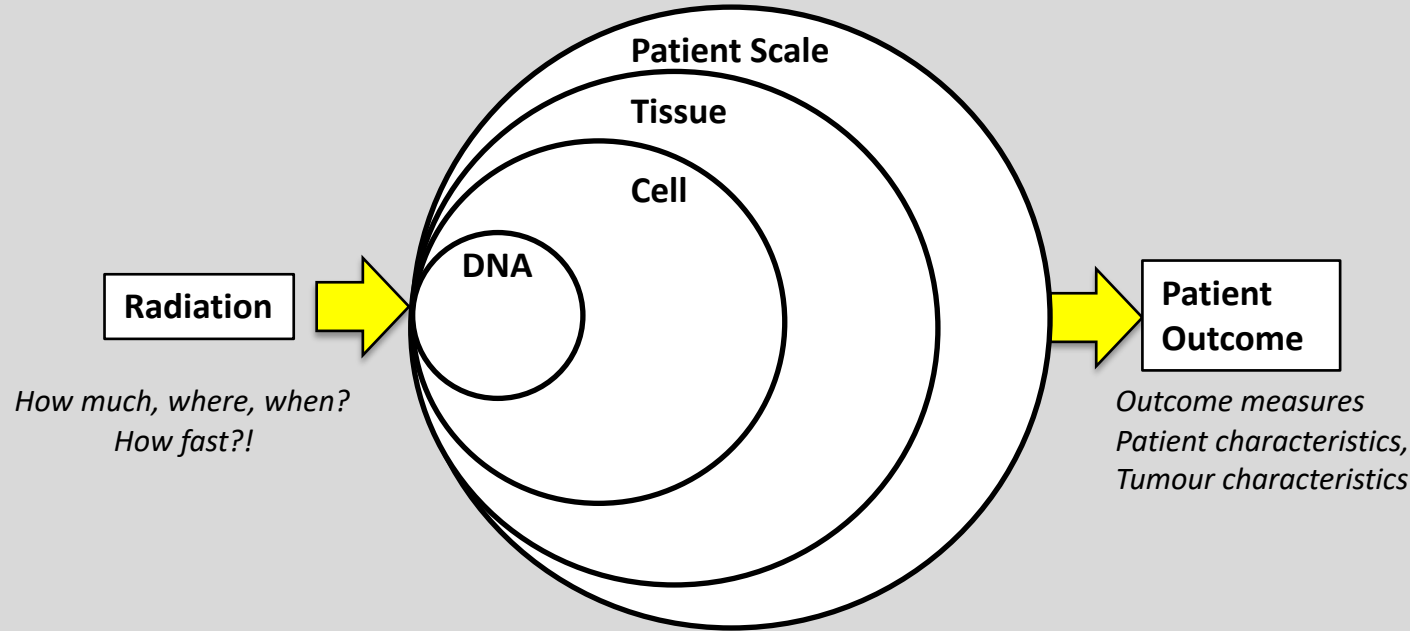


Mechanistic modelling of DNA damage and repair for Proton Therapy

Mike Merchant,

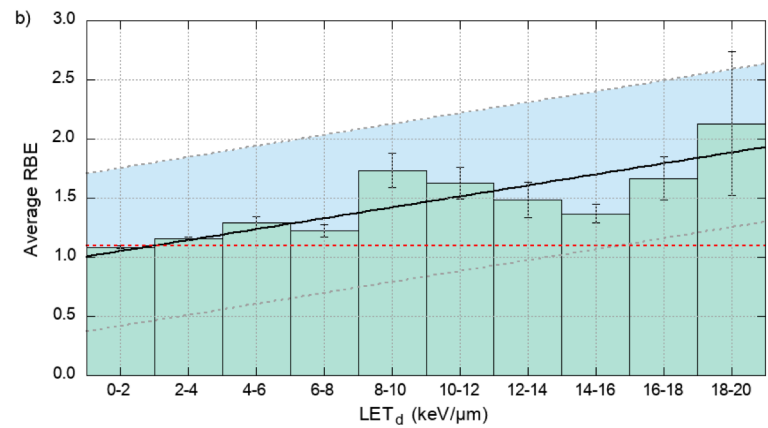
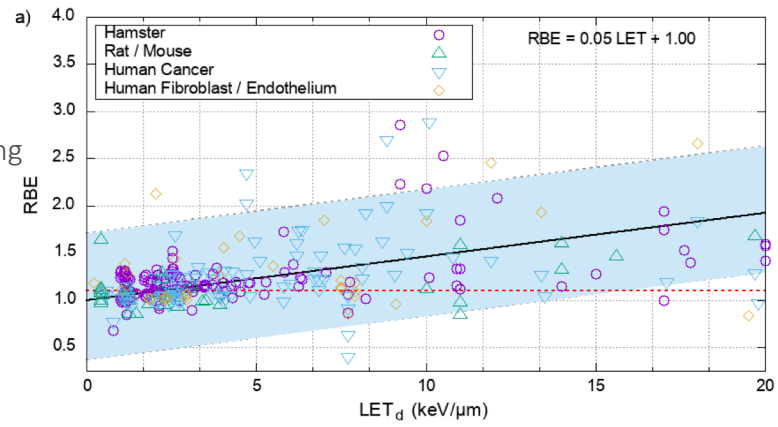
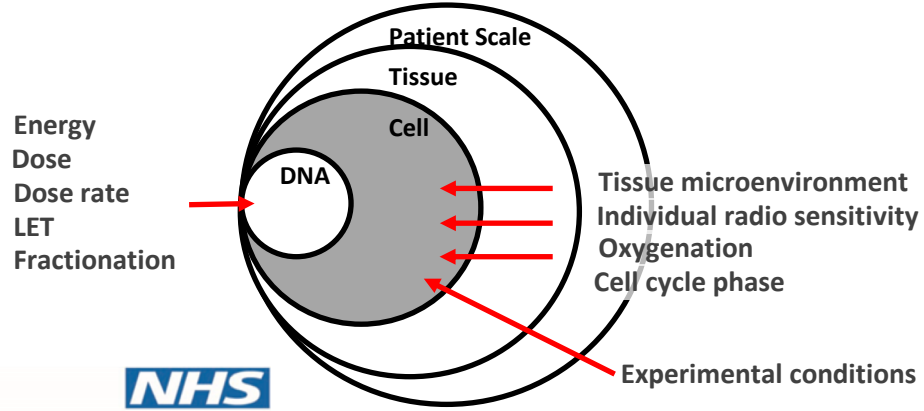
Division of Cancer Science, University of Manchester

michael.merchant@manchester.ac.uk



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)

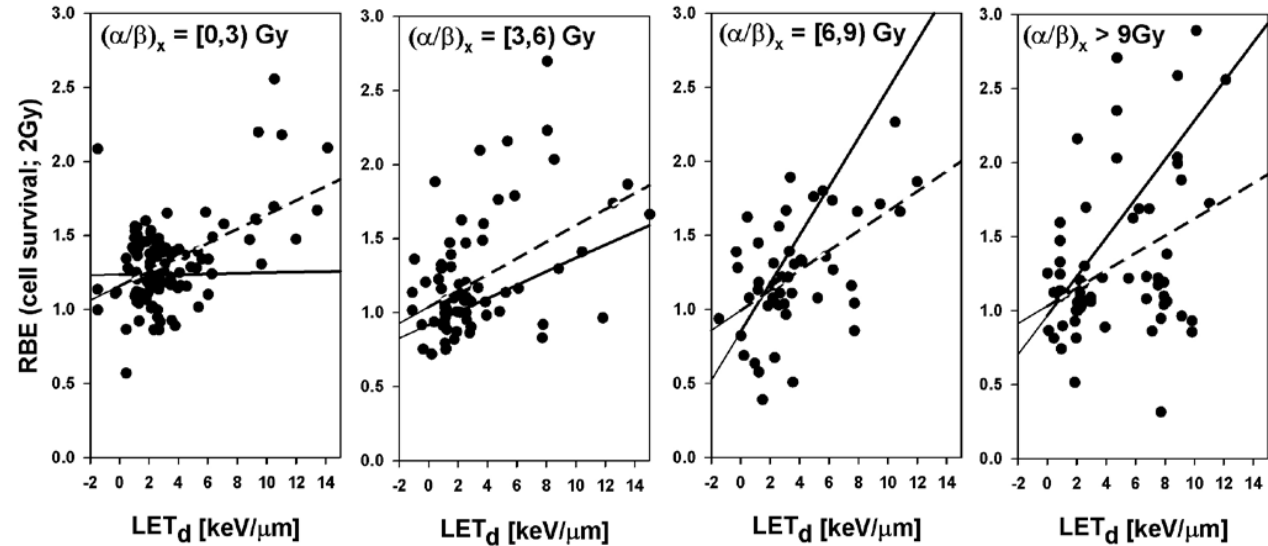
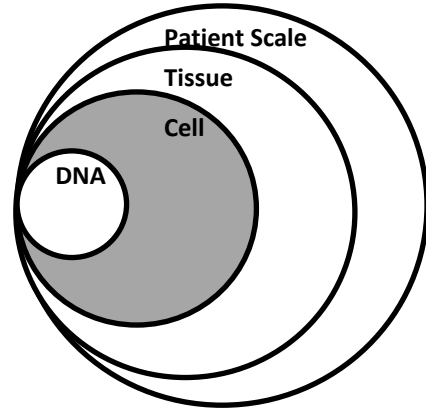


Data reproduced from Paganetti, *Phys. Med. Biol.*, 2014

Why do we need a model?

