Radiation track structure - the beginning of the end

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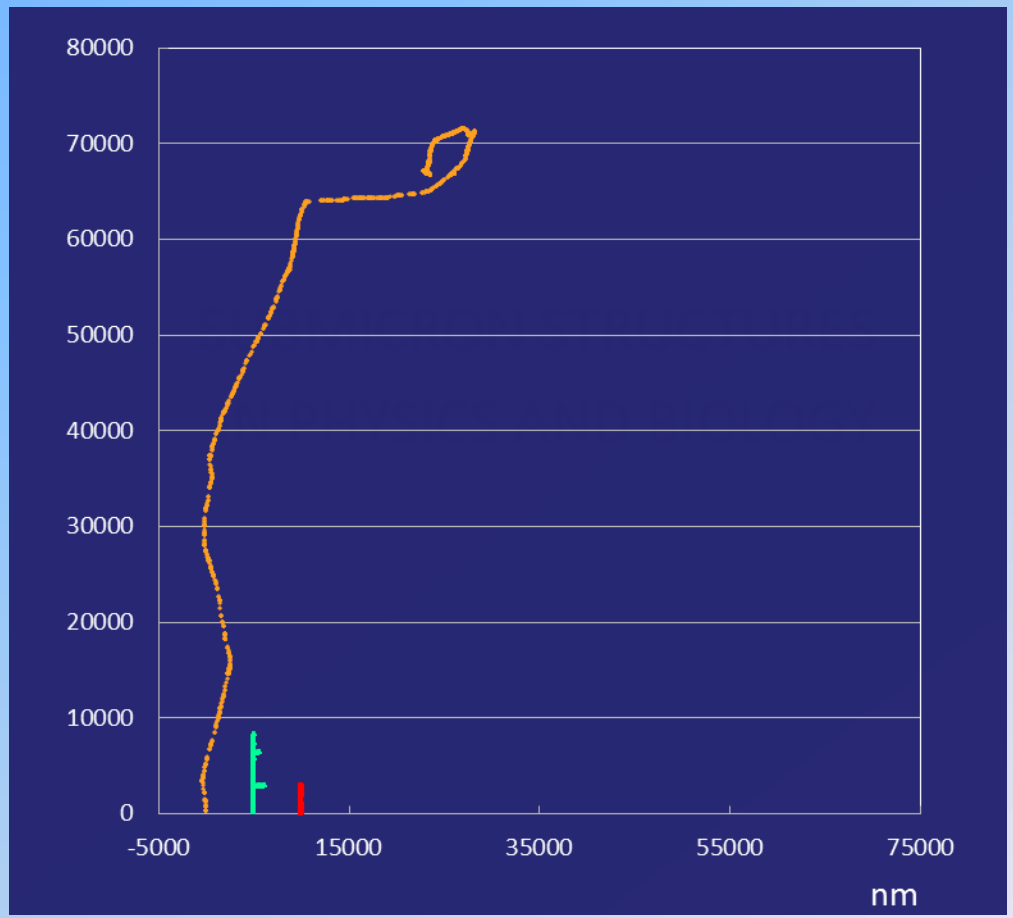
Particle track

= all energy depositions (ionizations, excitations) produced by primary particle and all secondary particles



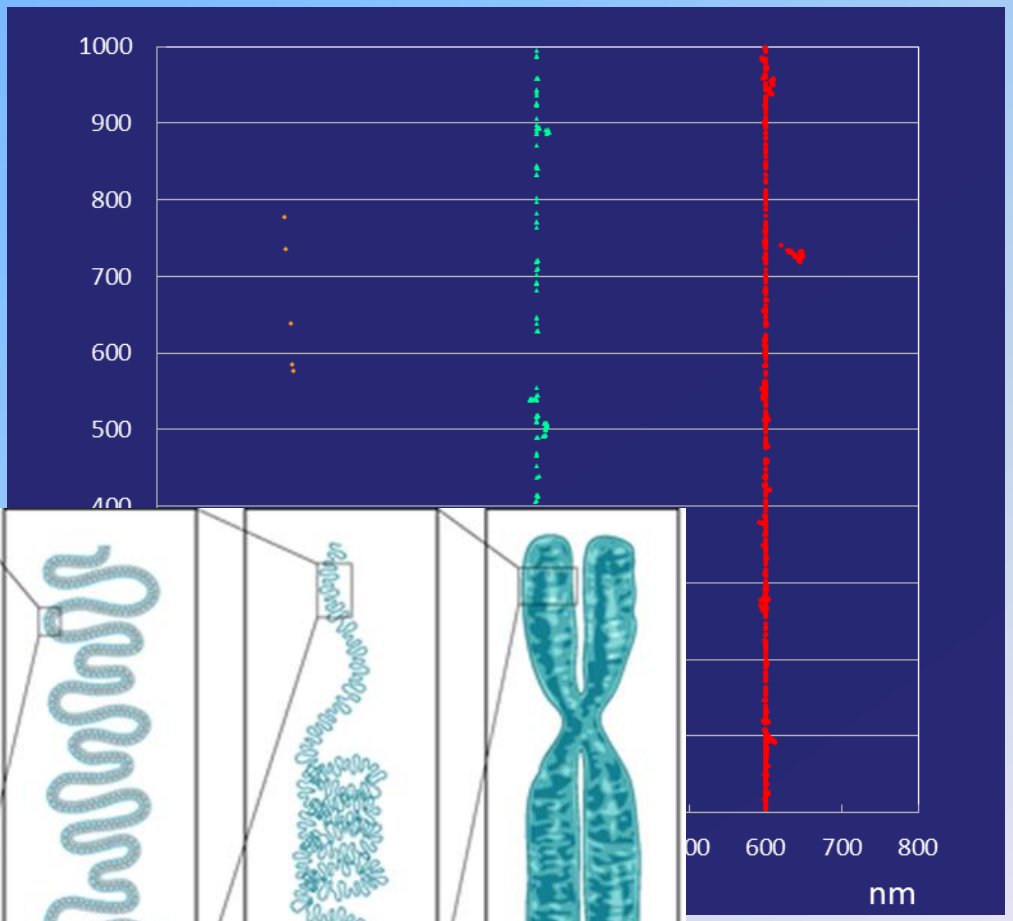
Electron 100 keV ~1,5keV/μm
C ion 480 MeV/n 14 keV/μm
Proton 1 MeV 24 keV/μm

