# *IN VITRO RADIOBIOLOGY AND RELATED TOPICS: description of some fundamental research performed with a low energy particle accelerator.*

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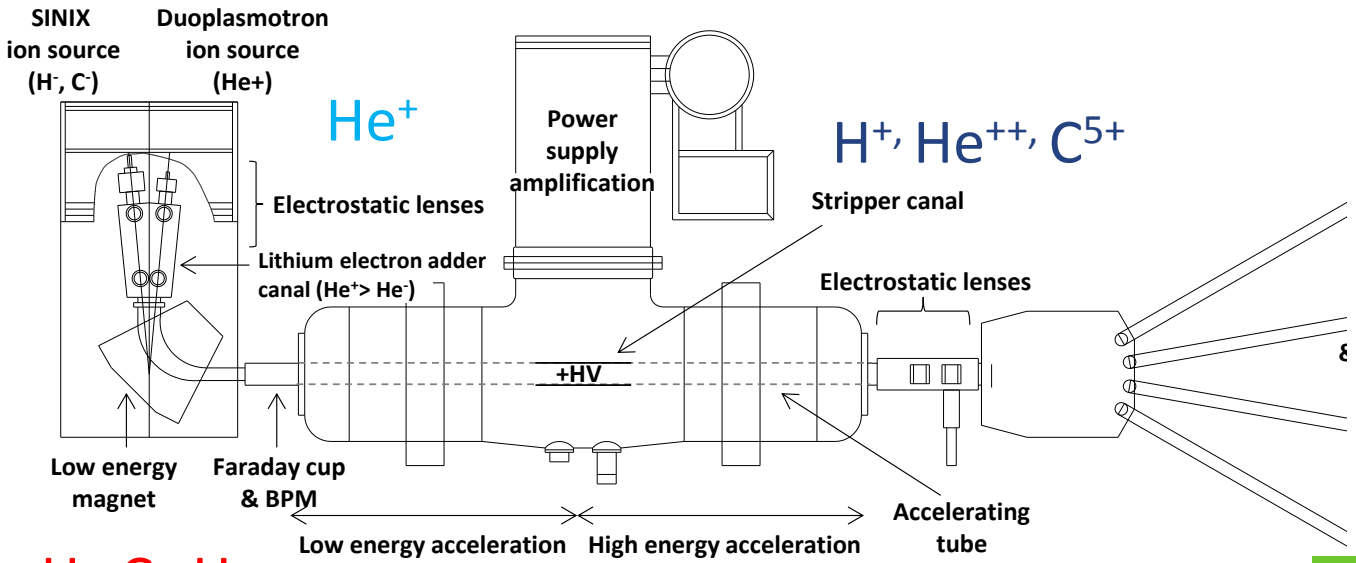
# Low energy particle accelerator ?

- 2 MV terminal voltage (4 MeV $H^+$, 6 MeV $He^{2+}$, 12 MeV $C^{5+}$)
- Multi-ions,
- DC beam: DC ion sources and 100 V ripple on 2 MV.
- "Originally" designed for material analysis