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

Proton RBE (survival) – in vitro



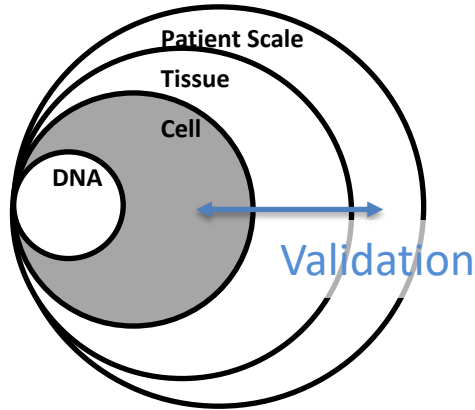
Paganetti, H. Relative Biological Effectiveness (RBE) Values for Proton Beam Therapy. Variations as a Function of Biological Endpoint, Dose, and Linear Energy Transfer. *Physics in Medicine and Biology* **2014**, 59, R419–R472.

Cell scale approaches to RBE

Phenomenological models

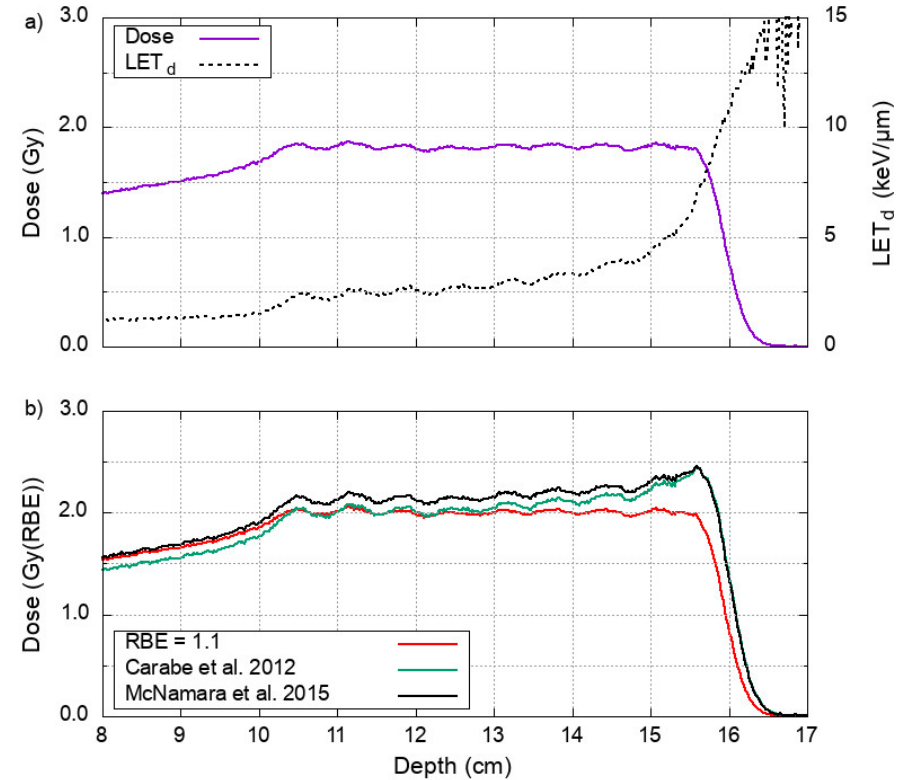
$$RBE = \frac{\sqrt{(\alpha/\beta)_x^2 + 4(\alpha/\beta)_x RBE_{max} D_p + 4(RBE_{min})^2 D_p^2} - (\alpha/\beta)_x}{2D_p}$$

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



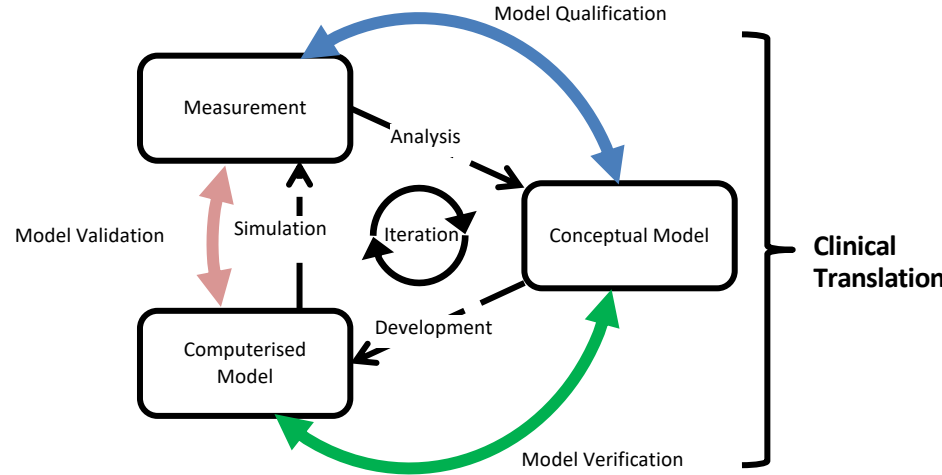
Many models proposed, but hard to validate using post-treatment imaging.

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

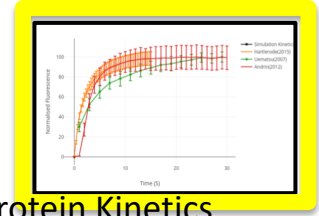
We need to understand what happens before cell death: What are the mechanisms?



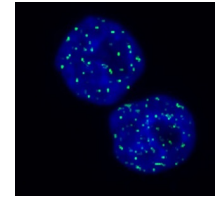
Multi-assay validation

Reduction of errors

- Equipment designed for experimental need



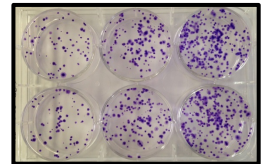
Protein Kinetics



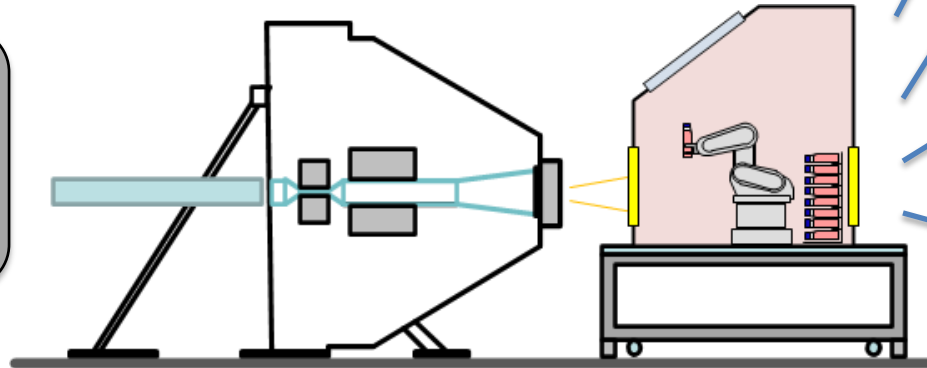
Foci counting



Chromosome Aberrations



Cell survival

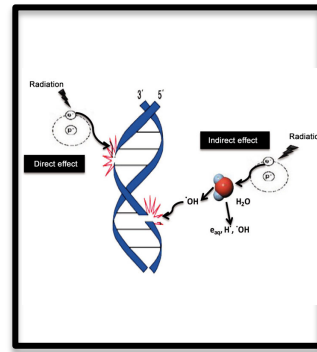
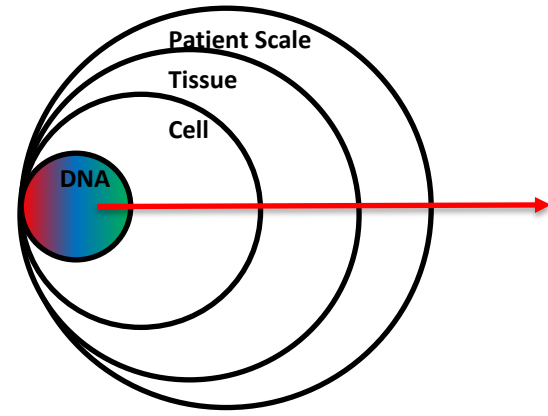


Understanding the error chain

- What is the required experiment to reduce overall uncertainty?

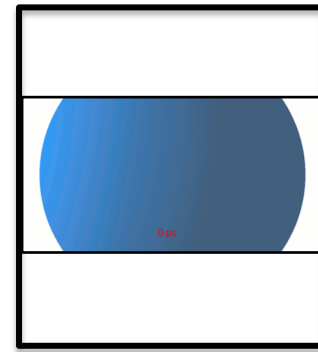
What happens at the DNA level?