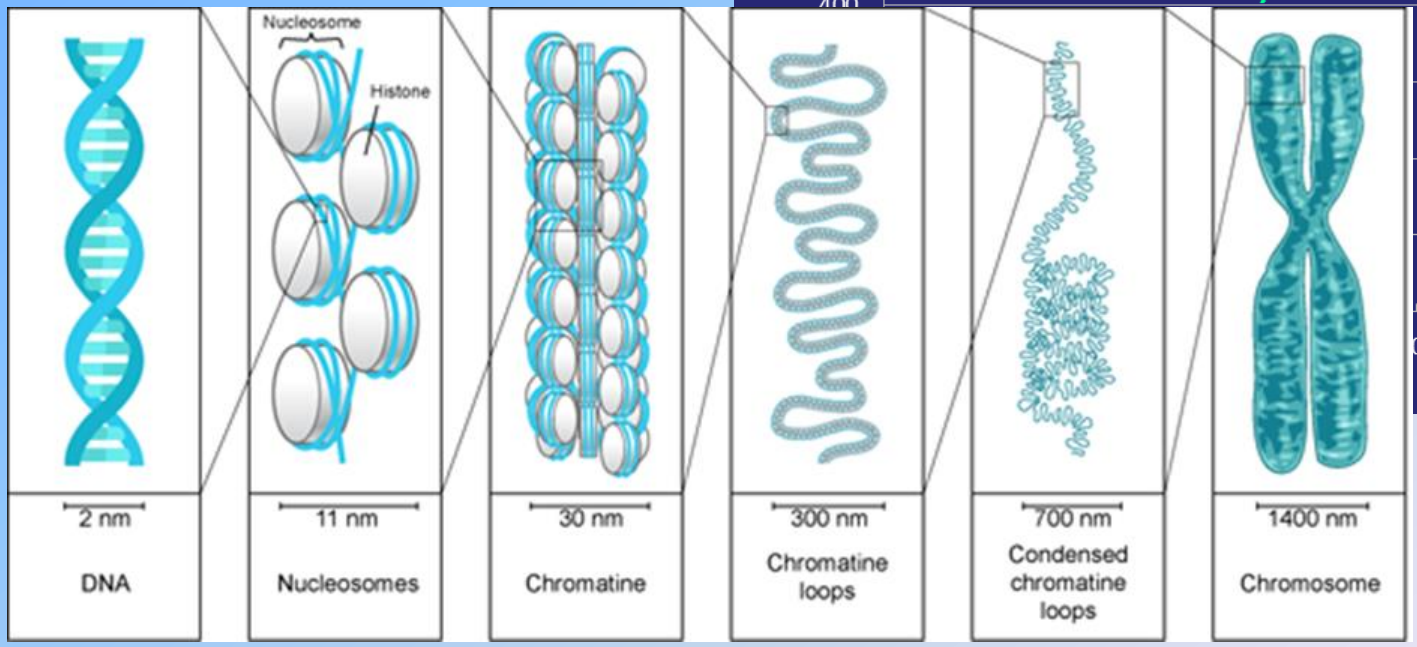
Deposited energy 100 keV

LET - linear energy transfer, $L_\Delta = (dE/dx)_\Delta$

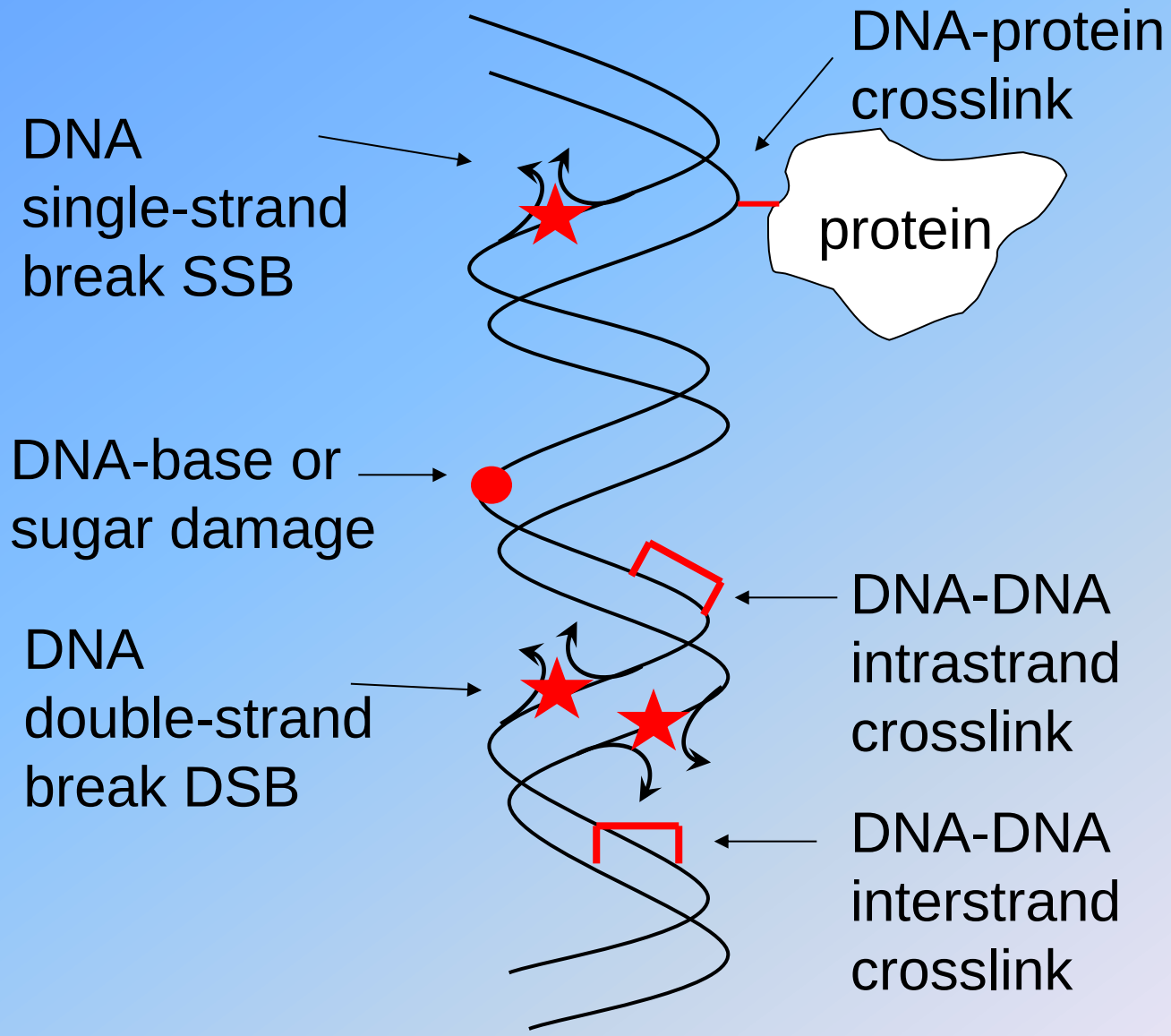
Track vs target structure

Electron 100 keV ~1,5 keV/μm
C ion 480 MeV/n 14 keV/μm
Proton 1 MeV 24 keV/μm



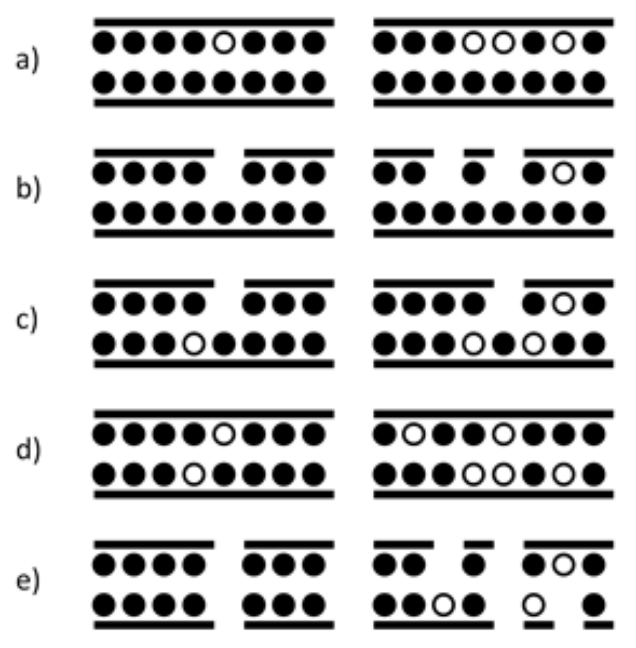


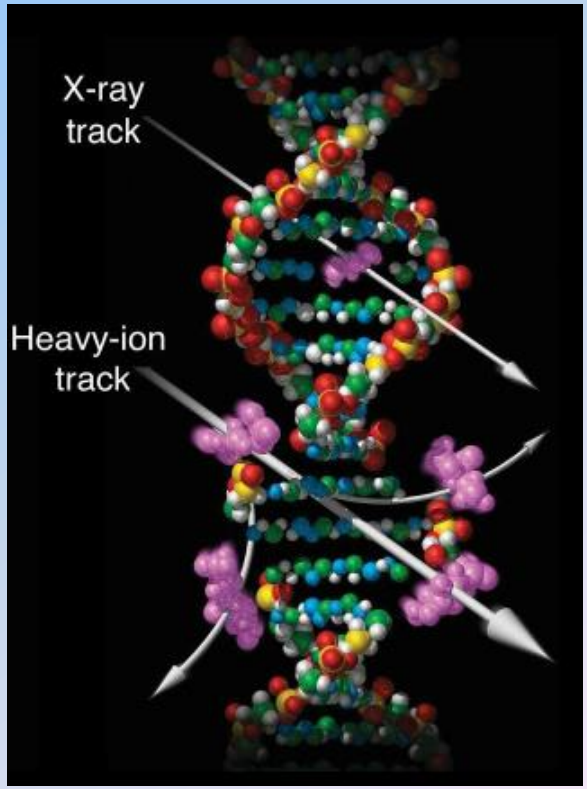
Radiation damage to DNA



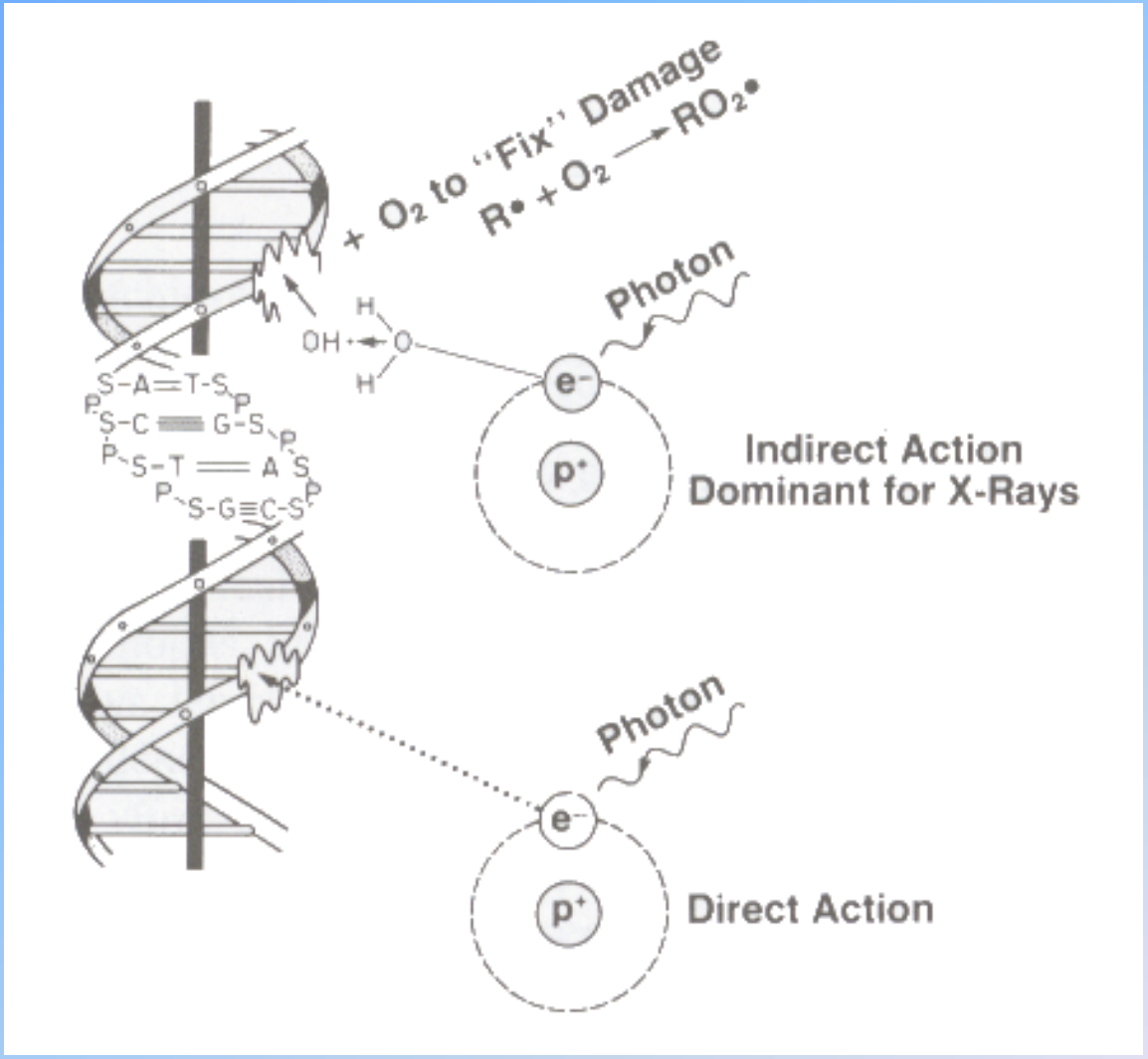
Cluster damage

- locally multiply damaged site = any combination of two or more types of DNA damage (SSB, DSB, oxidized bases)
- Reduction, or inhibition of repair → chromosomal abnormalities → cancer cells
- Two types of clusters: non-DSB clusters > DSB clusters
- Dependent on microscopic radiation quality factors (localization of effects linked to ionization density) – usually simplified approach of linear energy transfer concept (LET)

