, B., Bender, M.A., Groudine, M., Gnirke, A., Stamatoyannopoulos, J., Mirny, L.A., Lander, E.S., and Dekker, J.; Comprehensive mapping of long-range interactions reveals folding priniciples of the human genome; **Science; 2009**

Using Hi-C data :
A spatial solver

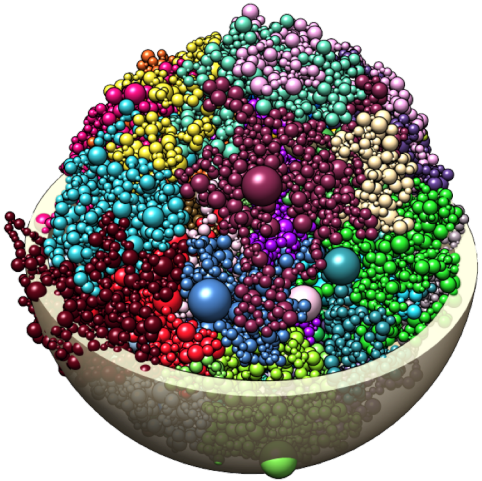




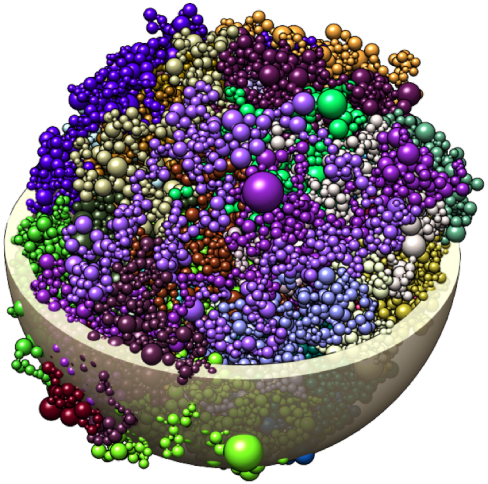
Solving:
46 Chromosomes
15,282 Beads
31,062 Constraints

Ingram, S.P., Henthorn, N.T., Warmenhoven, J.W., Kirkby, N.F., Mackay, R.I., Kirkby, K.J. and Merchant, M.J; Hi-C implementation of genome structure for in silico models of radiation-induced DNA damage. **Submitted 2020**.

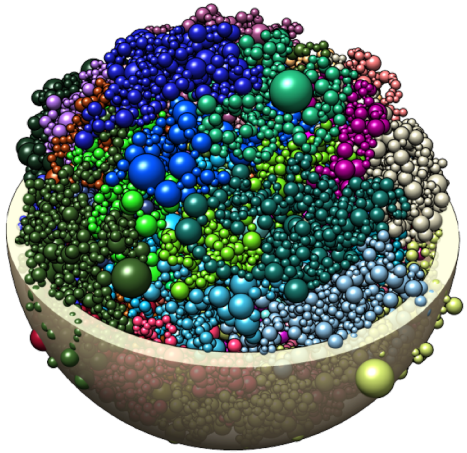
Using Hi-C data: Cell line specific models