3 stages to mechanism of DNA response



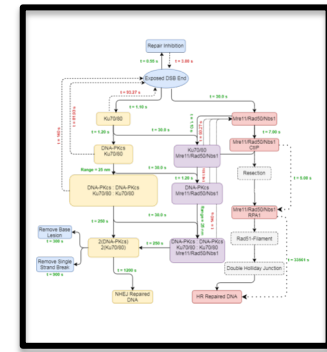
Physics

Ionisations



**Radiation-
Chemistry**

Strand breaks

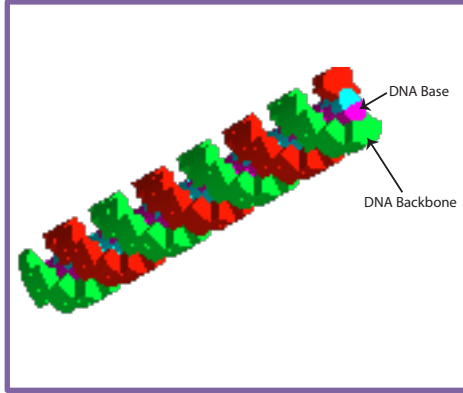


Repair

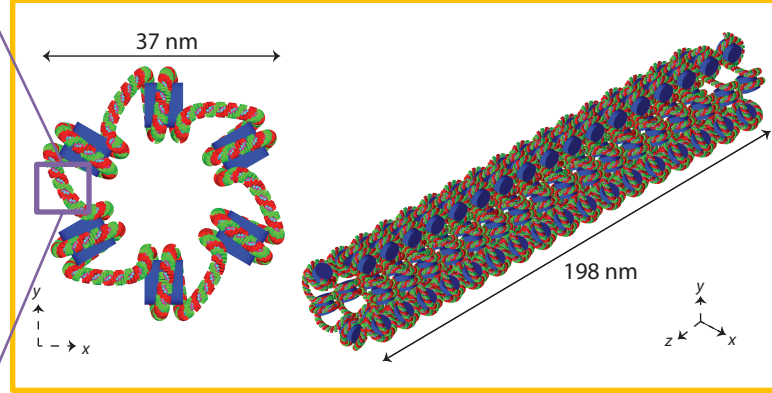
Repair fidelity

DNA Damage

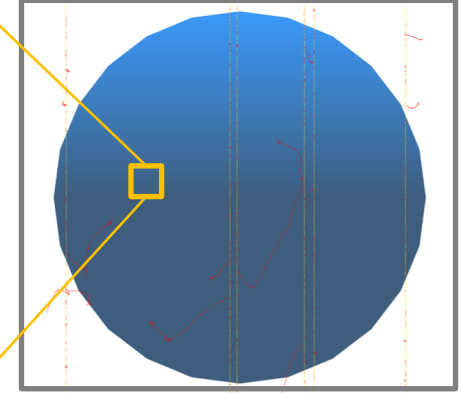
DNA Double Helix



Chromatin Fibre

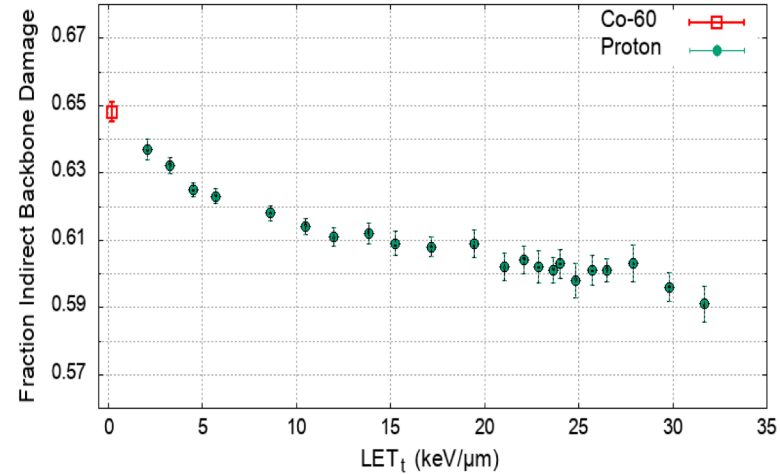
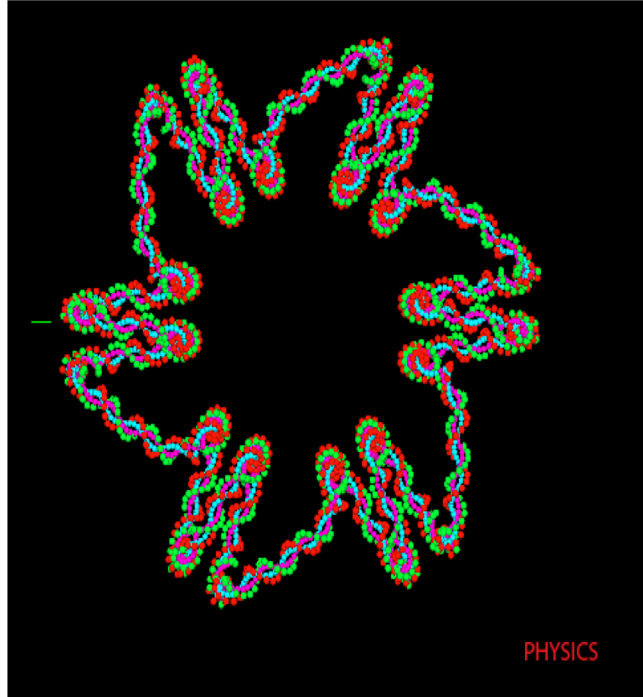


Cell Nucleus



- 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

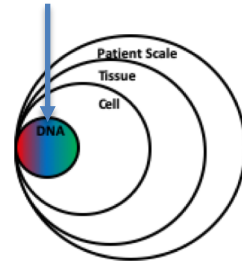


- For Co-60 irradiation 65% of the strand breaks are from indirect effects*

Modelling Assumption

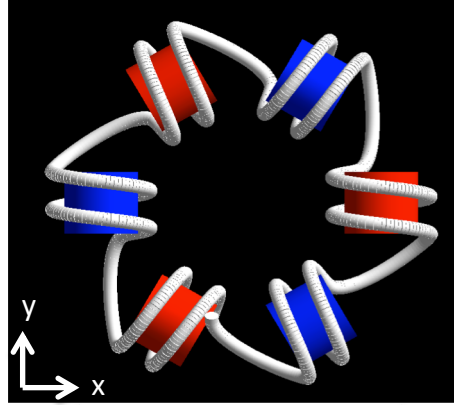
- If an OH radical steps into a DNA backbone it reacts, set a probability that the reaction causes damage
- P=0.5 gives 65% indirect damage

*Ward, Radiat. Res., 1985



Chromatin Models

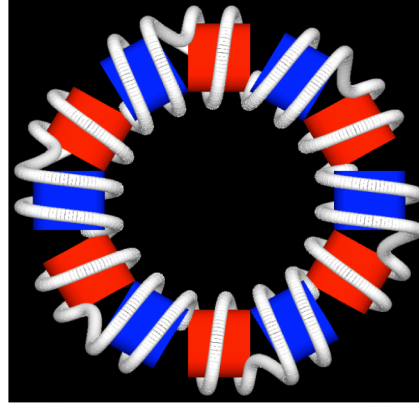
Solenoid



37 nm

61 Nucleosomes
4.2 Nuc/11nm
10.8 kbp DNA

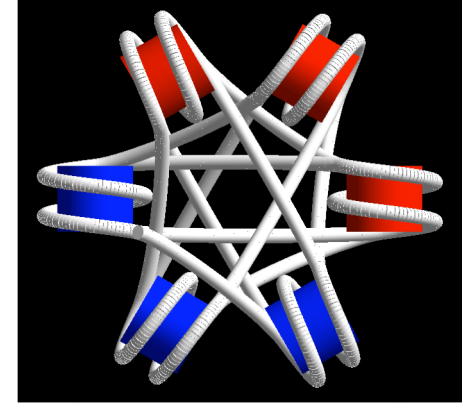
Zig-Zag



40 nm

61 Nucleosomes
4.2 Nuc/11nm
10.5 kbp DNA

Cross-Linked



37 nm

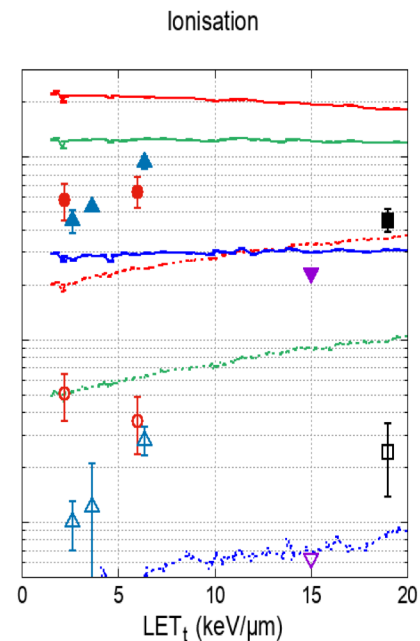
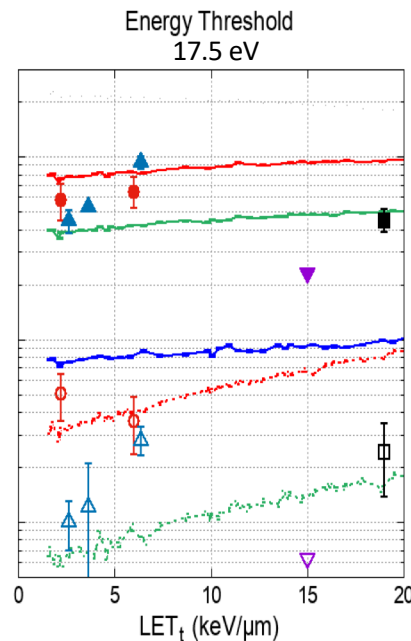
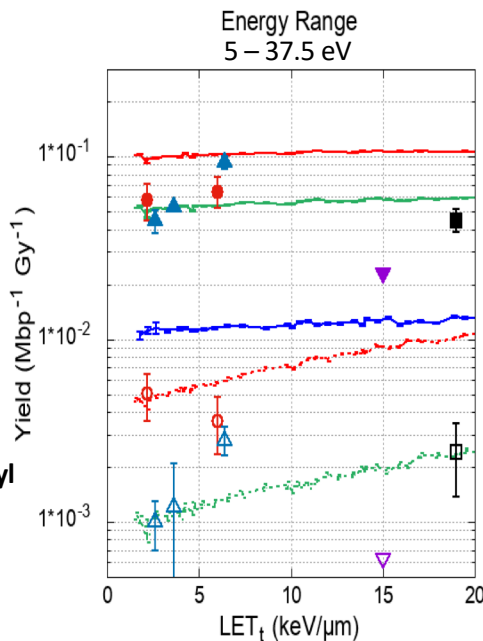
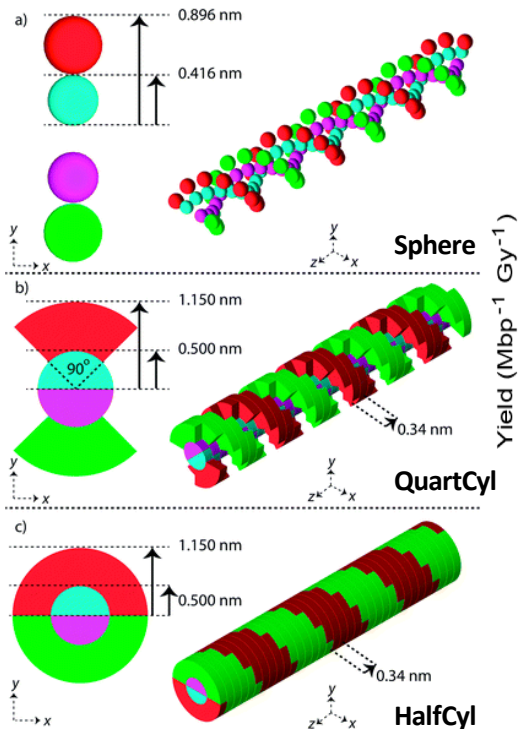
61 Nucleosomes
4.2 Nuc/11nm
12.4 kbp DNA