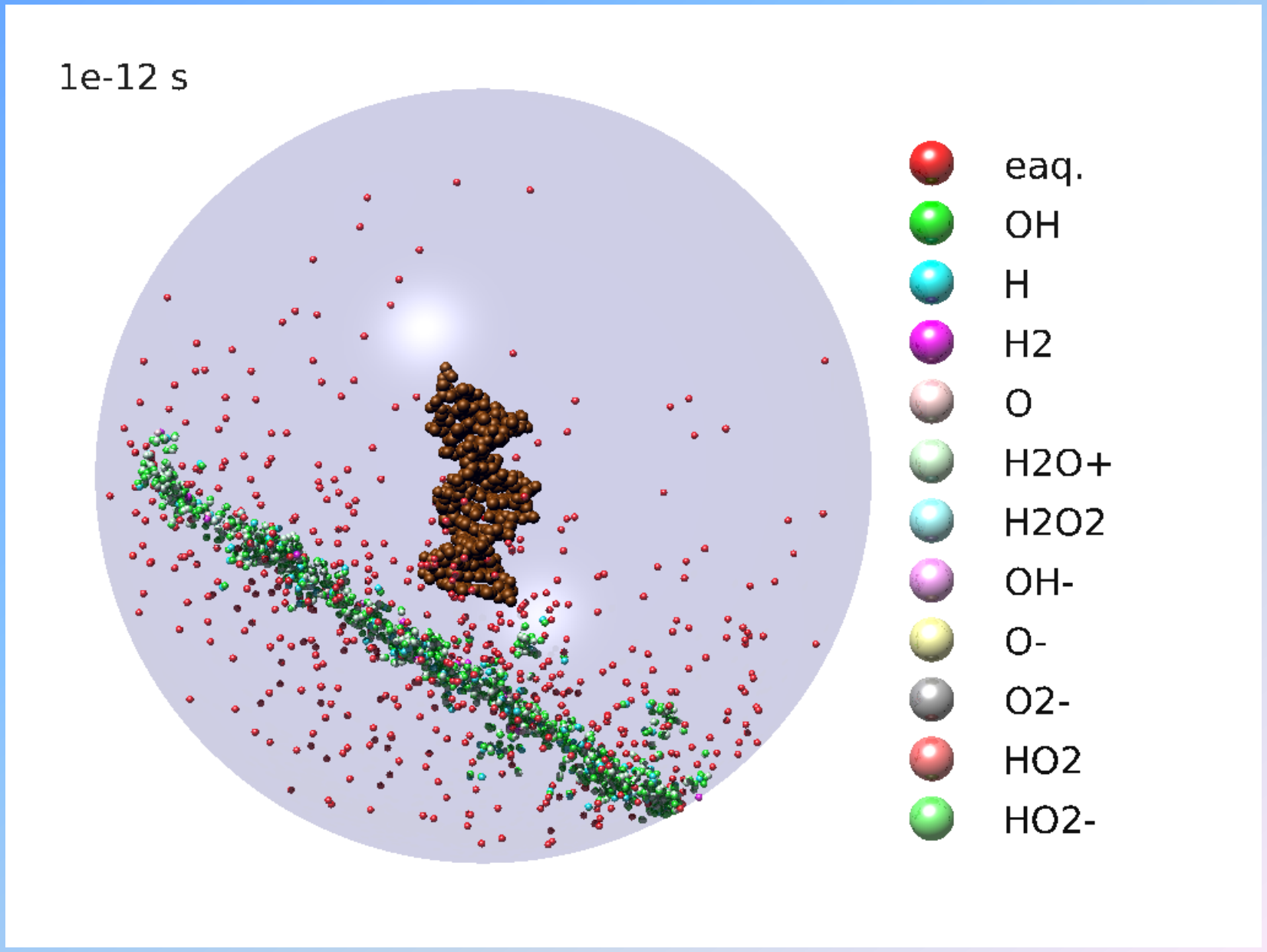


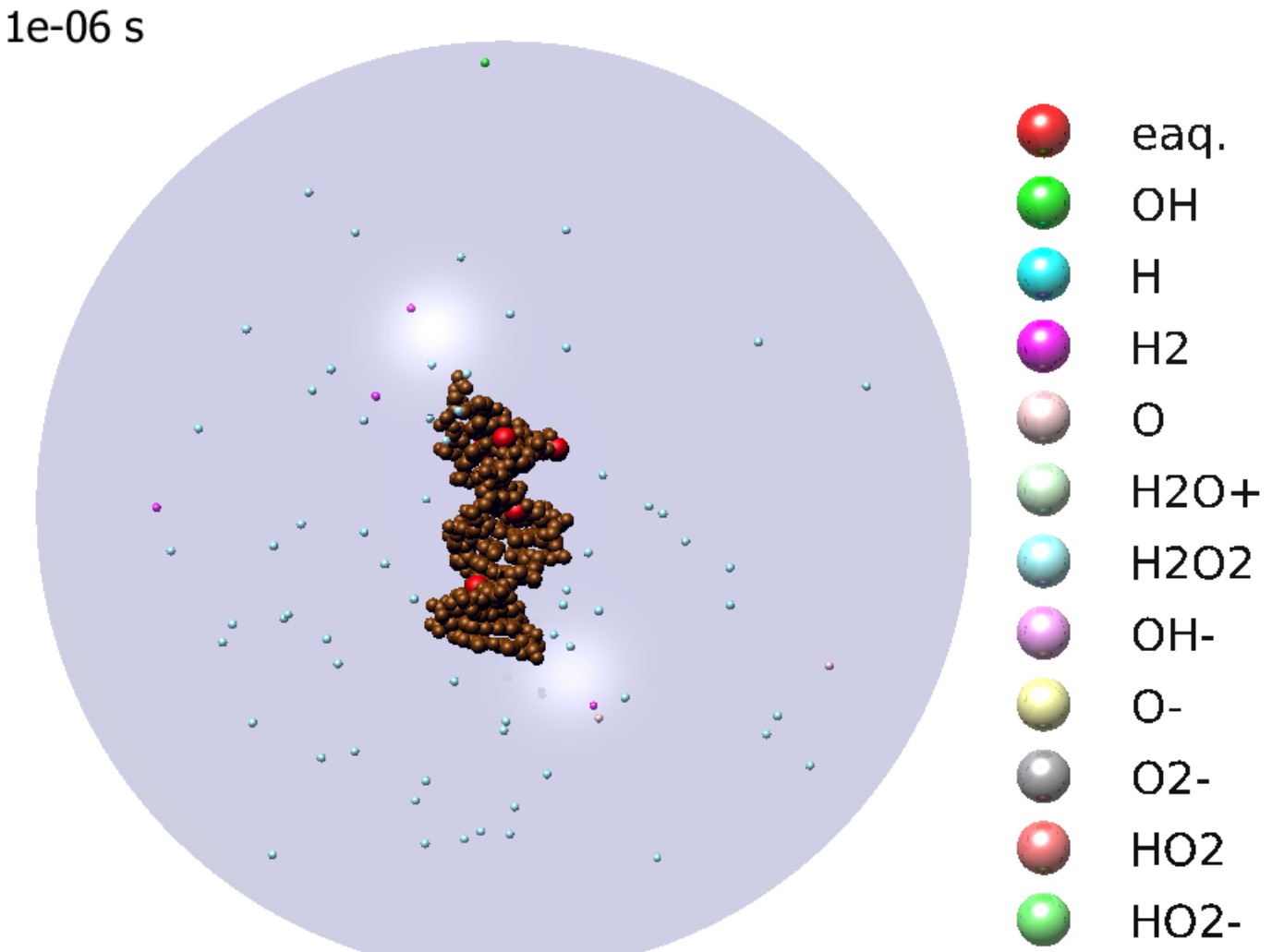


Direct vs indirect effect of ionizing radiation



Radiation damage to DNA




1e-06 s
eaq.
OH
H
H2
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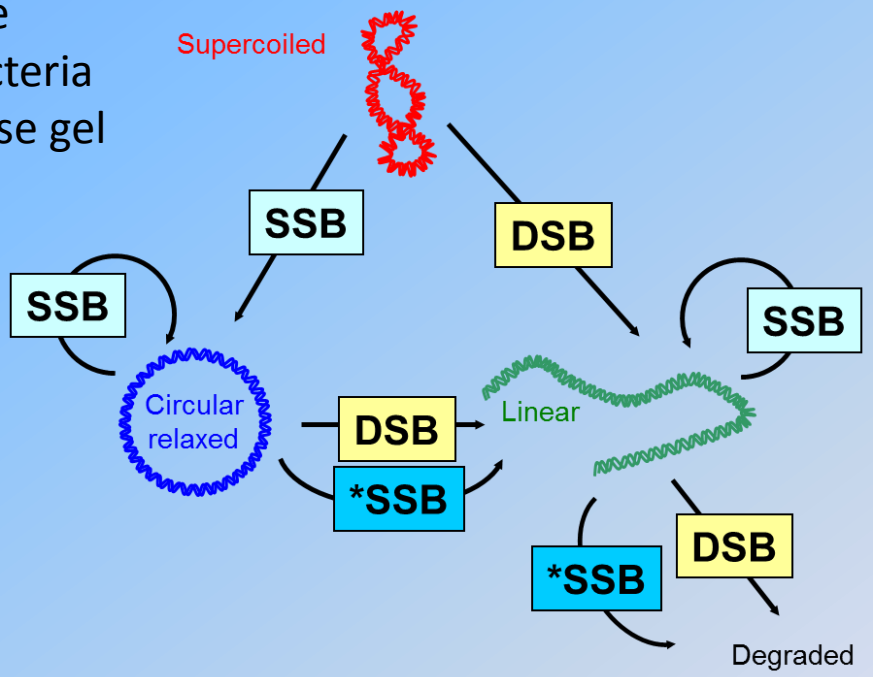
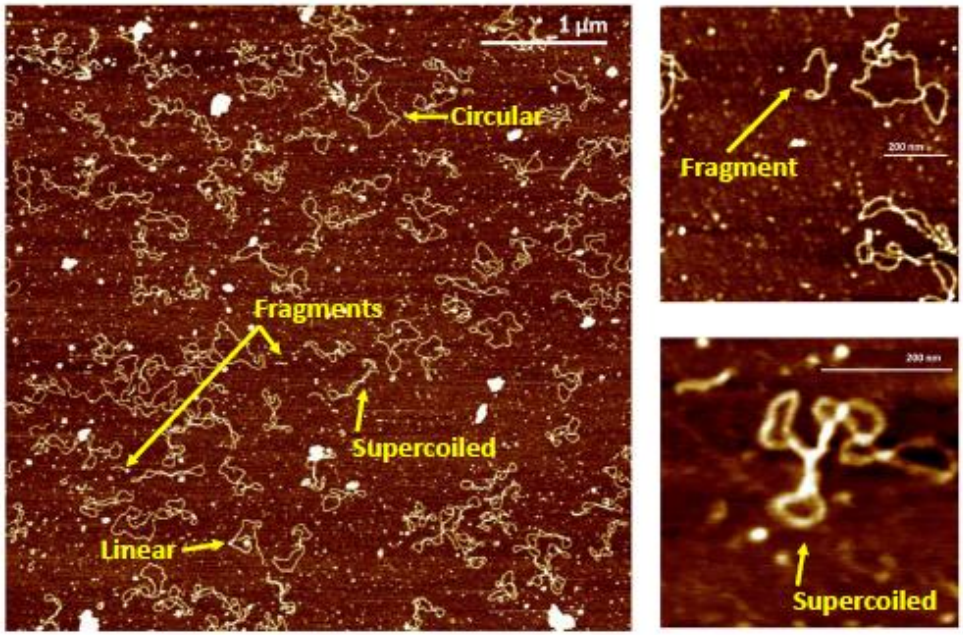
H2O+
H2O2
OH-
O-
O2-
HO2
HO2-

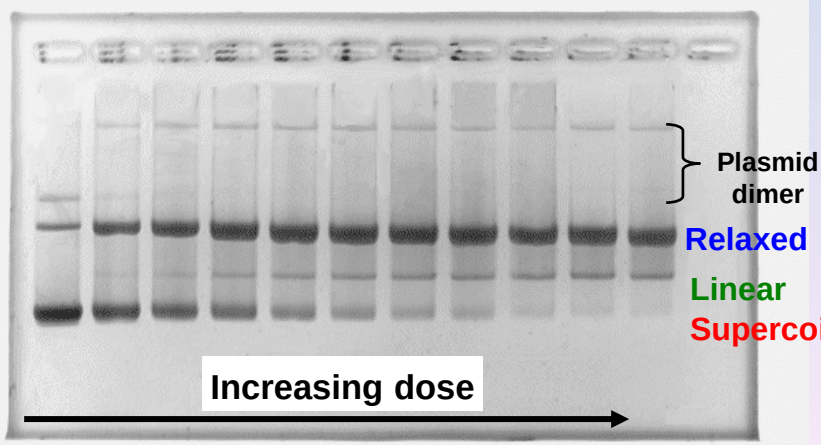
Model system – DNA plasmid

- Plasmid DNA (0.5-10 kbp, e.g. pBR322, pUC) are circular double-stranded DNA purified from bacteria
- Plasmid forms can be easily separated by agarose gel electrophoresis



Plasmid DNA (pBR322), adsorption from solution with 1 ng/µl in water with only residual TE buffer, all forms are included and marked. On the right the details of the same sample.





Proton irradiation

- U120-M cyclotron, Nuclear Physics Institute CAS
- DNA plasmid pBR322
- Oxidized bases detected with Fpg (formamidopyrimidine DNA glycosylase) and Nth (Endonuclease III) - optimized reaction buffers and concentration due to titration
- Agarose gel electrophoresis → separation of supercoiled (SC), relaxed (R) and linear (L) form
- Yields of SSB and DSB (Parameters **μ** and **ϕ** are the average numbers of single and double strand breaks per plasmid and Gy) calculated according to two statistical models:

Cowan, Collis, Grigg, 1987, J. Theor. Biol. 127, 229

$$S(D) = e^{-(\mu_0+\mu D)}/(1+\phi_0+\phi D),$$

$$C(D) = (1-e^{-(\mu_0+\mu D)})/(1+\phi_0+\phi D),$$

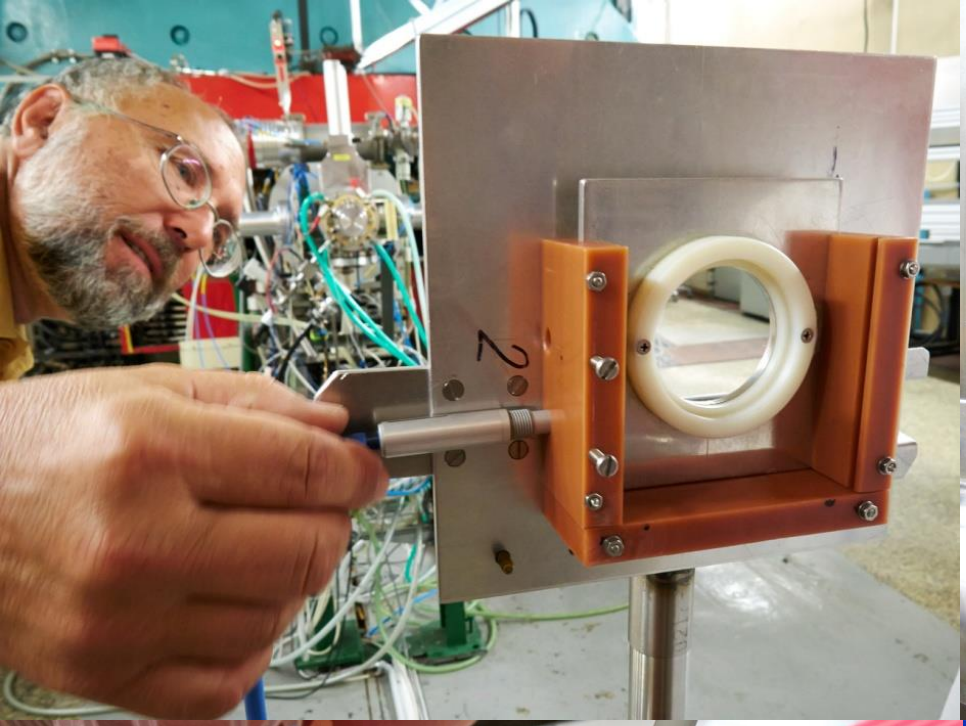
$$L(D) = (\phi_0+\phi D)/(1+\phi_0+\phi D),$$

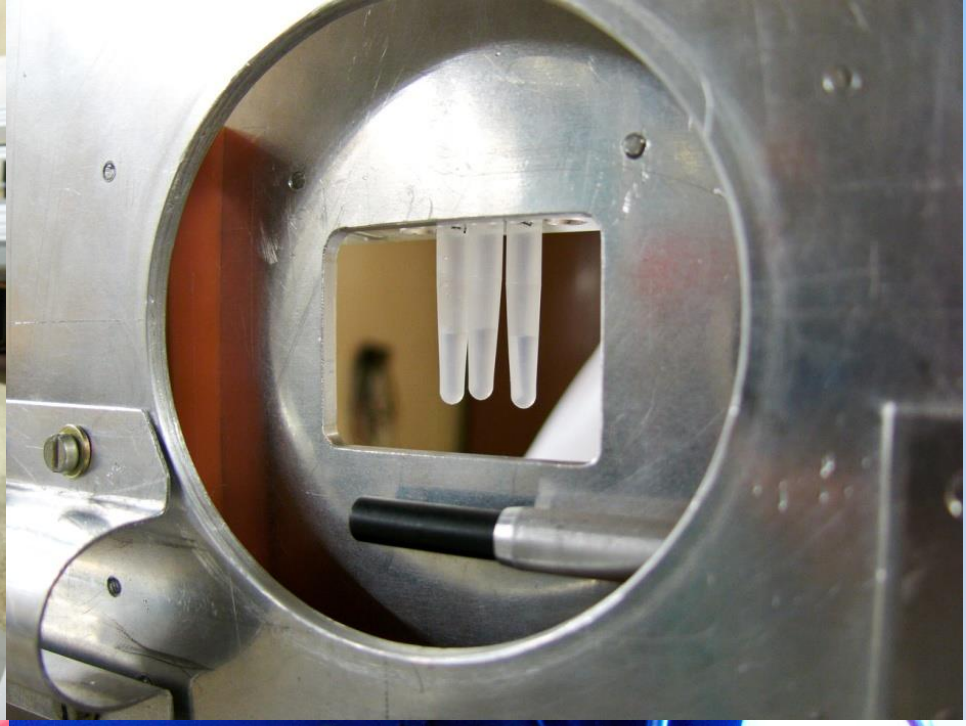
McMahon and Currell, 2011, Radiat. Res. 175, 797