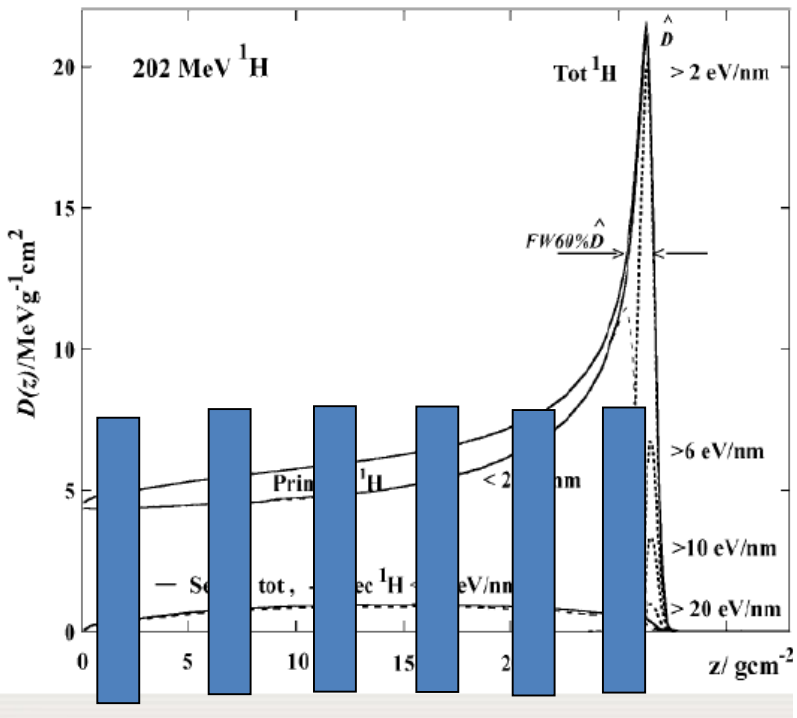
$TiH_2$ + (C) + Cs



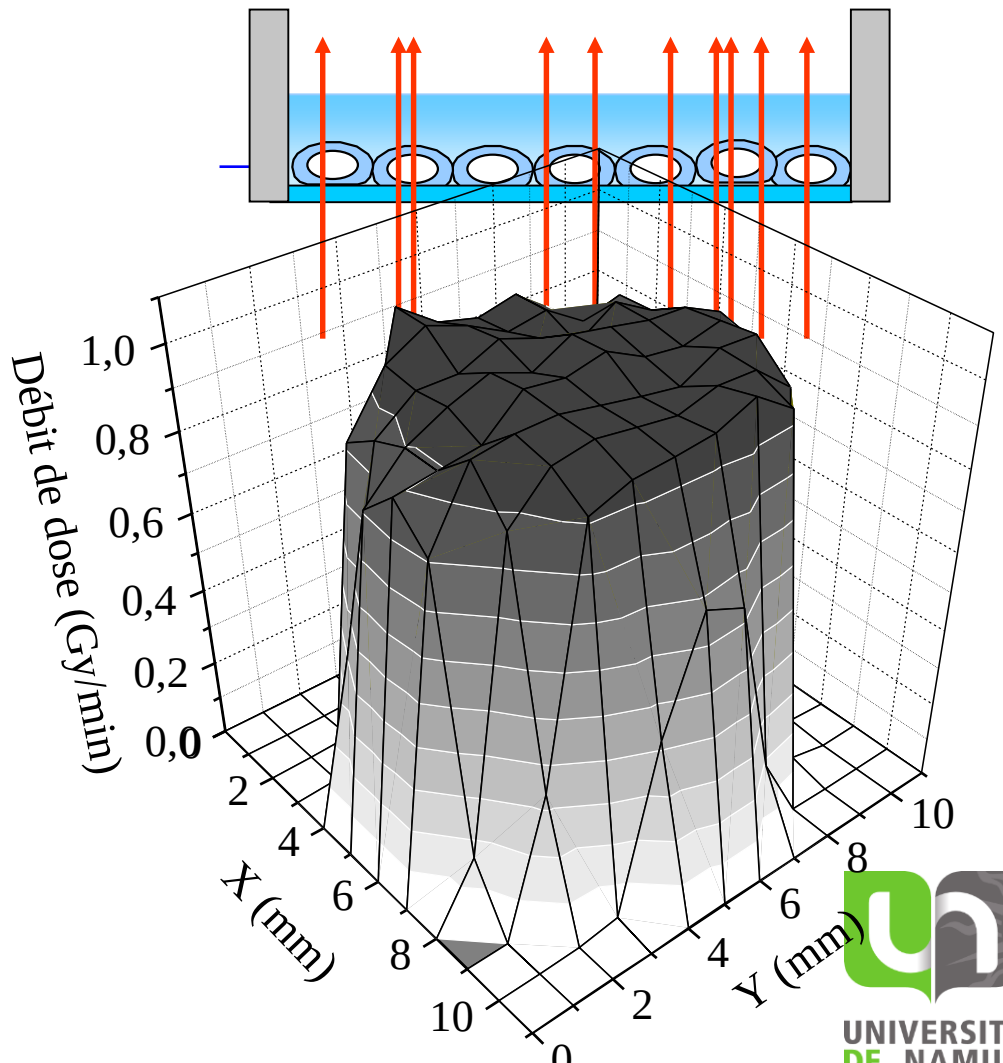

$H^-$, $C^-$, $He^-$

# Low energy ?

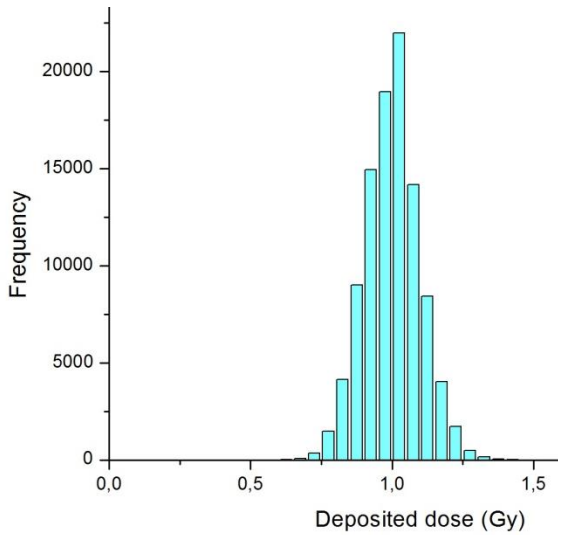
We “play” with LET