Henthorn et al. Rad Res 2017

Even modelling chromatin is not simple –
we need to know what model of
chromatin to use!

Is the damage model accurate?

Plasmid data:



HalfCyl SSB ———
HalfCyl DSB - - - -
QuartCyl SSB ———
QuartCyl DSB - - - -

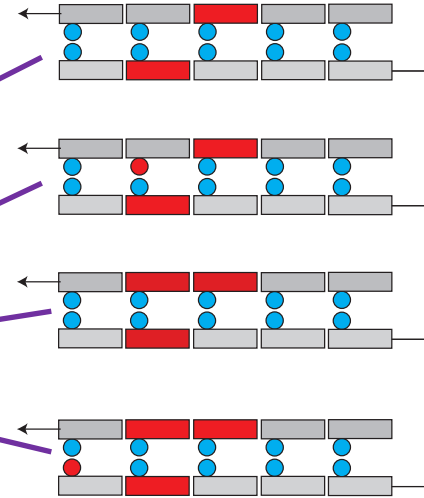
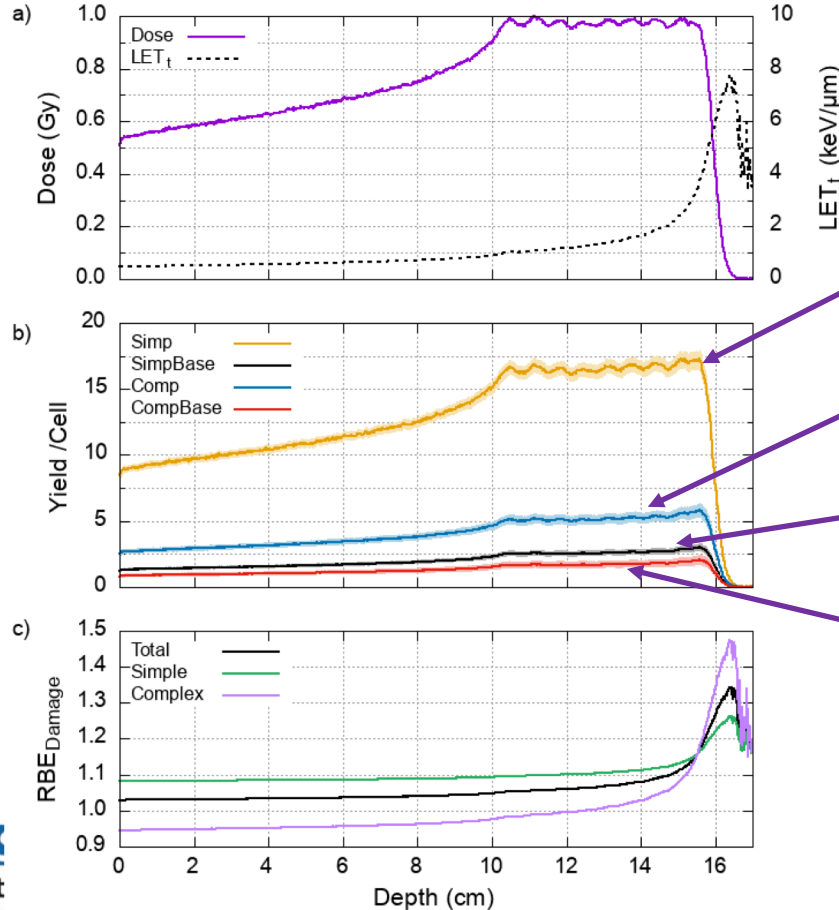
Sphere SSB ———
Sphere DSB - - - -

Urushibara (2009) α SSB —■—
Urushibara (2009) α DSB —□—
Ushigome (2012) α SSB —●—
Ushigome (2012) α DSB —○—

Vysin (2015) p SSB —▲—
Vysin (2015) p DSB —△—
Souici (2017) p SSB —▼—
Souici (2017) p DSB —▽—

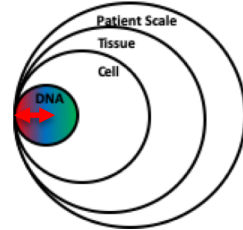
Henthorn, N.T., Warmenhoven, J.W., Sotiropoulos, M., Aitkenhead, A.H., Smith, E.A.K., Ingram, S.P., Kirkby, N.F., Chadwick, A.L., Burnet, N.G., Mackay, R.I., Kirkby, K.J. and Merchant, M.J.; Clinically relevant nanodosimetric simulation of DNA damage complexity from photons and protons; RSC advances;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

RBE of Damage Complexity

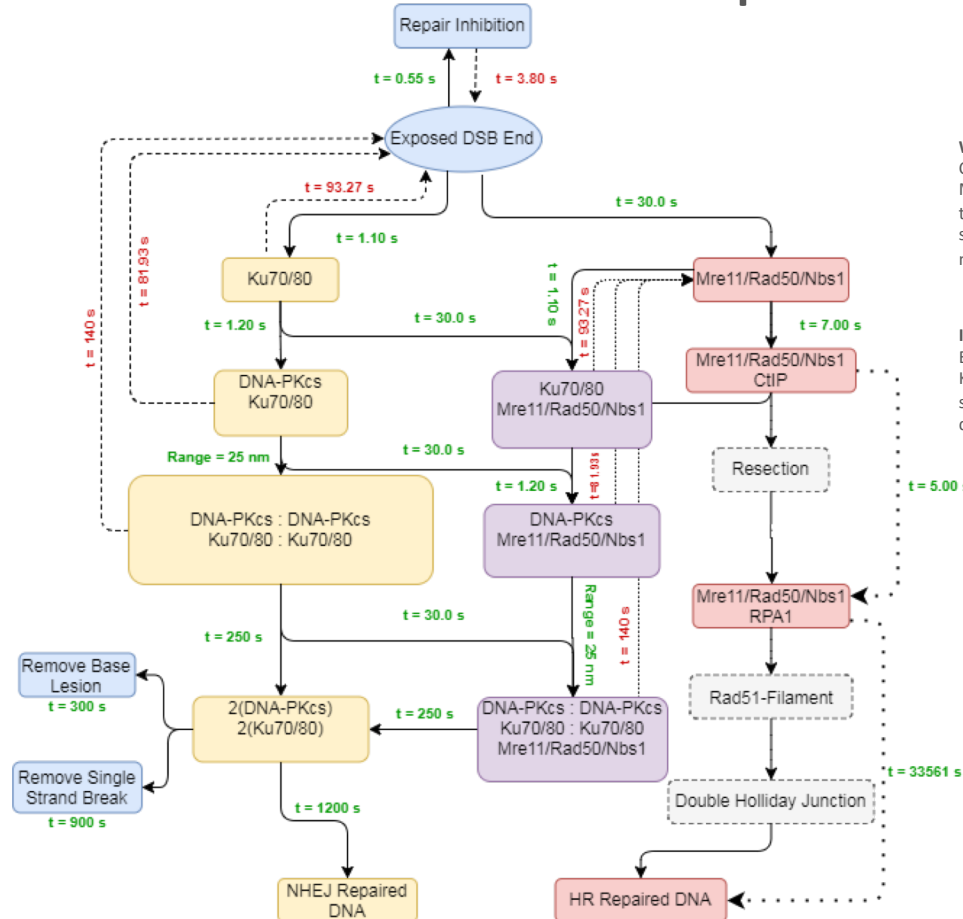


“Simple”

“Complex”