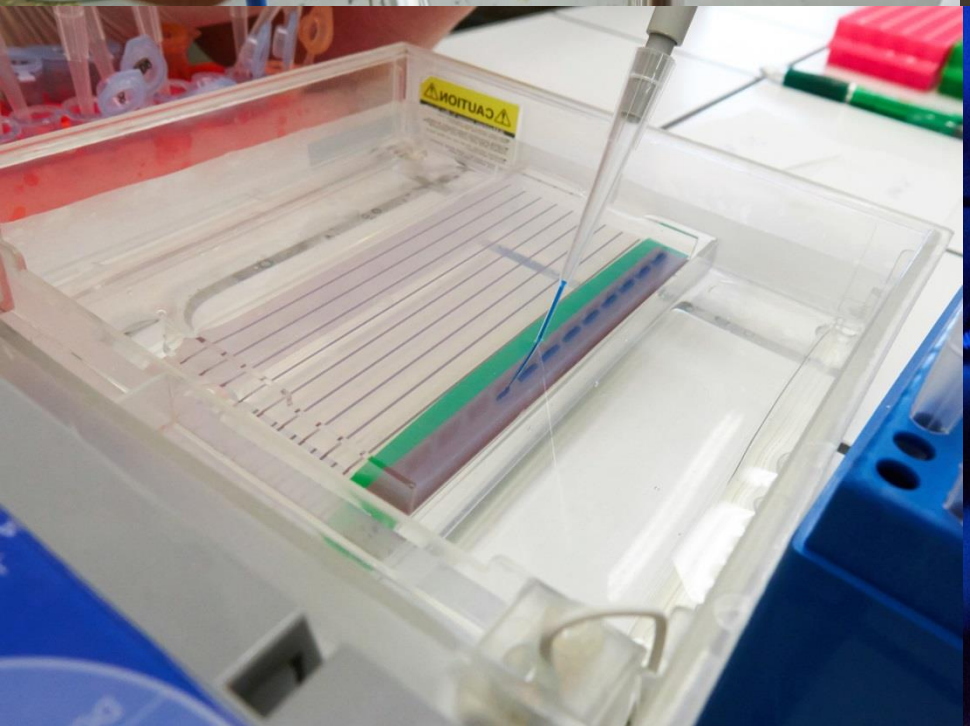
$$S(D) = S_0 e^{-(\mu D+\phi D)},$$

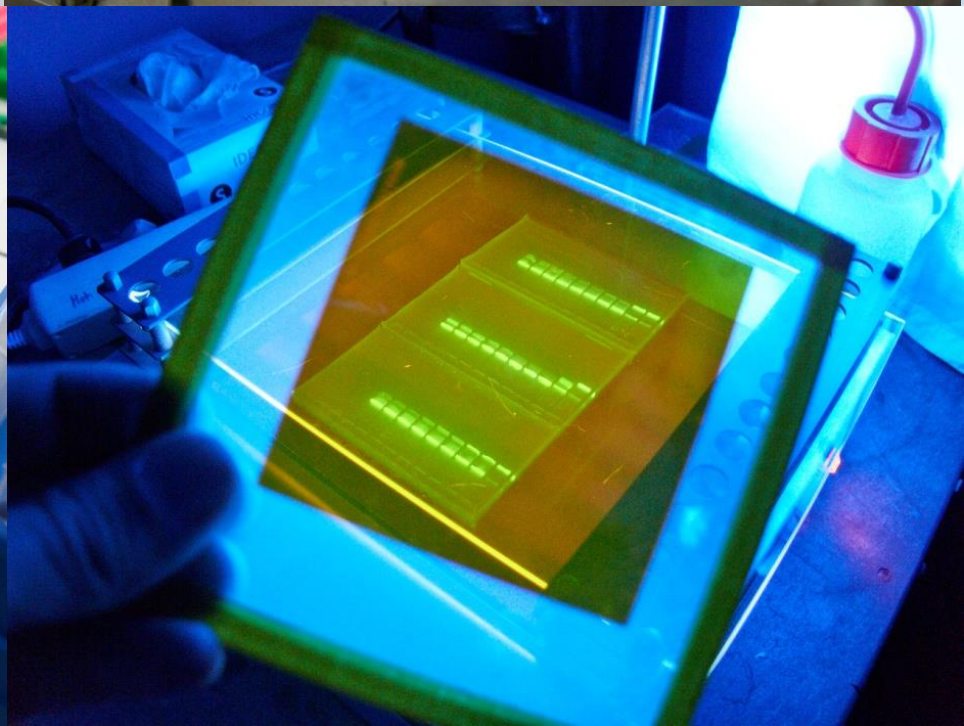
$$C(D) = e^{-\phi D}[C_0 e^{-0.5\mu\rho D^2} + S_0 e^{-0.5\mu^2\rho D^2} - S_0 e^{-\mu D}],$$

$$L(D) = 1-(S_0+C_0)e^{-(\phi D+0.5\mu^2\rho D^2)}.$$

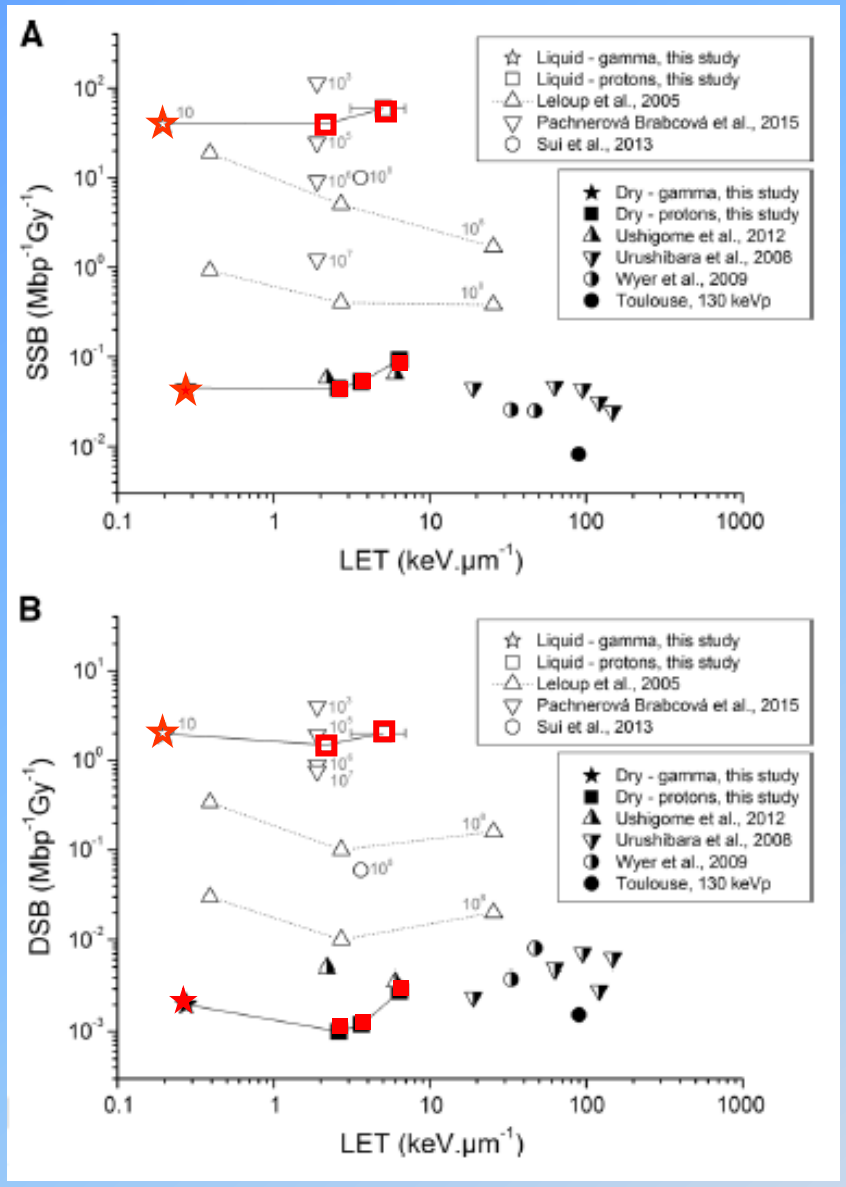


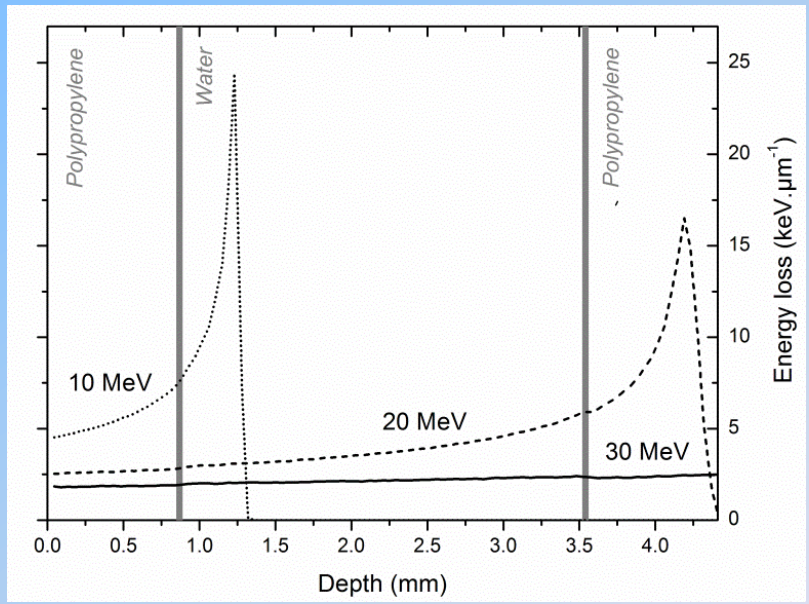



CAUTION



DNA: dry and in solution





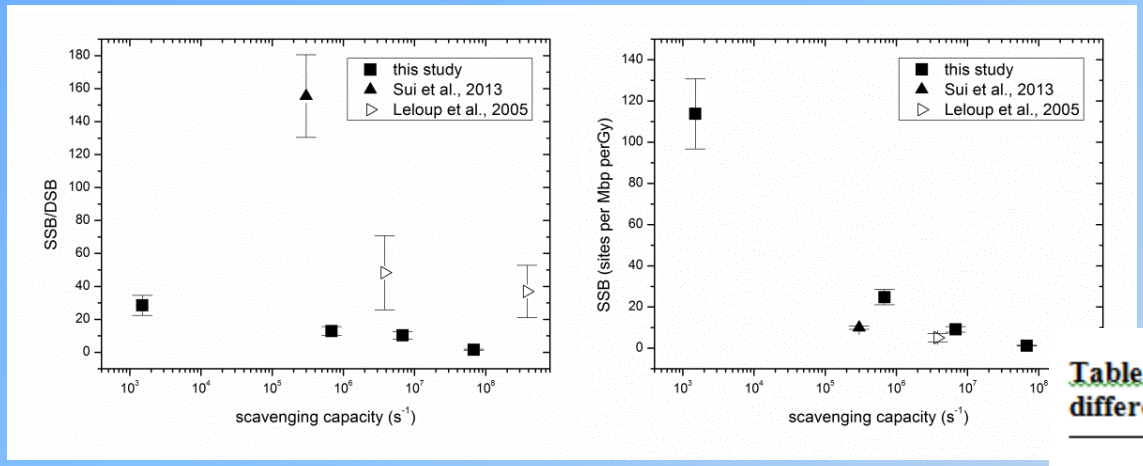
LET in water and in the dry samples in keV/µm of proton beam

Proton energy	Water	Dry sample
30 MeV	1.903	2.612
20 MeV	2.648	3.636
10 MeV	4.657	6.394

L. Vyšín et al., Radiat Environ Biophys, 2015,
DOI 10.1007/s00411-015-0605-6

DNA: scavengers (coumarin-3-carboxylic acid - C3CA)

- Isolated events: SSB (without enzymatic treatment) + isolated bases (increase of SSB after enzymatic treatment compared to SSB without enzymes)
- Cluster events: DSB (without enzymatic treatment) + cluster bases (increase of DSB after enzymatic treatment compared to DSB without enzymes)



SSB/DSB ratio as function of scavenging capacity (a) and SSB sites per 10^6 bp per Gy (b); comparison of different experimental results: this study with C3CA, 30 MeV protons (squares), data with Tris-HCl buffer, 15 MeV protons (closed triangles, Sui et al., 2013), and data with glycerol, 19.3 MeV protons (open triangles, Leloup et al., 2005).