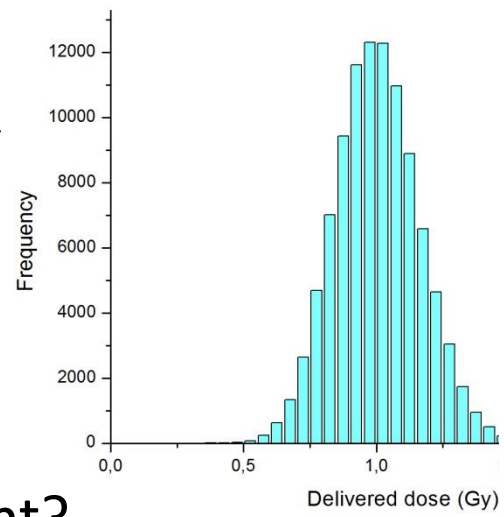


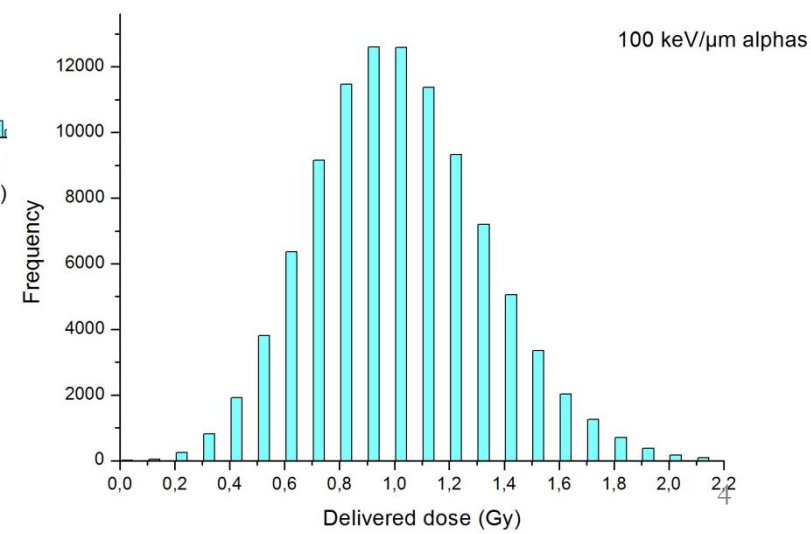

***Broad beam: Statistical hit of cells***



# Broad beam dose delivery




Why is this important?