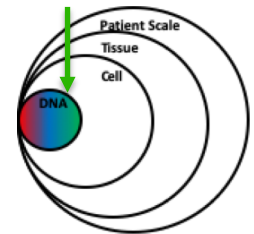


Henthorn, N.T., Warmenhoven, J.W., Sotiropoulos, M., Aitkenhead, A.H., Smith, E.A.K., Ingram, S.P., Kirkby, N.F., Chadwick, A.L., Burnet, N.G., Mackay, R.I., Kirkby, K.J. and Merchant, M.J.; Clinically relevant nanodosimetric simulation of DNA damage complexity from photons and protons; *RSC advances*;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

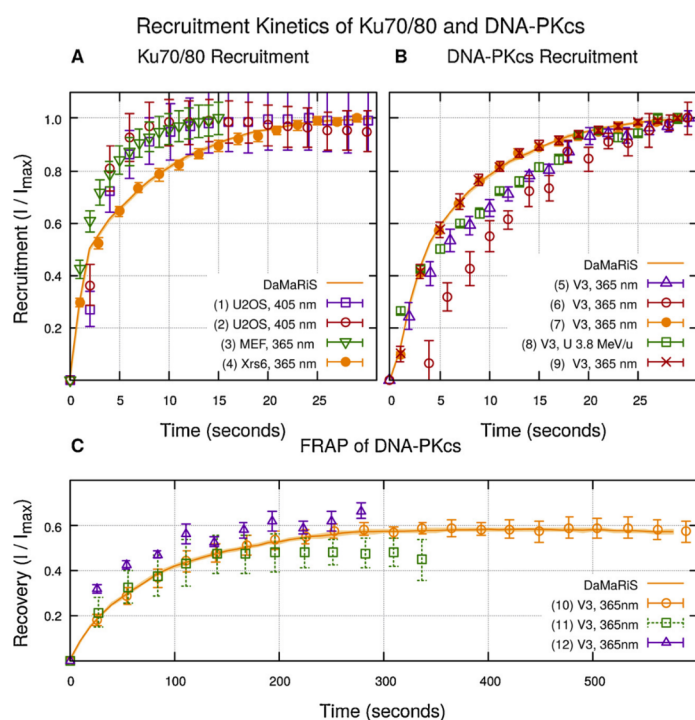


Warmenhoven, J.W., Henthorn, N.T., Ingram, S.P., Chadwick, A.L., Sotiropoulos, M., Korabel, N., Fedotov, S., Mackay, R.I., Kirkby, K.J. and Merchant, M.J.; Insights into the non-homologous end joining pathway and double strand break end mobility provided by mechanistic *in silico* modelling; **DNA repair**;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

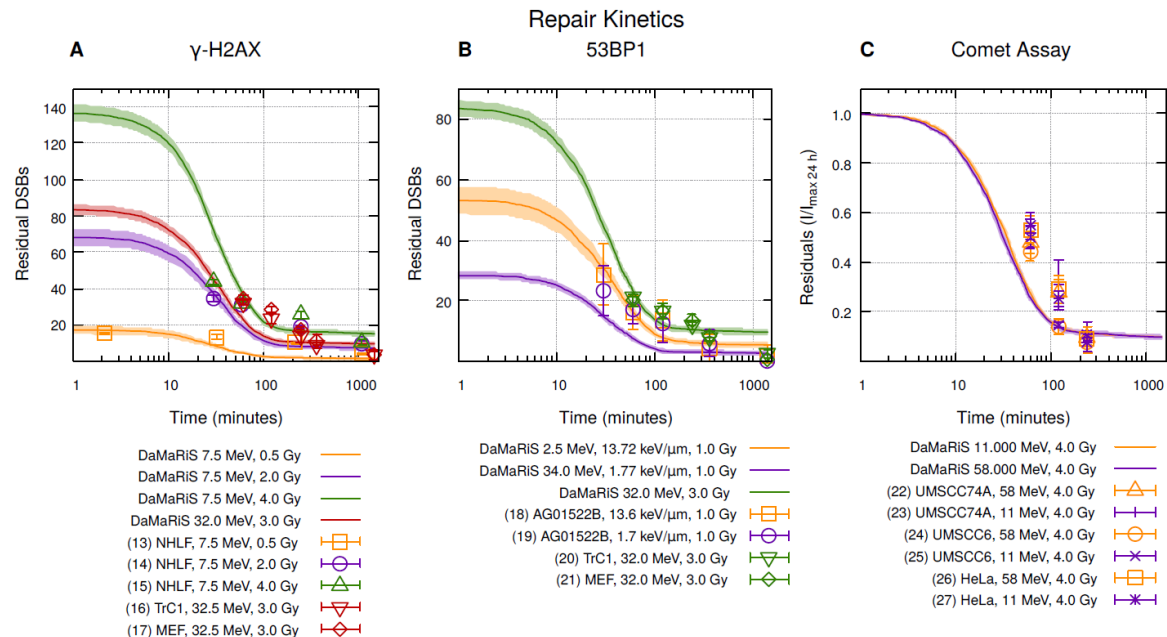
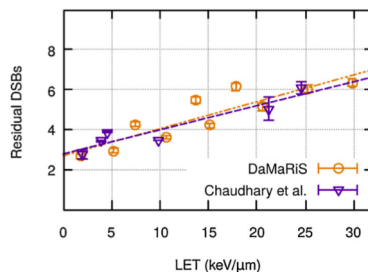
Ingram, S.P., Warmenhoven, J.W., Henthorn, N.T., Smith, E.A.K., Chadwick, A.L., Burnet, N.G., Mackay, R.I., Kirkby, N.F., Kirkby, K.J. and Merchant, M.J.; Mechanistic modelling supports entwined rather than exclusively competitive DNA double-strand break repair pathway; **Scientific reports**; 2019.



DNA Repair: Fitting Protein Kinetics



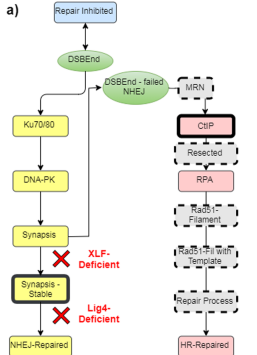
A LET vs. Residual DSBs at 24 h



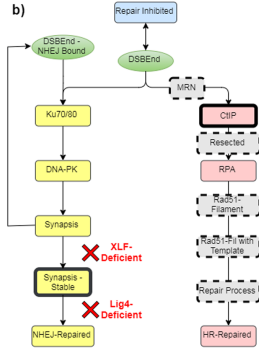
Warmenhoven, J.W., Henthorn, N.T., Ingram, S.P., Chadwick, A.L., Sotiropoulos, M., Korabel, N., Fedotov, S., Mackay, R.I., Kirkby, K.J. and Merchant, M.J.; Insights into the non-homologous end joining pathway and double strand break end mobility provided by mechanistic *in silico* modelling; **DNA repair**;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

DNA Repair: Pathway interactions

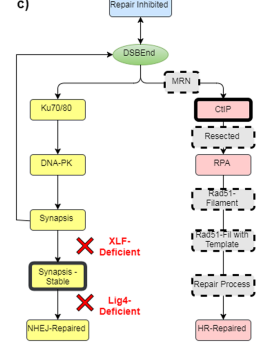
NHEJ First



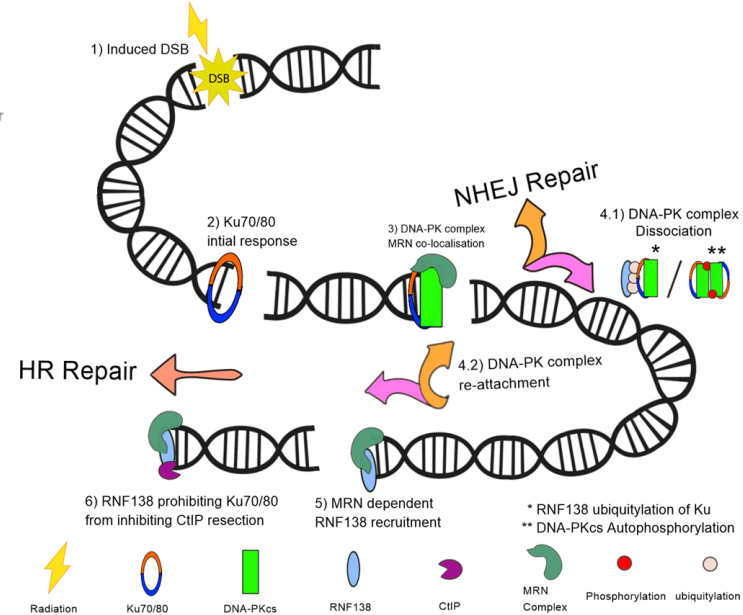
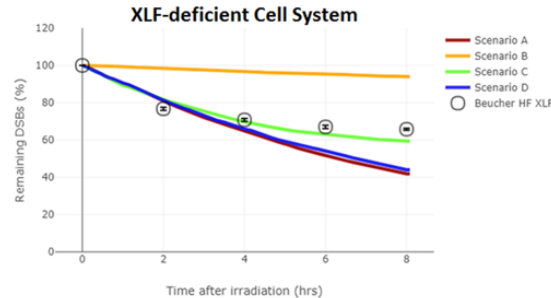
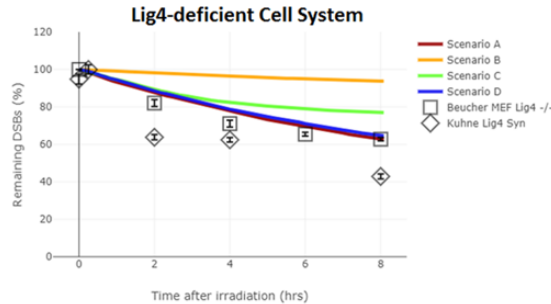
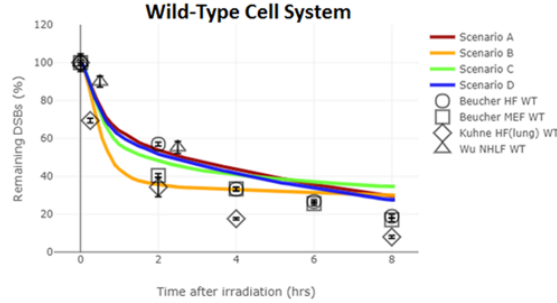
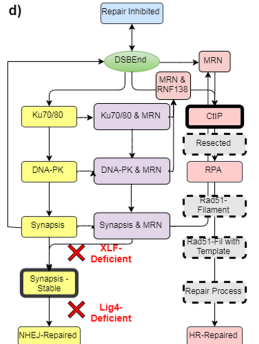
Competition