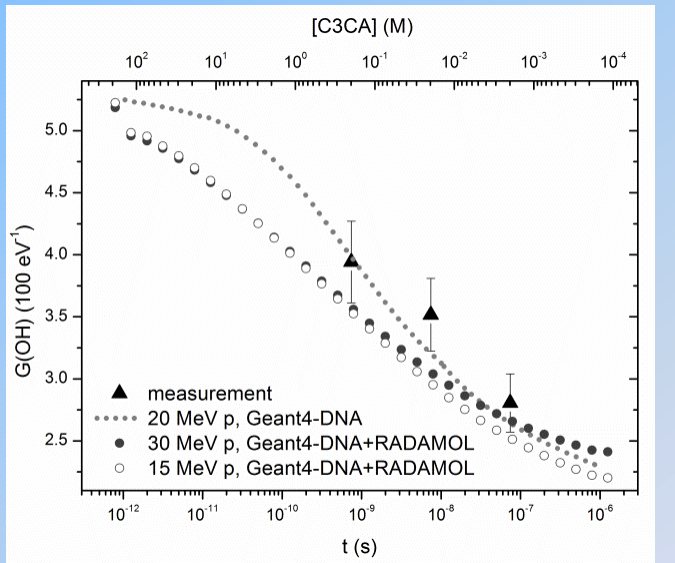
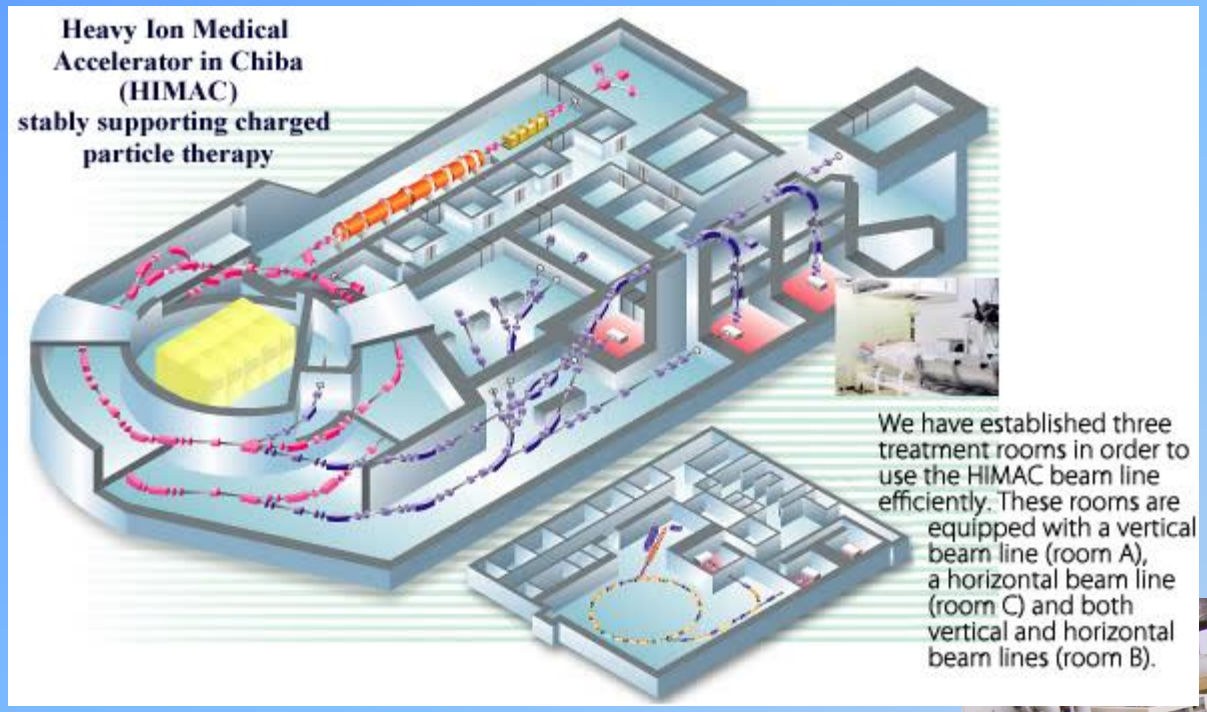


Table 1. Yields of DNA damage per 10^6 bp per Gy for different concentrations of C3CA scavenger

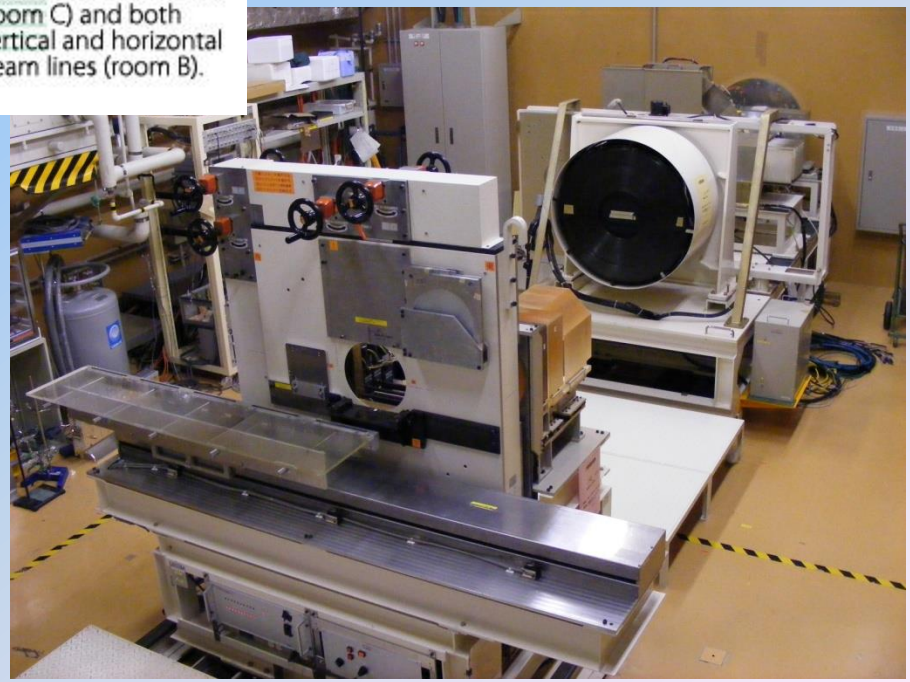
[C3CA], mM	0	2	20	200
SSB	113.76	24.79	9.17	1.22
Isolated oxidized bases	113.09	18.02	13.32	3.39
Total isolated events	226.85	42.81	22.49	4.61
DSB	3.99	1.93	0.89	0.76
Clustered oxidized bases	14.58	1.54	0.41	0.00
Total clustered events	18.57	3.46	1.31	0.76

Brabcova et al. Radiation Protection Dosimetry, 2015, DOI:10.1093/rpd/ncv159

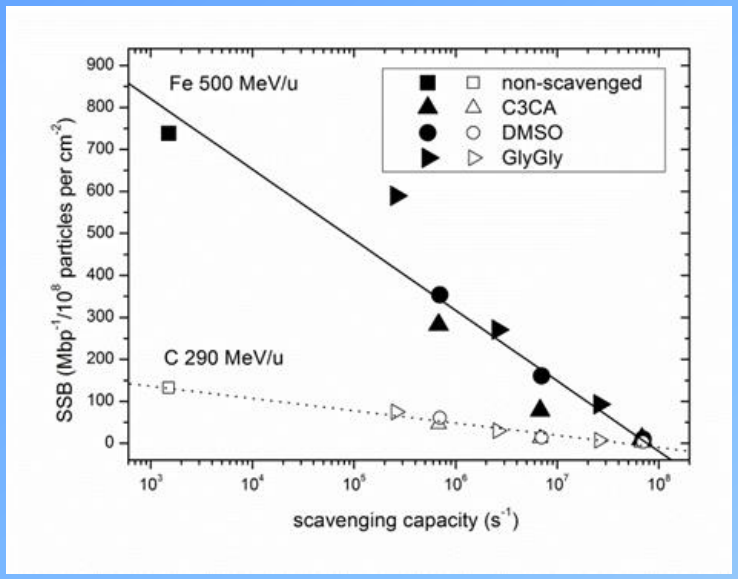
Ion irradiation at HIMAC, Japan

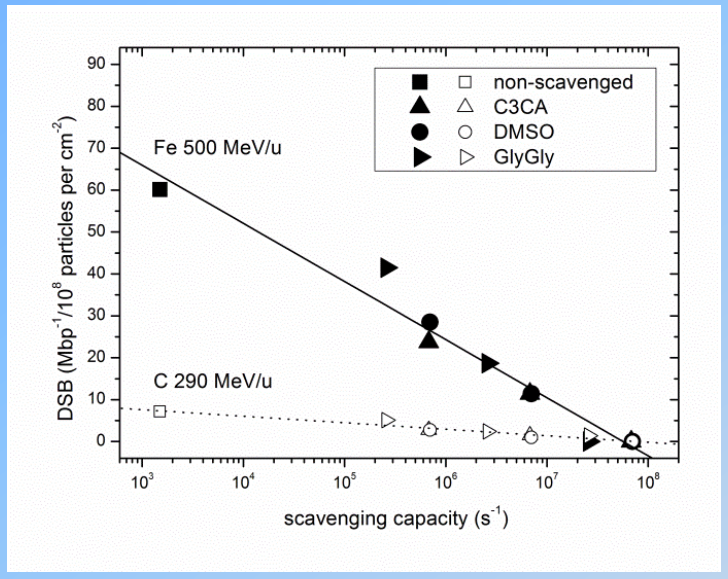


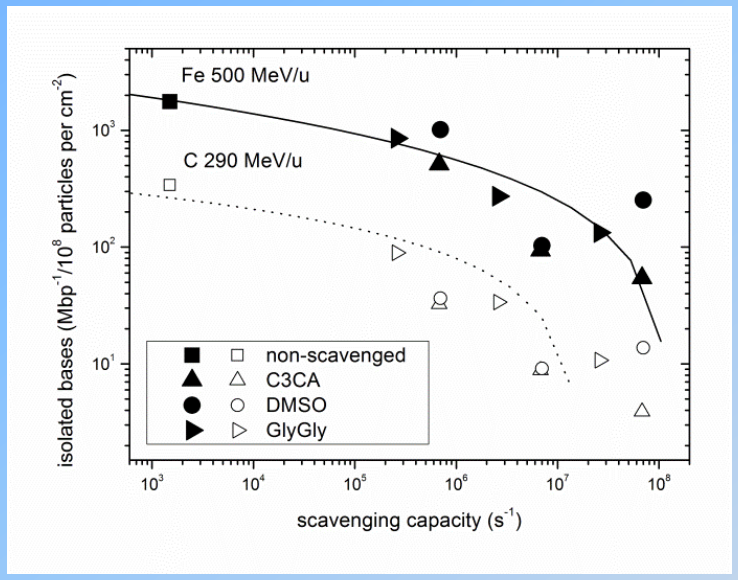
We have established three treatment rooms in order to use the HIMAC beam line efficiently. These rooms are equipped with a vertical beam line (room A), a horizontal beam line (room C) and both vertical and horizontal beam lines (room B).