Our spot size ~ 1cm

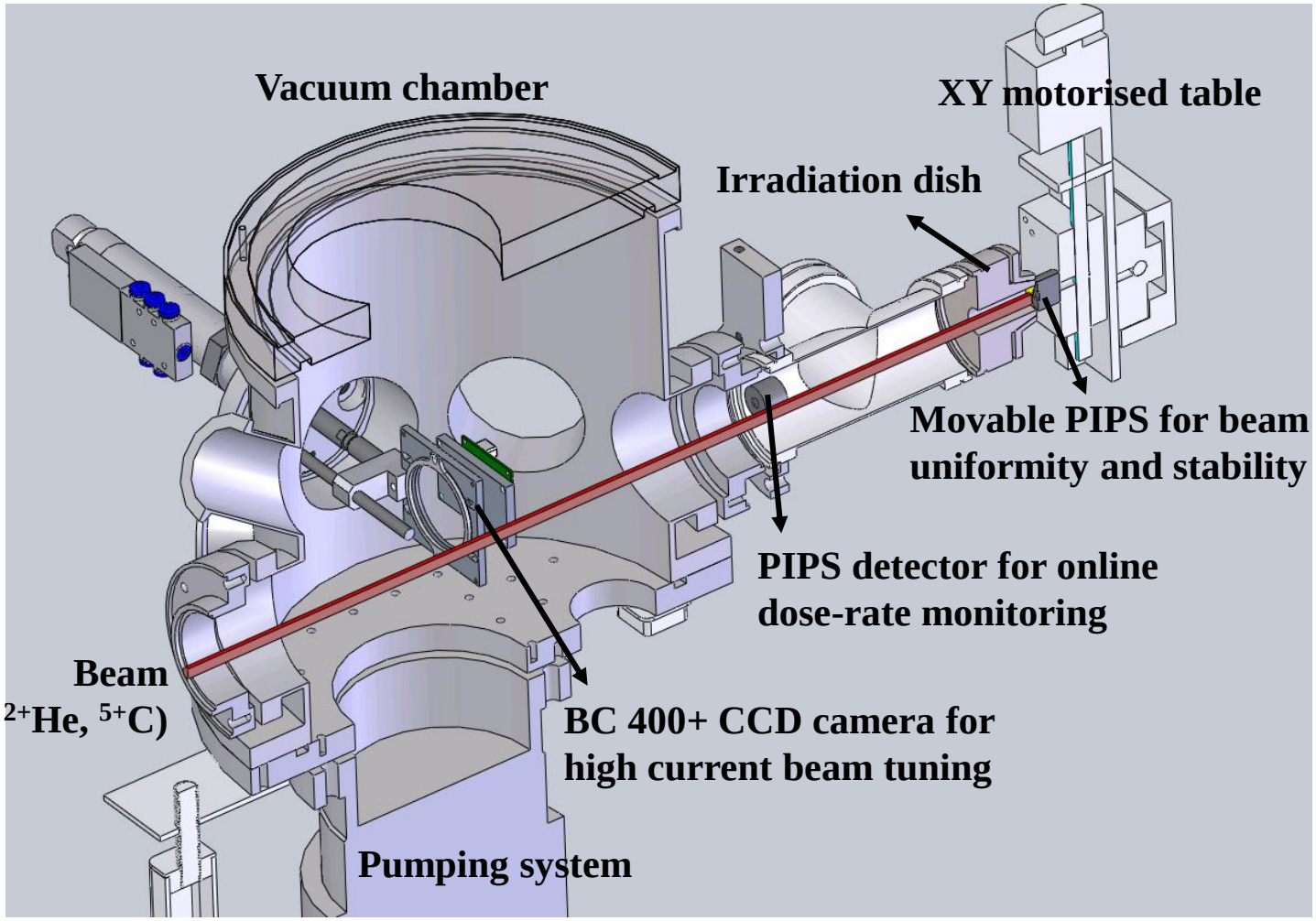
PBS ~ 3-9 mm

Worse effect with higher LET

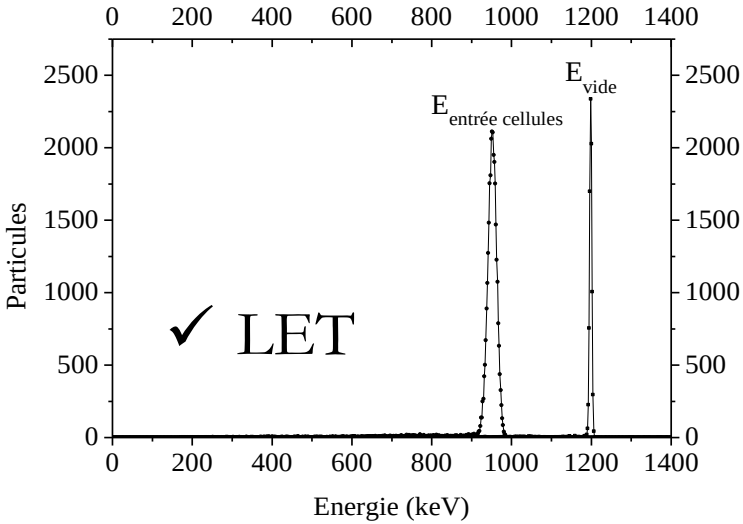
# General overview

narlis
Namur Research Institute for Life Sciences

# Irradiation facility



# Energy/dose rate qualification




$^{+}$**H**: 700 keV (30 keV/µm) to 4 MeV (10 keV/µm)

$^{2+}$**He**: 2,7 MeV (140 keV/µm) to 6 MeV (90 keV/µm)