IMR90
Human, Normal
Fibroblast



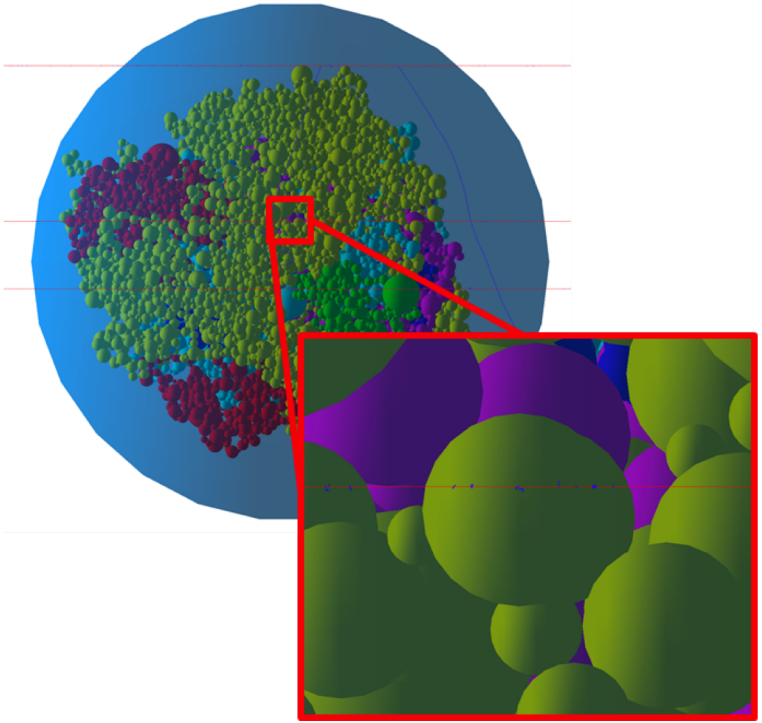
GM12878
Human, Normal
B-Lymphocyte

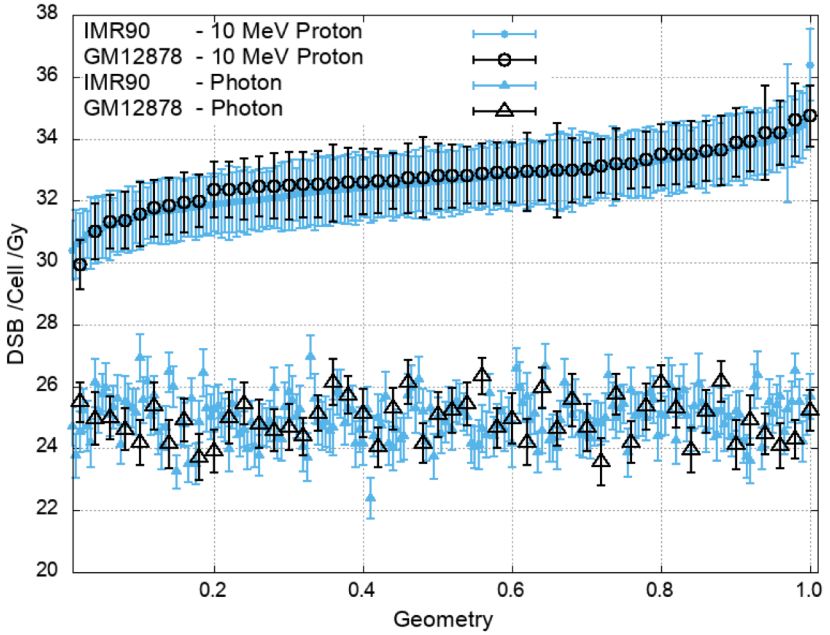


HMEC
Human, Normal
Dermal Endothelium

Ingram, S.P., Henthorn, N.T., Warmenhoven, J.W., Kirkby, N.F., Mackay, R.I., Kirkby, K.J. and Merchant, M.J; Hi-C implementation of genome structure for in silico models of radiation-induced DNA damage. **Accepted 2020**.

Radiation interactions at the nucleus scale:
Cell line specific prediction of DSB





Ingram, S.P., Henthorn, N.T., Warmenhoven, J.W., Kirkby, N.F., Mackay, R.I., Kirkby, K.J. and Merchant, M.J; Hi-C implementation of genome structure for in silico models of radiation-induced DNA damage. **PLOS Computational Biology 2020**.

MANCHESTER 1824
The University of Manchester

The Precise Group

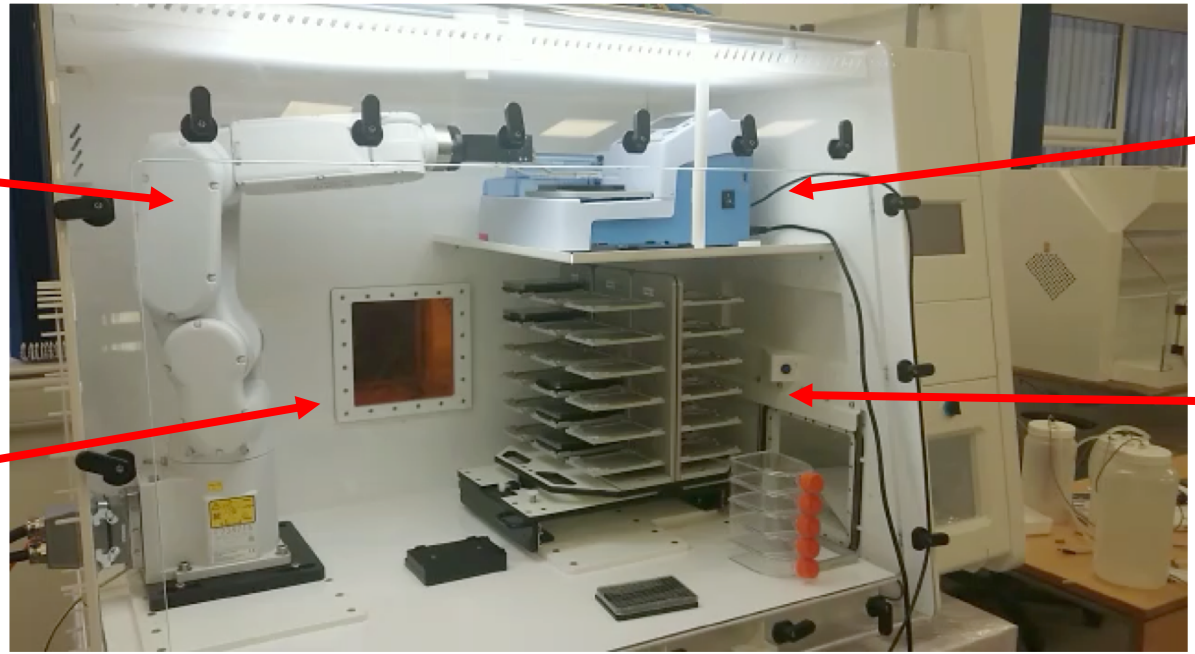
The Christie Research Beamline

The Christie NHS Foundation Trust

Protons: Hypoxia Radiobiology End-station

FANUC 6-axis robot arm

Proton beam entry window



Automation for liquid handling: Cell fixing.

'Hotel' for 36 flasks or well plates

Automated hypoxia cabinet for proton irradiation.

Designed in collaboration with Don Whitley Scientific Ltd

Thanks for listening!

DNA Damage and Repair work from PRECISE

- Dr Nick Henthorn
- Dr John Warmenhoven
- Dr Nickolay Korabel
- Sam Ingram
- Ed Smith
- Yaping Qi
- Charlotte Heaven
- Bethany Rothwell
- Hannah Wantsall
- Prof. Ranald MacKay
- Prof. Norman Kirkby
- Prof. Sergei Fedotov
- Dr Amy Chadwick
- Dr Elham Santina
- Dr Adam Aitkenhead
- Prof. Karen Kirkby

VHEE work led by Prof. Roger Jones

- K. L. Small
- D. Angal-Kalinin
- R. J. Smith
- M. Surman
- J. Jones
- W. Farabolini
- R. Corsini, D. Gamba
- A. Gilardi