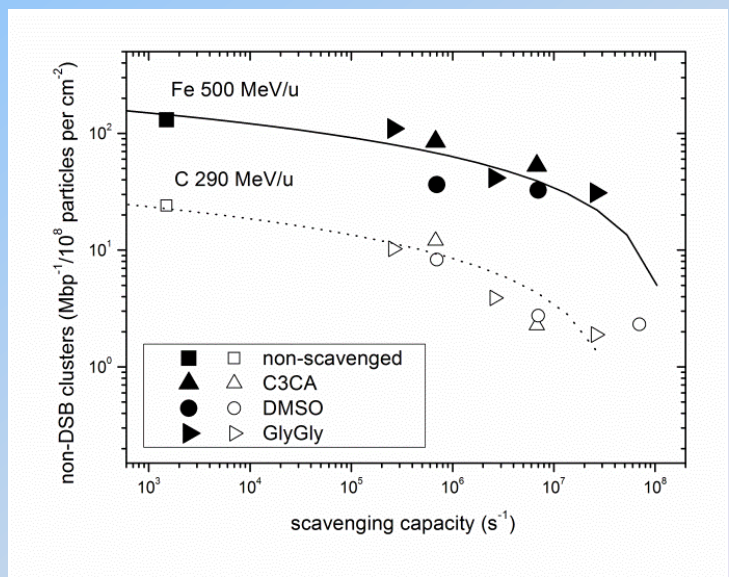


500 MeV/u Fe, 290 MeV/u C ions









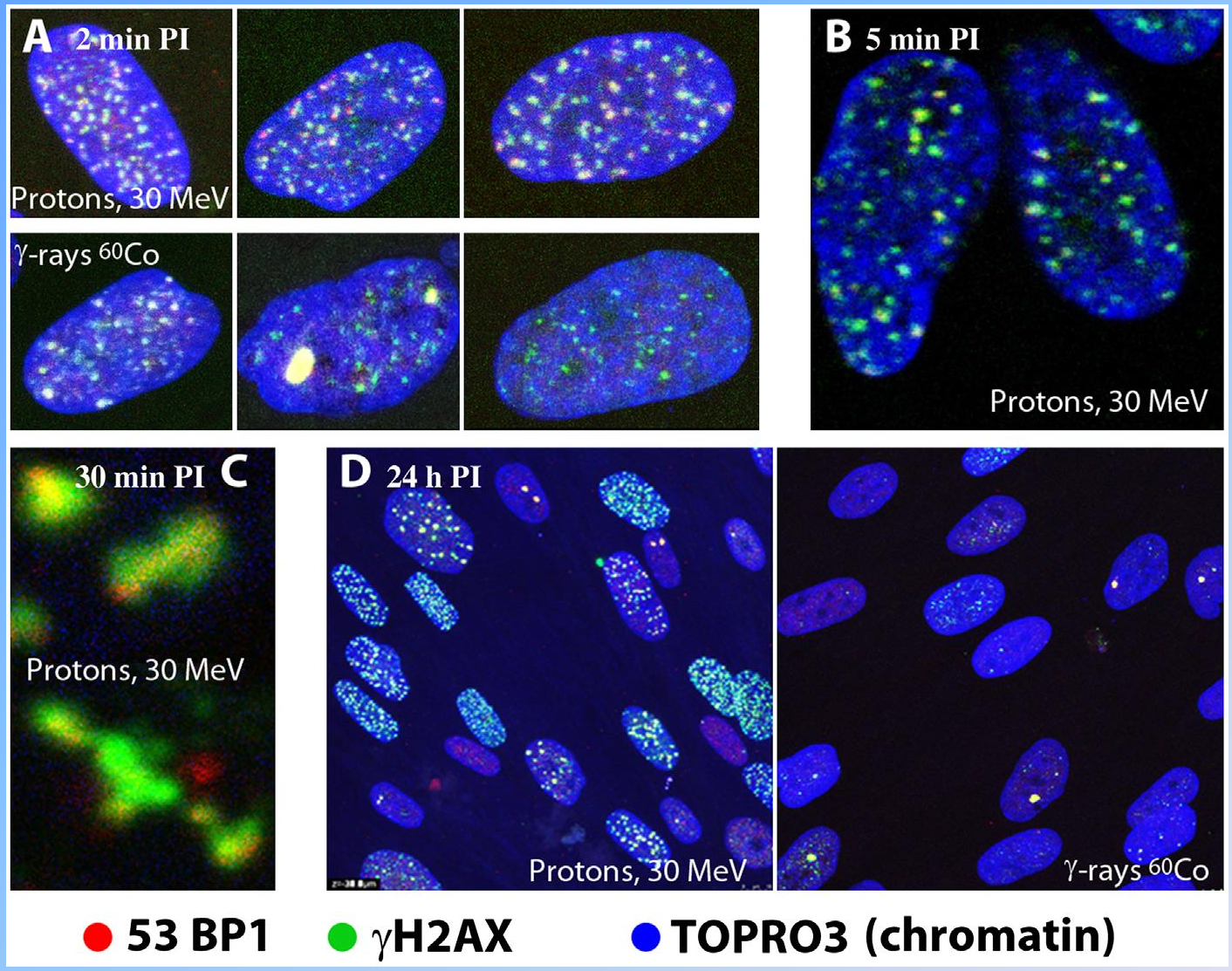
K. Pachnerova Brabcova et al., Radiat Environ Biophys (2014) 53:705–712

Conclusions 1.

- Yields of both simple and clustered DNA damages are suppressed in presence of scavengers.
- With increasing LET, the unscavengeable effect become more important.

- Extremely careful control of experimental conditions is necessary

What about cells?



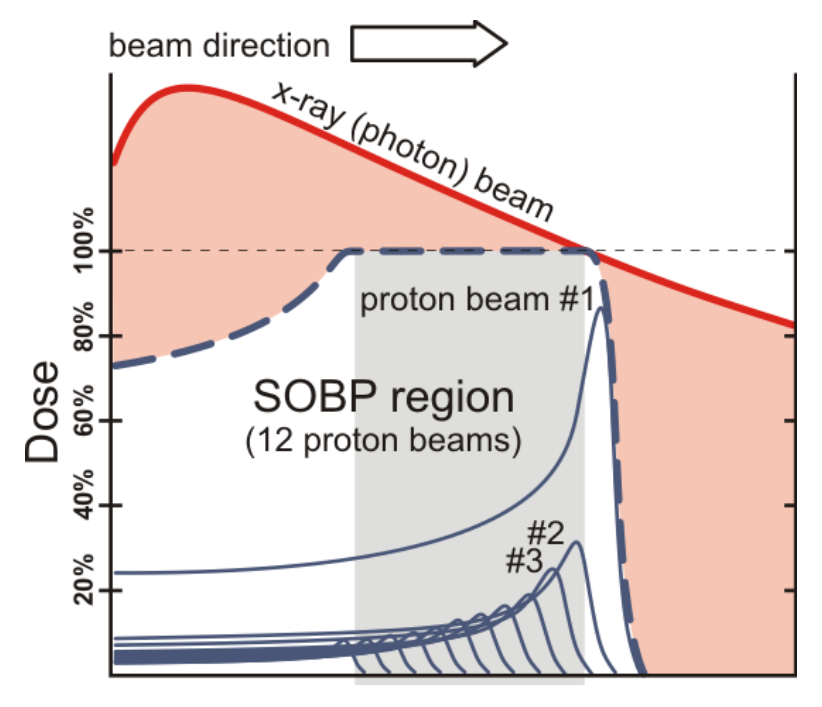
Jezkova et al. Applied Radiation and Isotopes 83(2014): 128–136

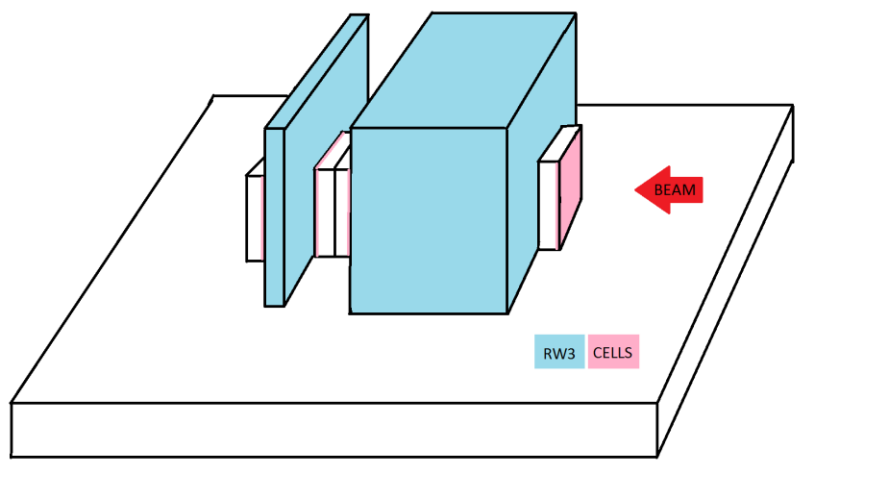
Proton Therapy Center Prague

- IBA cyclotron provides proton beams with energy up to 226 MeV
- Irradiation modes: single scattering, double scattering, uniform scanning, pencil beam scanning

- First patient on 12/12/12

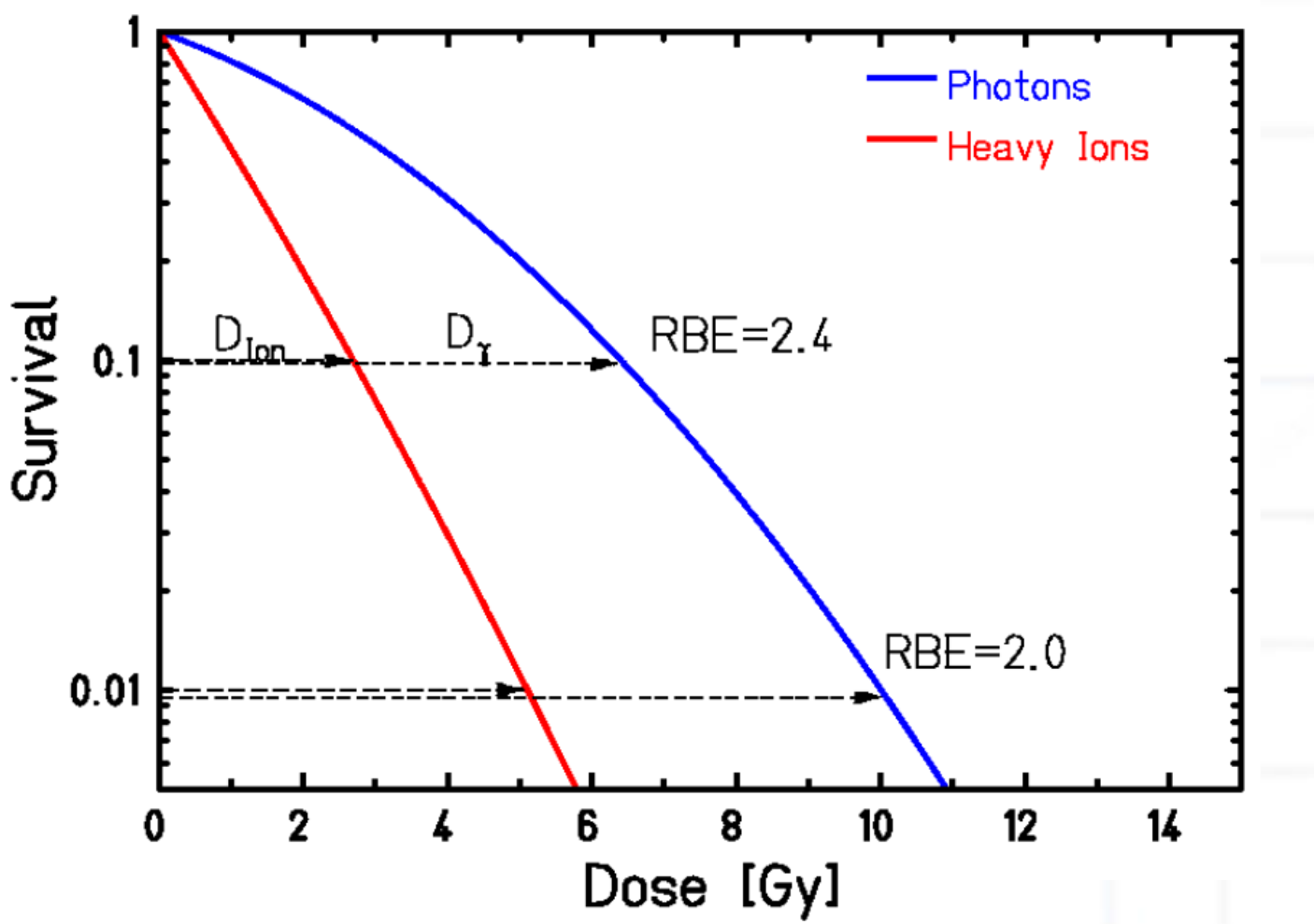
Relative Biological Effectiveness in a proton SOBP





- Irradiation plans were prepared in XIO treatment planning system using the PBS model used for patient treatment for target volume 16 cm x 8 cm x 10 cm. The different positions in the SOBP were adjusted using different thicknesses of RW3 plastic.
- Samples were placed at the beam entrance, in the proximal region of SOBP, in the middle and before the distal edge of SOBP .
- The dose at the beam entrance was 80 % of the dose maximum. The homogeneity of the dose in the PTV was ±2 %.