$^{5+}$**C**: 12 MeV ($LET_{mean}$: 282 keV/µm) (model for distal edge)

$$\dot{D}\,(Gy/s) = 1.6\,10^{-9}\,\frac{LET\,.\,\Phi}{\rho}$$

LET: particle energy loss / cell tickness

$\phi$: nber of part/cm$^2$.s

$\Phi$: $10^4 - 10^6$ part/s/cm$^2$

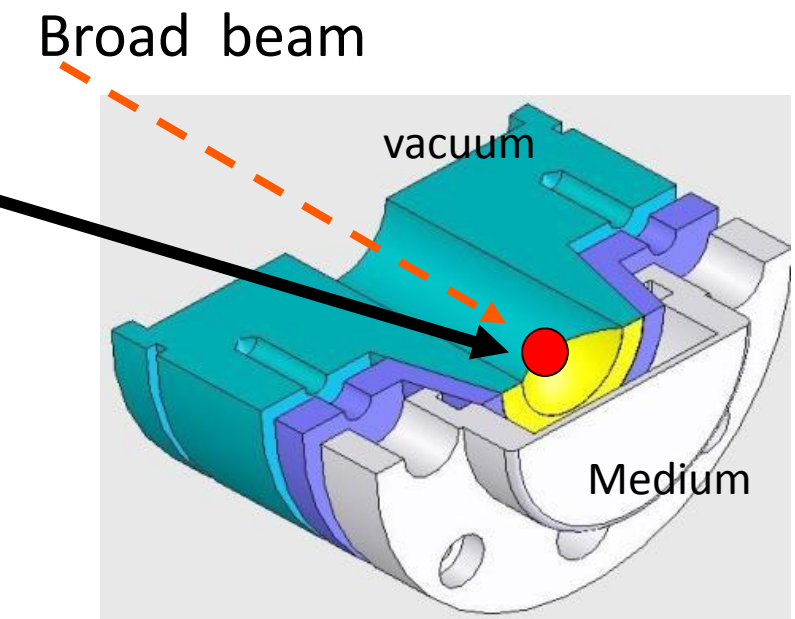
**Dose rate:** 0,01 to 100 Gy/min

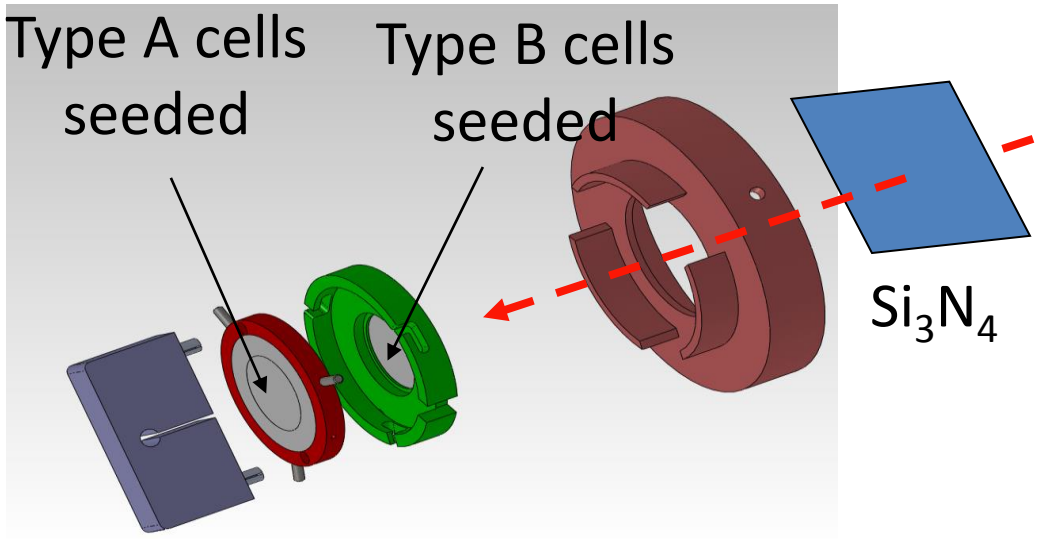
Wéra *et al.*, NIMB 269 (2011) 3120-3124