Re Competition

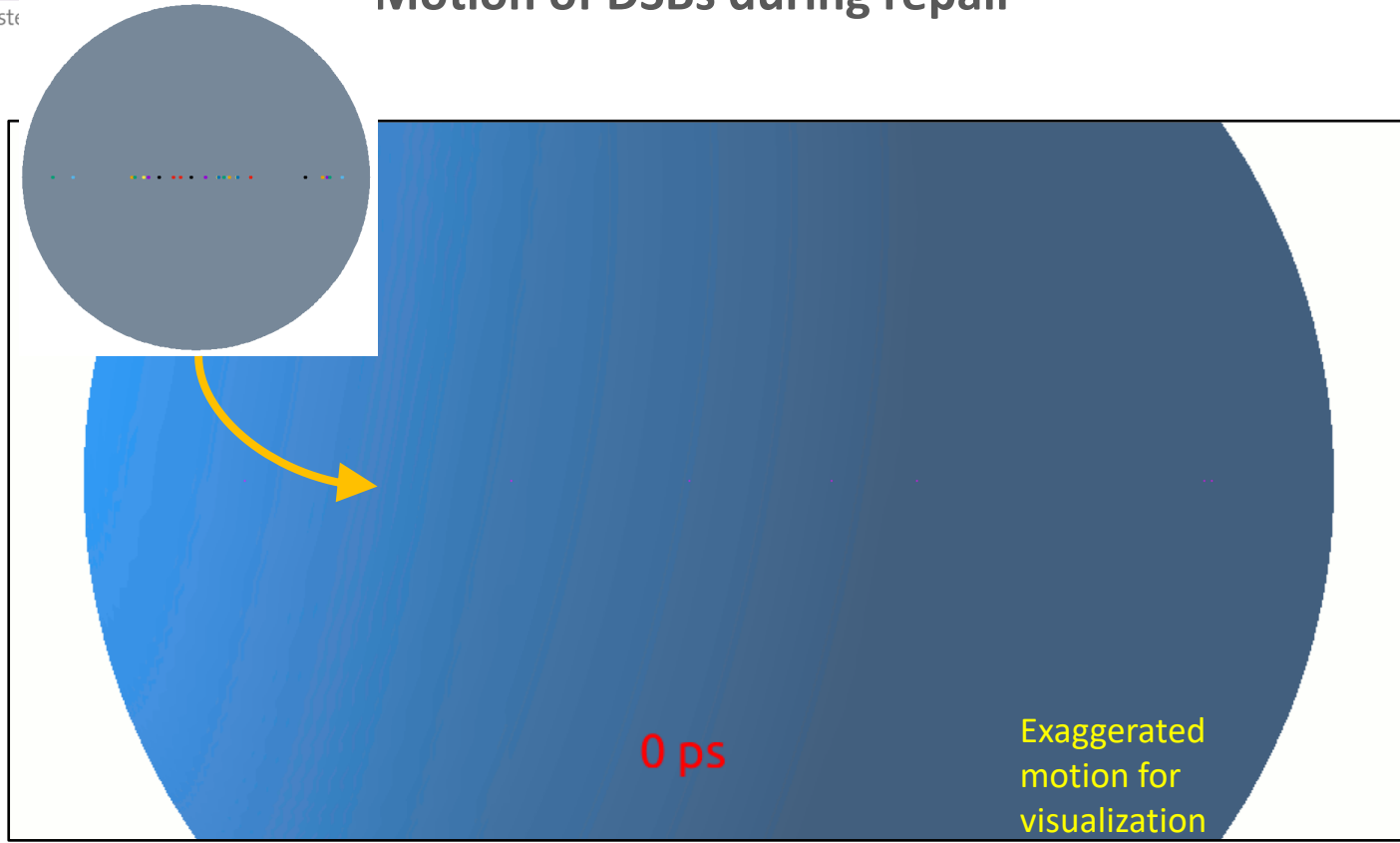


Entwined

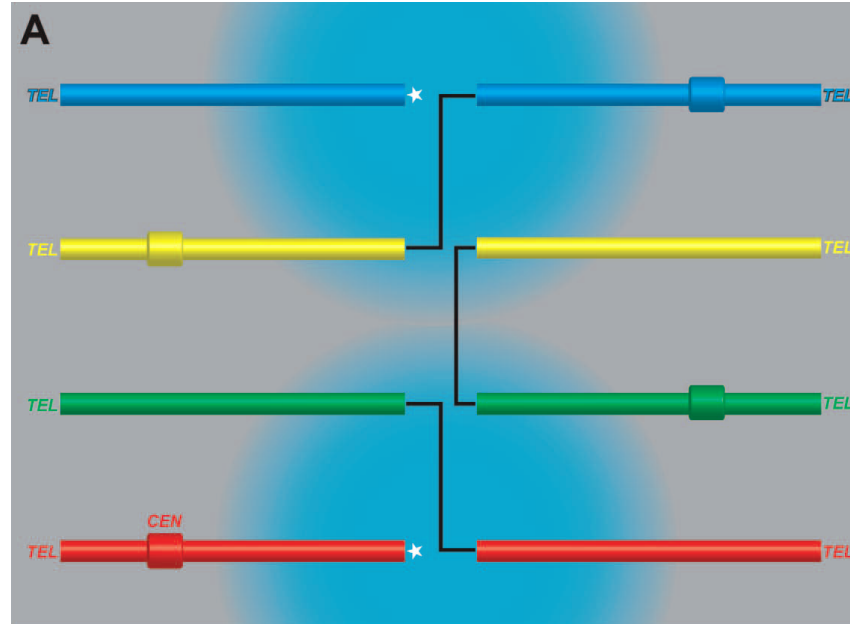


Ingram, S.P., Warmenhoven, J.W., Henthorn, N.T., Smith, E.A.K., Chadwick, A.L., Burnet, N.G., Mackay, R.I., Kirkby, N.F., Kirkby, K.J. and Merchant, M.J.; Mechanistic modelling supports entwined rather than exclusively competitive DNA double-strand break repair pathway; *Scientific reports*; 2019.

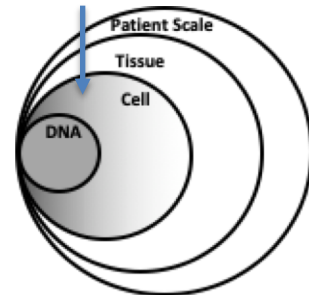
DNA Repair: Motion of DSBs during repair



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

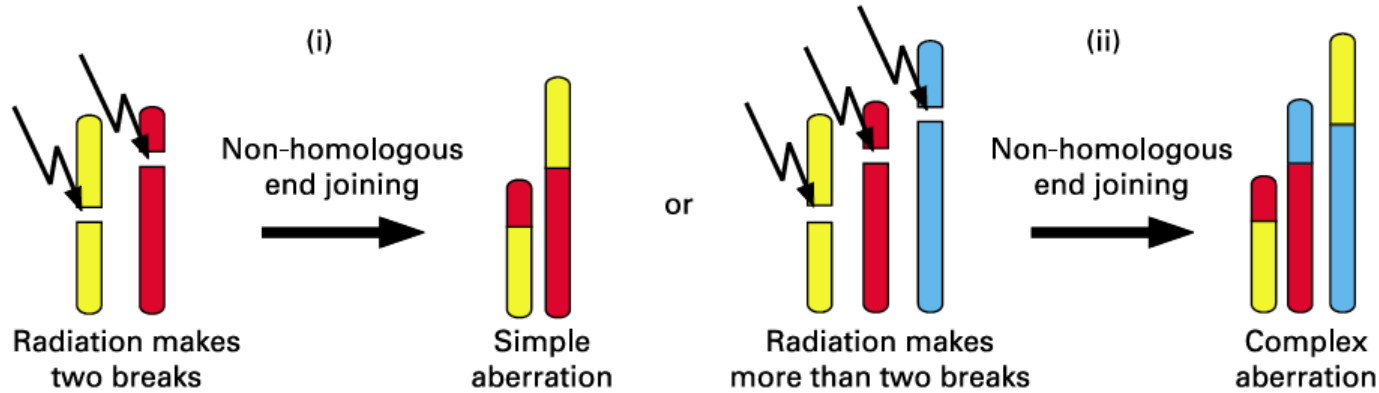
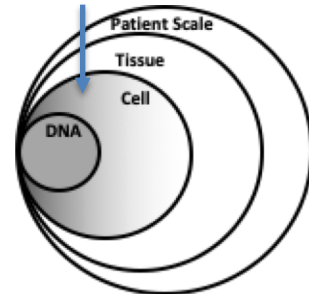


Figure from: Sachs, RK; Levy, D; Hahnfeldt, P; Hlatky, L Quantitative Analysis of Radiation-Induced Chromosome Aberrations. *Cytogenetic and genome* ... **2004**



Anomalous diffusion of DSB Ends

nature
cell biology

LETTERS

Positional stability of single double-strand breaks in mammalian cells

Esther Schmitt¹, David F. Suck², Sandra Imort², Martin Jais², Andre Stenning², Thomas Rief², Gerd Kramer² and Tim Blumberg¹

Formation of a protein-protein complex requires the presence of a specific binding site. In the case of a double-strand break (DSB), the broken DNA ends are the binding site for the repair machinery. We show that DSB ends are positioned within the cell nucleus, thus, we have quantified and characterized the mobility of DSB ends.