Relative biological efficiency - RBE

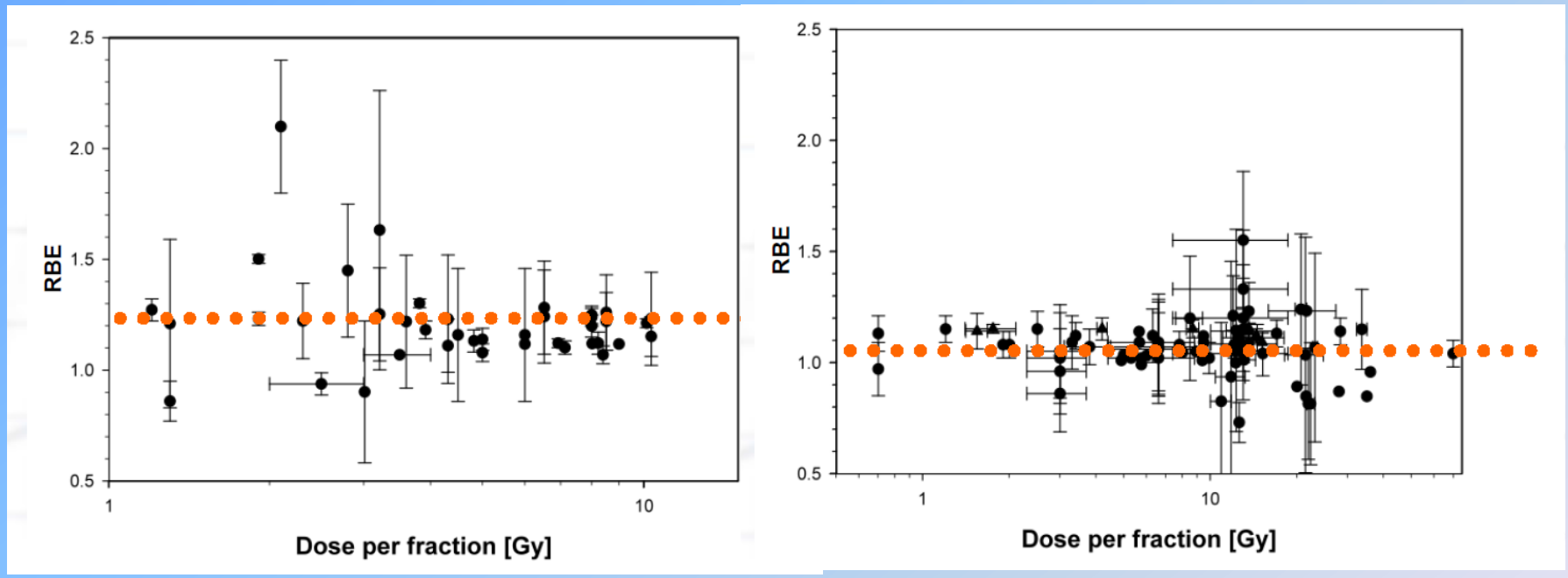


$$RBE = \frac{D_\gamma}{D_{ion}} \langle effect \rangle$$

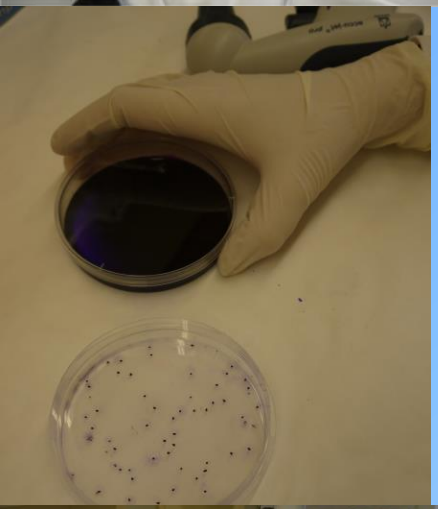
RBE in SOBP

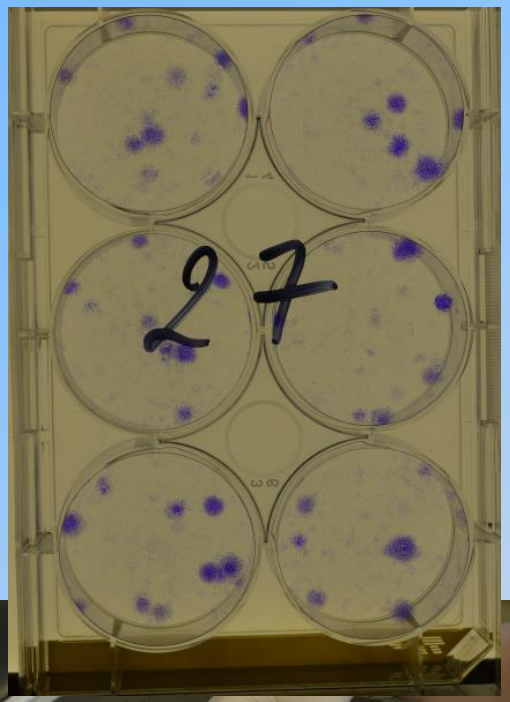
In vitro 1.21 ± 0.2

In vivo 1.07 ± 0.17



Paganetti et al., IJROBP 53, 407-21, 2002

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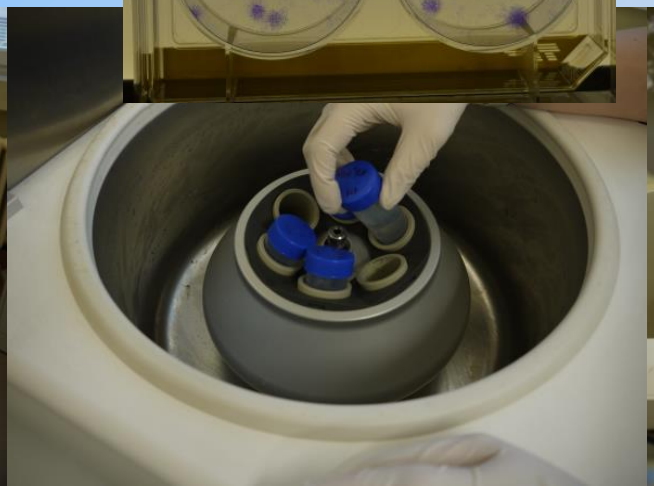
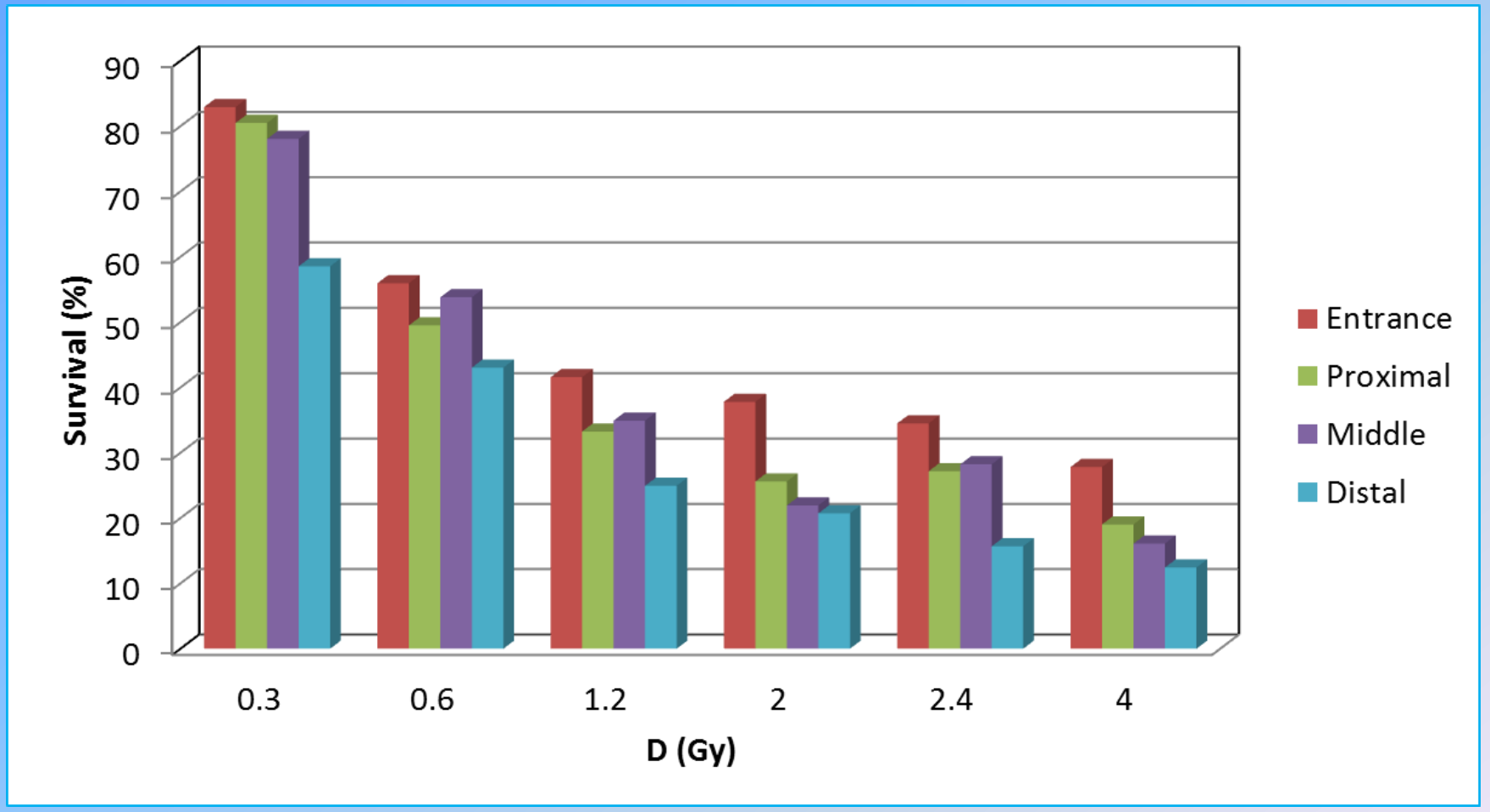
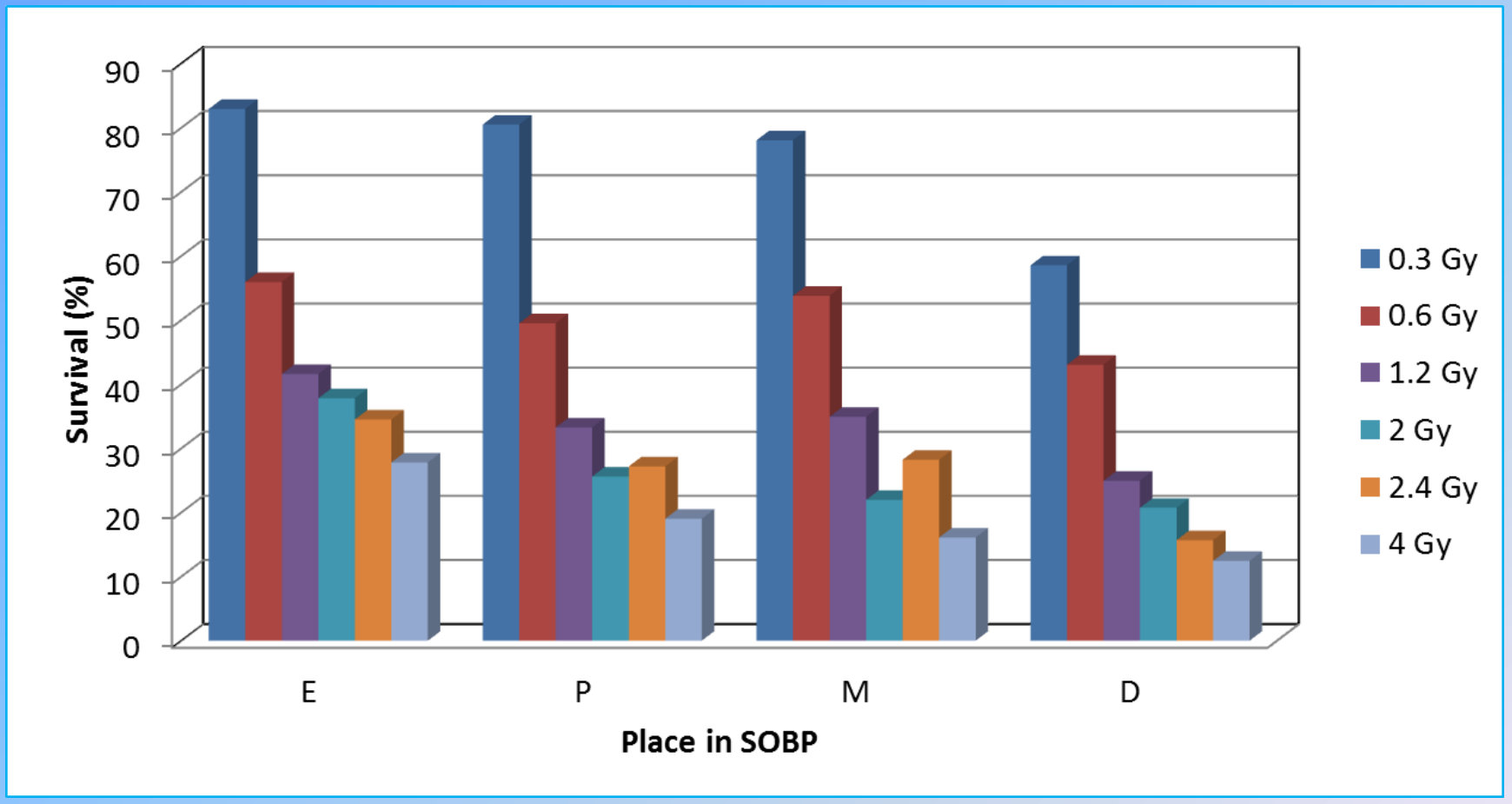