# The U. Of Namur's broad beam setup

## ***The cell dishes***

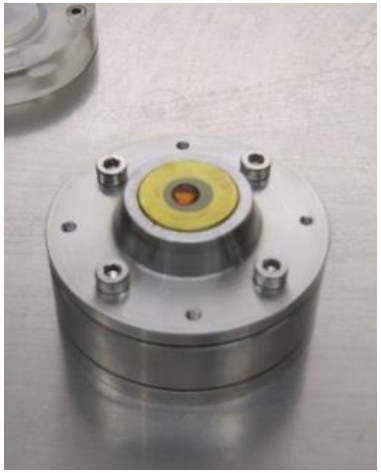
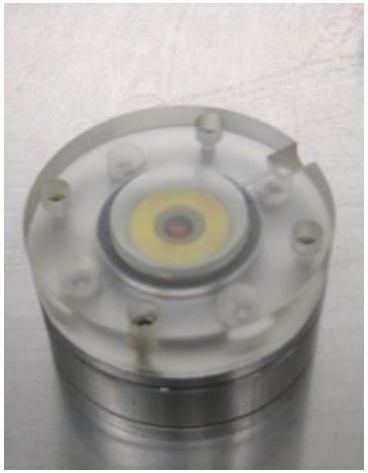
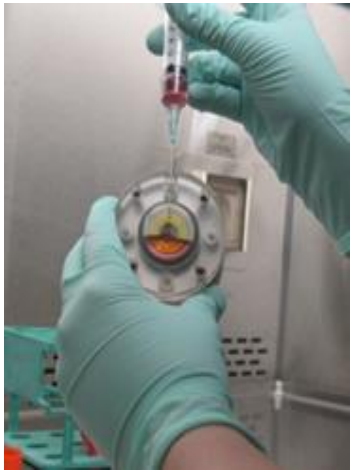
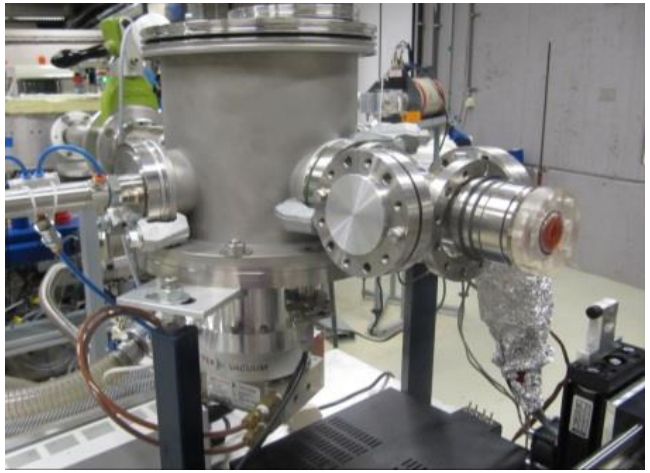
100,000 cells seeded as a drop on a polymer foil (8 µm)



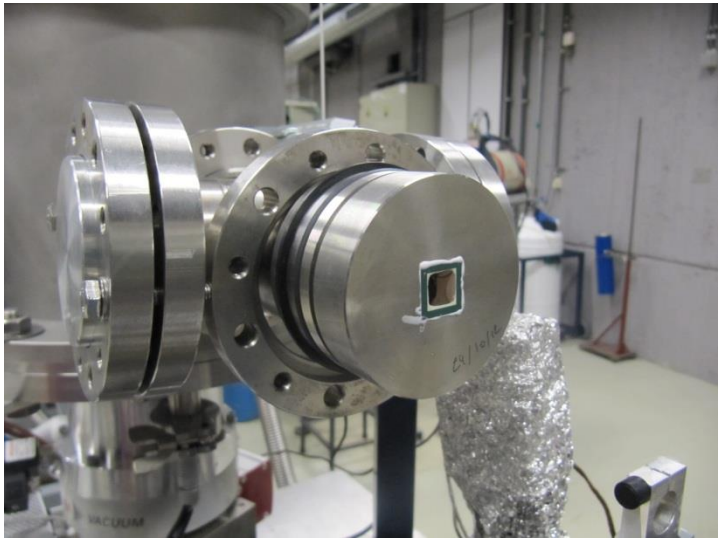
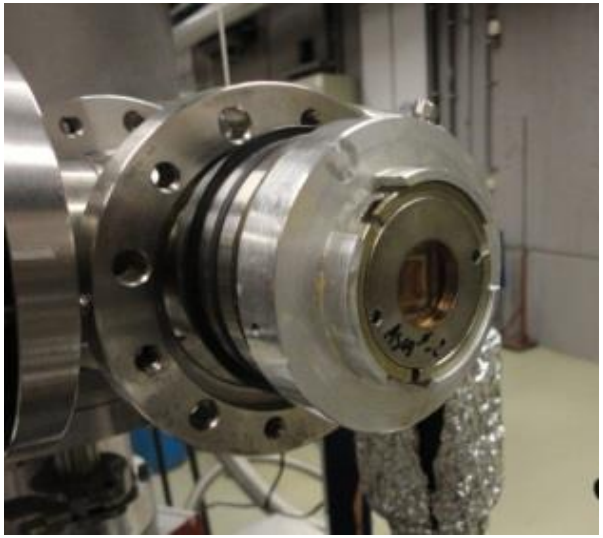