DSB ends are positioned within the cell nucleus, thus, we have quantified and characterized the mobility of DSB ends. DSB ends are positioned within the cell nucleus, thus, we have quantified and characterized the mobility of DSB ends.

SCIENTIFIC REPORTS

Subdiffusion Supports Joining Of Correct Ends During Repair Of DNA Double-Strand Breaks

S. Gier¹, V. Hübner¹, G. A. Diederich¹, C. Grottel¹, C. Scharfetter¹, M. Hauer¹, A. A. Friedl¹ & D. Dillinger¹

¹Department of Physics and Institute for Physics, University of Würzburg, 97074 Würzburg, Germany

Received: 20 April 2017

Accepted: 18 July 2017

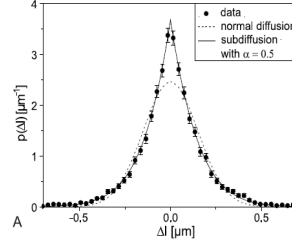
Published: 27 August 2017

Correspondence and requests for materials should be addressed to S.G. (s.gier@physik.uni-wuerzburg.de)

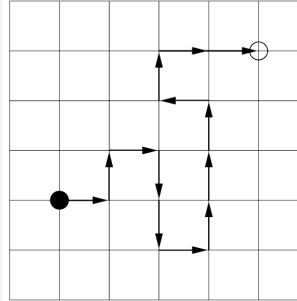
Reprints and permissions information is available at www.nature.com/reprints

DOI: 10.1038/s41598-017-07000-0

SCIENTIFIC REPORTS | 7: 10000 | DOI: 10.1038/s41598-017-07000-0



○ ‘Standard’ diffusion



○ Anomalous diffusion

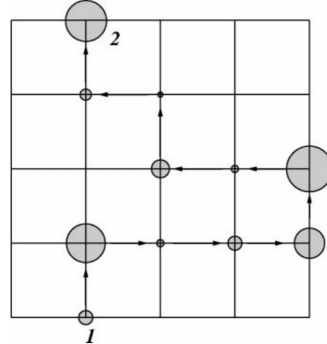


Figure 4. & 5. Metzler, R. and Klafter, J (2000) *The Random Walk's Guide to Anomalous Diffusion: A Fractional Dynamics Approach*, Physics Reports

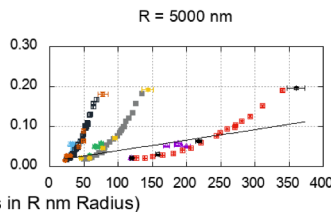
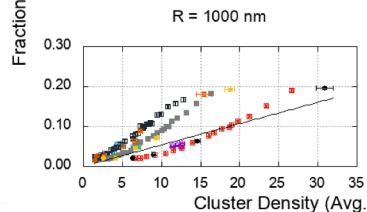
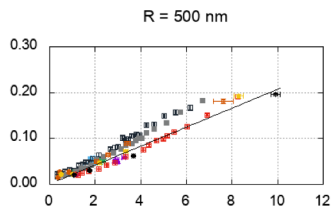
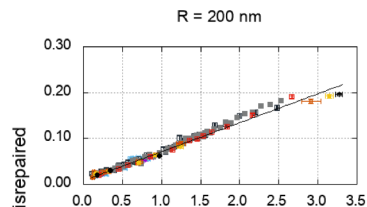
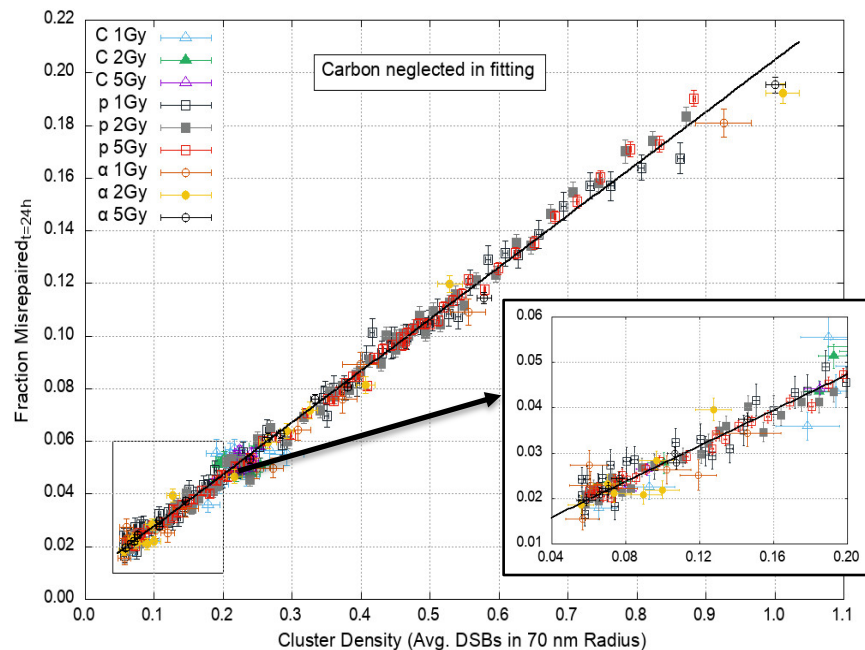
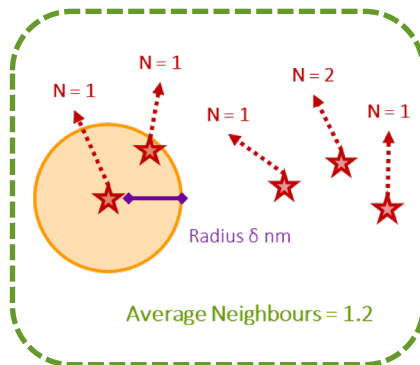
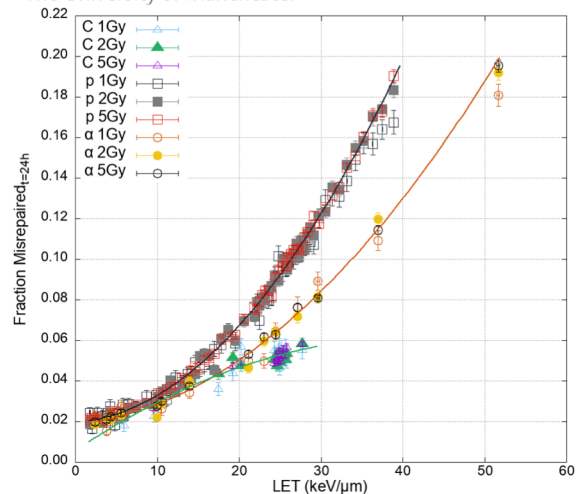
some. Similarly to single DSBs, the broken DNA ends were positionally stable (Fig. 2c). No coalescence was observed at times up to 24 h after breakage, even between arrays separated by less than 400 nm (data not shown). We conclude that DSBs are positionally immobile within the mammalian cell nucleus.

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

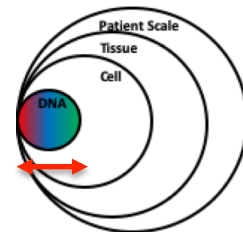
BIO
PROTON

DNA Damage & Repair:

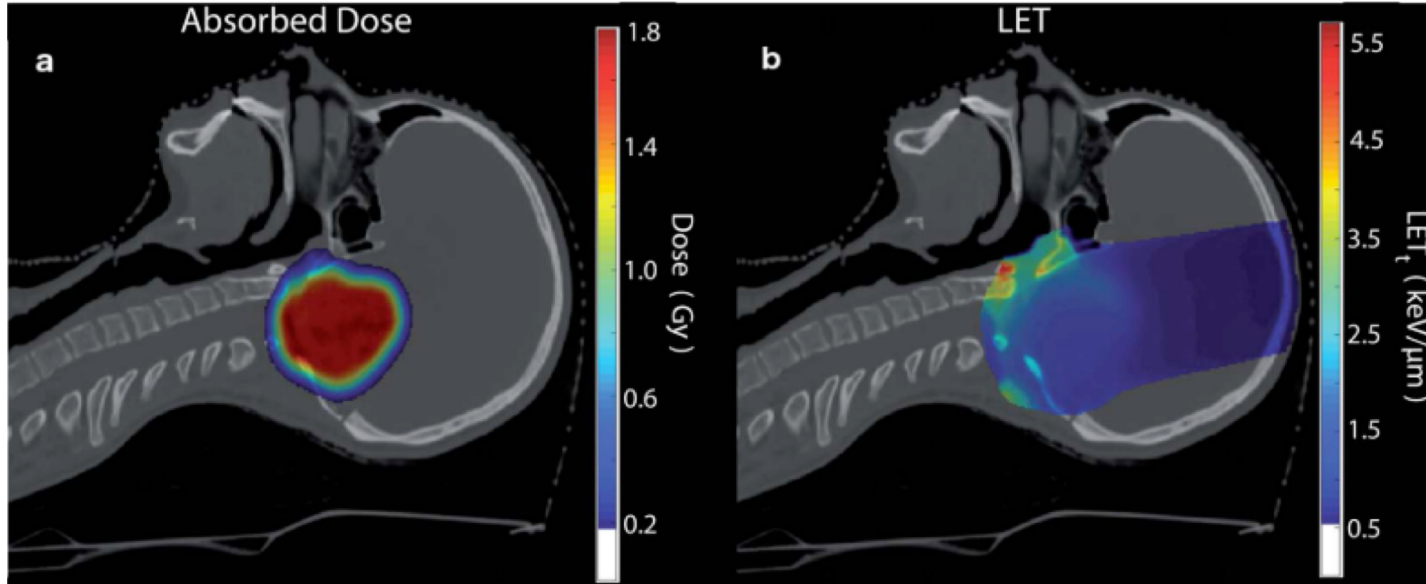
DSB cluster size – a better predictor than LET?