Human neonatal dermal fibroblasts - cell survival

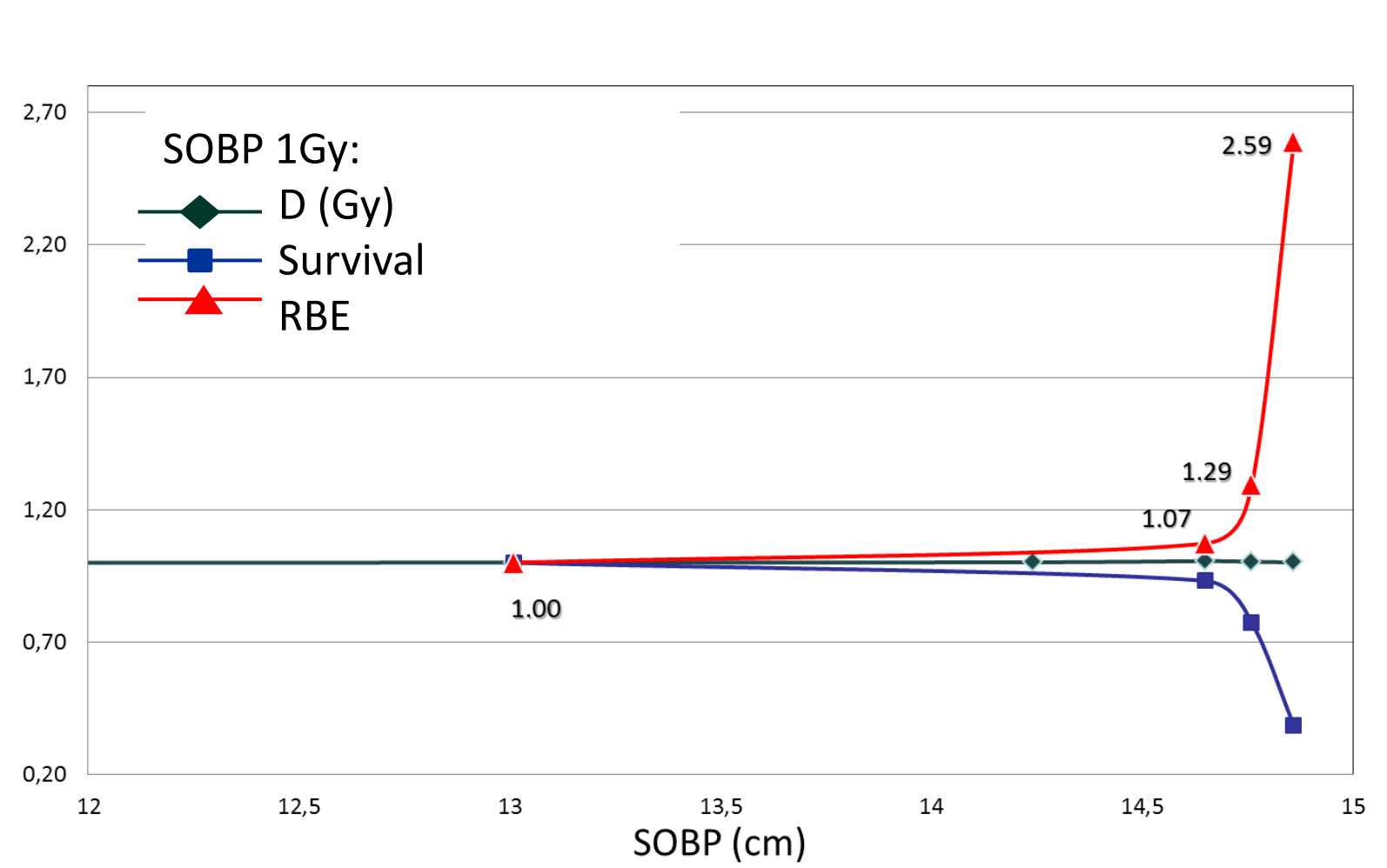


Human neonatal dermal fibroblasts - cell survival

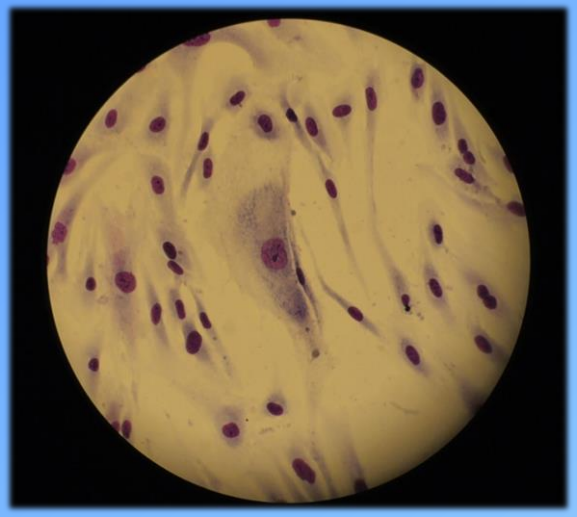


The survival level in the middle position in comparison to the distal position 1.31±0.22 times higher in average.

Human neonatal dermal fibroblasts - cell survival



DNA damage – Micronuclei formation



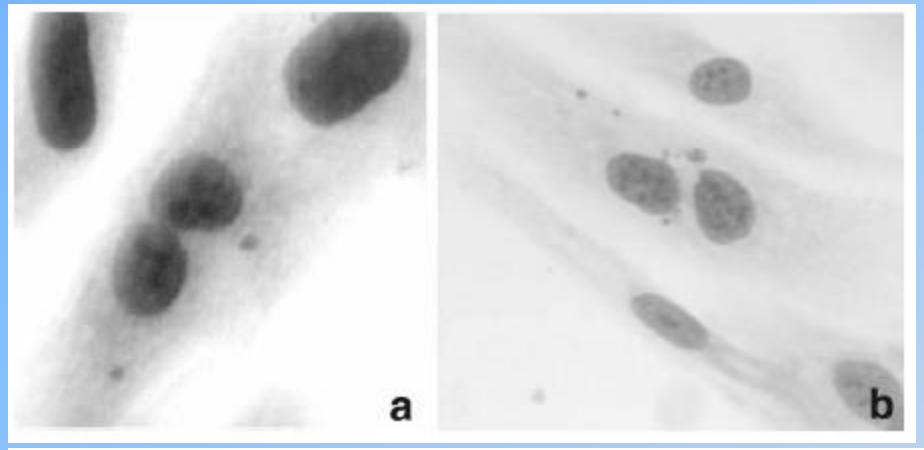
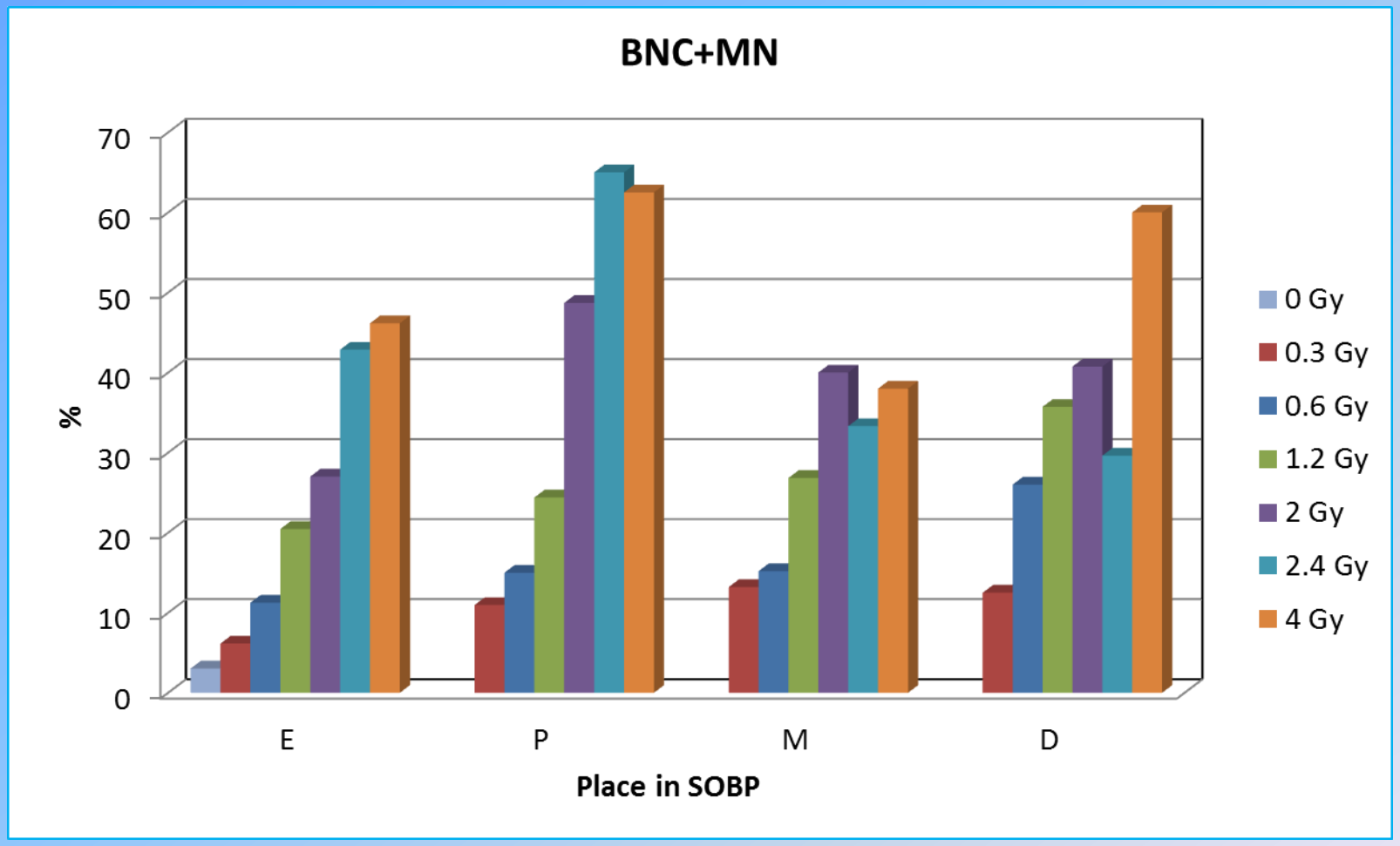


Fig. 1 Examples of micronuclei formation in human neonatal fibroblasts at first division after irradiation by 3 Gy using **a** Co-60 gamma rays, **b** scanning pristine 30-MeV proton beam

Cytokinesis-block micronucleus assay - Cells were reseeded just after the irradiation, 24 hrs after irradiation Cytochalasin B was added in concentration 1 µg/ml in medium and cells were incubated at 37 °C and 5% CO_2 atmosphere for 24 hours. After the incubation, cells were fixed by Methanol:acetic acid (9:1) and stained by Giemsa. The total amount of calculated cells was 500-1000 per case. The formation of binuclei (BNC), mononuclei (MNC), micronuclei (MN) and giant cells was followed.

Cytokinesis-block micronucleus assay



Conclusions 2

RADIATION TRACK MATTERS

- RBE in a proton SOBP increases significantly towards a distal edge of the SOBP
- Further extensive studies needed (other cell lines, dose rates, fractionation regimes, …)
- Necessary to follow in the same experiment multiple parameters of the cell culture: survival, apoptosis, necrosis, DNA repair

Acknowledgements

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OPVK CZ.1.07/2.3.00/30.0030

Czech Science Foundation

Centres of Excellence P108/12/G108 and P302/12/G157

COST MP1002 Nano-scale insights in ion beam cancer therapy (Nano-IBCT)

Cell irradiations have been performed at U120M cyclotron, Tandetron 4130 MC accelerator of the Center of Accelerators and Nuclear Analytical Methods (CANAM infrastructure, project No. LM2011019) and IBA cyclotron in the Proton Therapy Center Prague.