Cells, Bacteria,
Rotifer, C-elegant,
µ-chips, new dosimeters, …

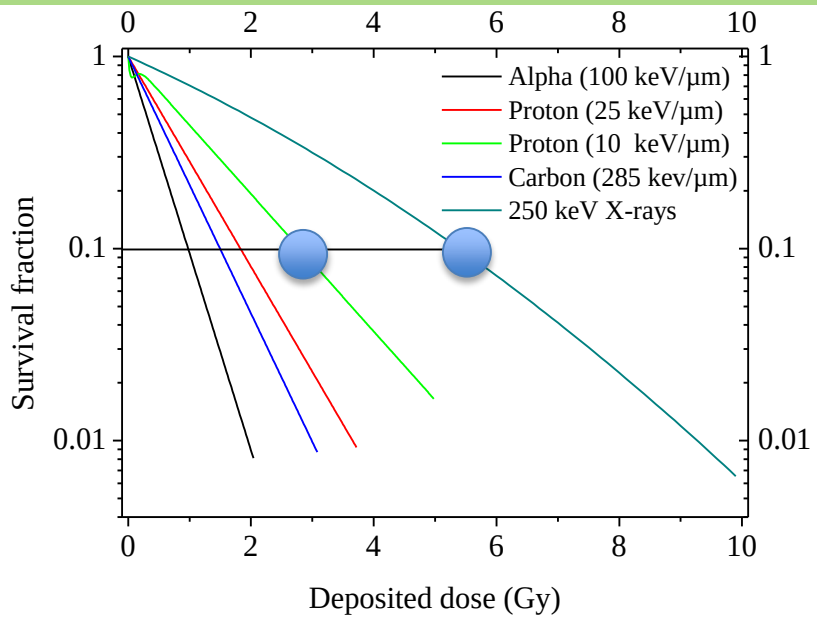
# One side cell dish

# Two sides cell dish

# Some outputs (A549 lung cancer cells)




1 Gy/min

**X-Rays**

α: 0,33 ± 0,04 $Gy^{-1}$

β: 0,018 ± 0,005 $Gy^{-2}$

**$^{+}$H 10 keV/µm**

α: 0,824 ± 0,029 $Gy^{-1}$

**$^{+}$H 25 keV/µm**

α: 1,26 ± 0,03 $Gy^{-1}$