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.

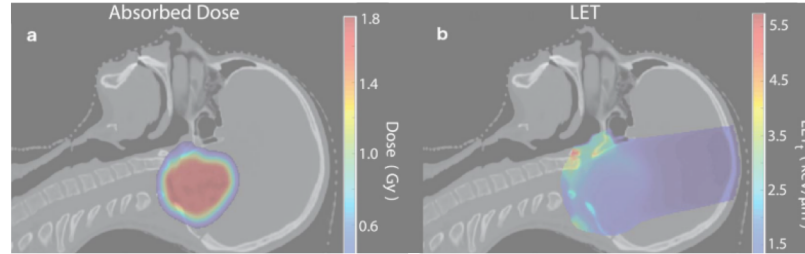


Model Translation: From DNA scale to Patient Scale



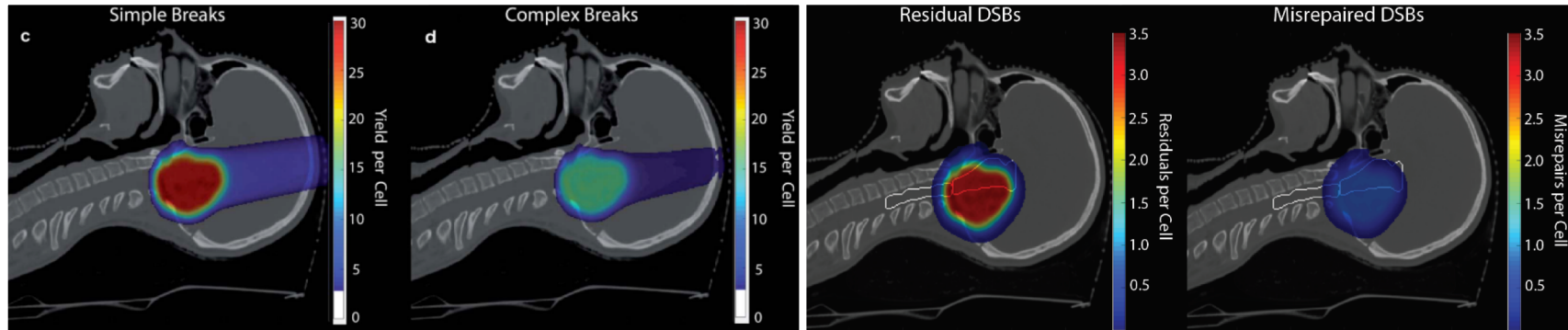
E. Smith et al., Nat. Sci. Rep 2019

Model Translation: From DNA scale to Patient Scale

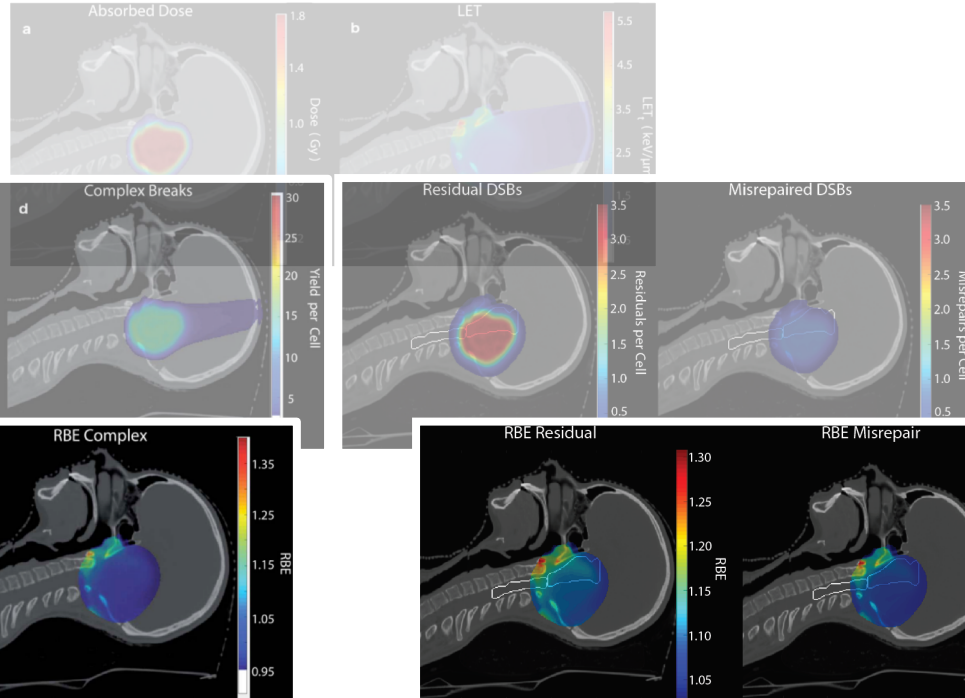


*N. Henthorn et al., RSC
Advances, 2019*

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

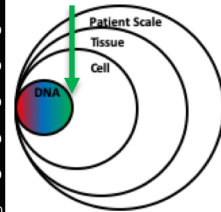
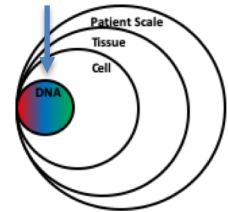


Model Translation: From DNA scale to Patient Scale



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

*N. Henthorn et al., RSC
Advances, 2019*



Biologically augmented treatment plans shown are for research only. These plans are not used clinically.

The Christie Research Beamline



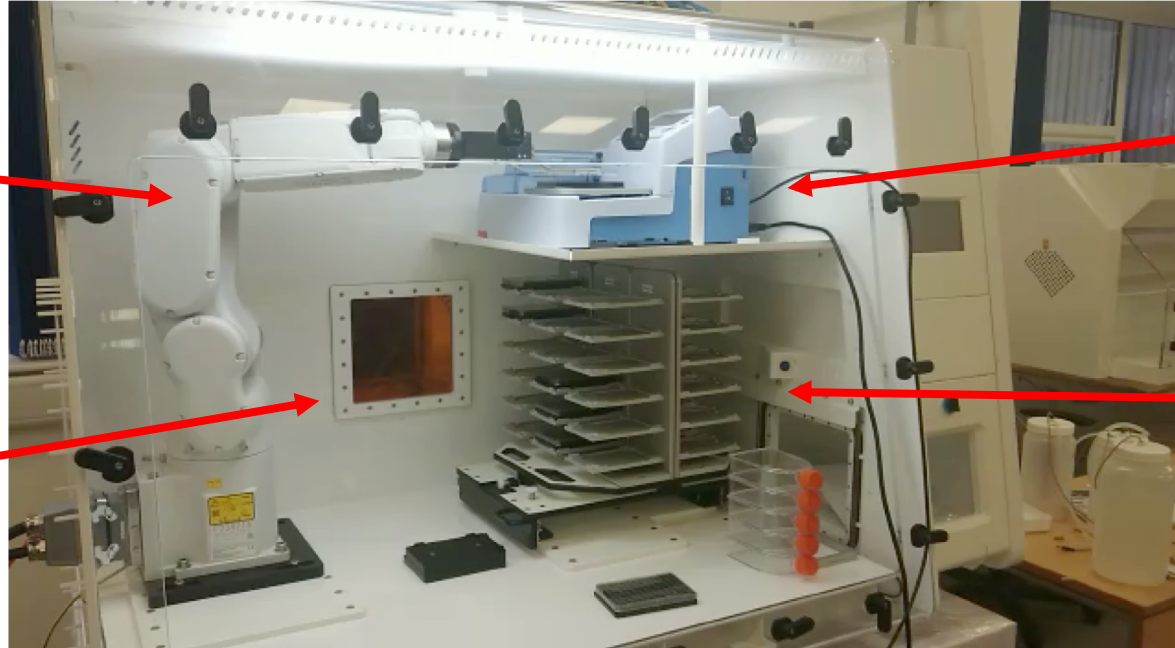
Protons: Hypoxia Radiobiology End-station

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!

The people who did most of the work

- Dr Nick Henthorn
- Dr John Warmenhoven
- Dr Nickolay Korabel
- Sam Ingram
- Ed Smith
- Yaping Qi
- Charlotte Heaven
- Bethany Rothwell
- Kristina Small
- Hannah Wantsall

Also thanks to:

- Prof Karen Kirkby
- Prof. Ranauld MacKay
- Prof. Norman Kirkby
- Prof. Sergei Fedotov
- Dr Amy Chadwick
- Dr Elham Santana
- Dr Adam Aitkenhead