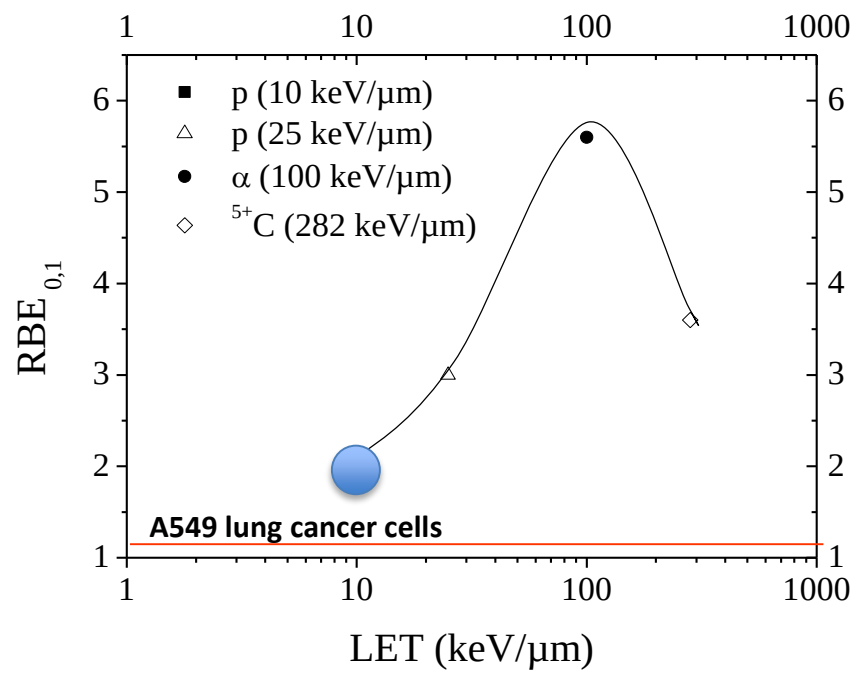
**$^{2+}$He 100 keV/µm**

α: 2,36 ± 0,08 $Gy^{-1}$

**$^{5+}$C 282 keV/µm**

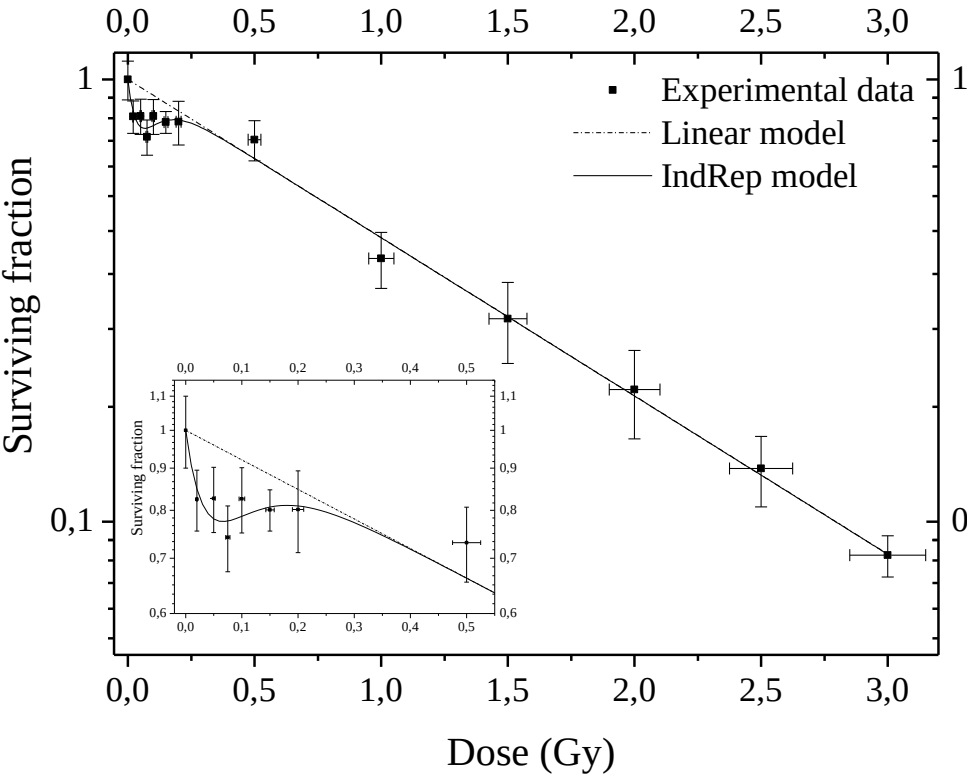
α: 1,54 ± 0,04 $Gy^{-1}$

- References: Heuskin, Wera, Michiels, Lucas, 2008-2015

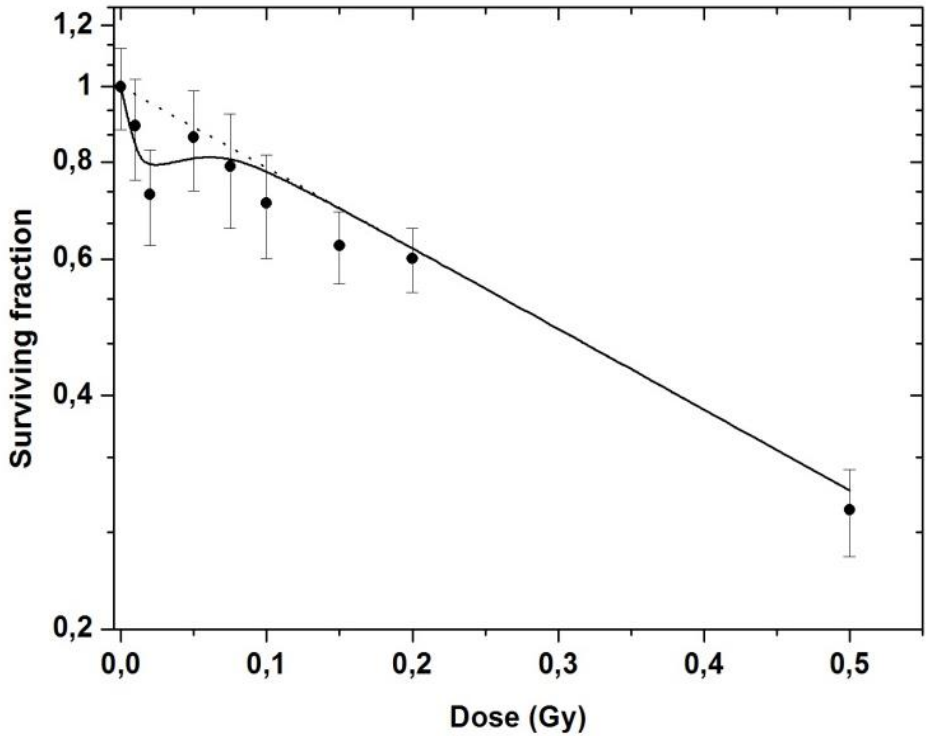


# Some outputs at low dose (A549)

**10 keV/µm protons: hypersensitivity**




**100 keV/µm alpha: bystander effect**

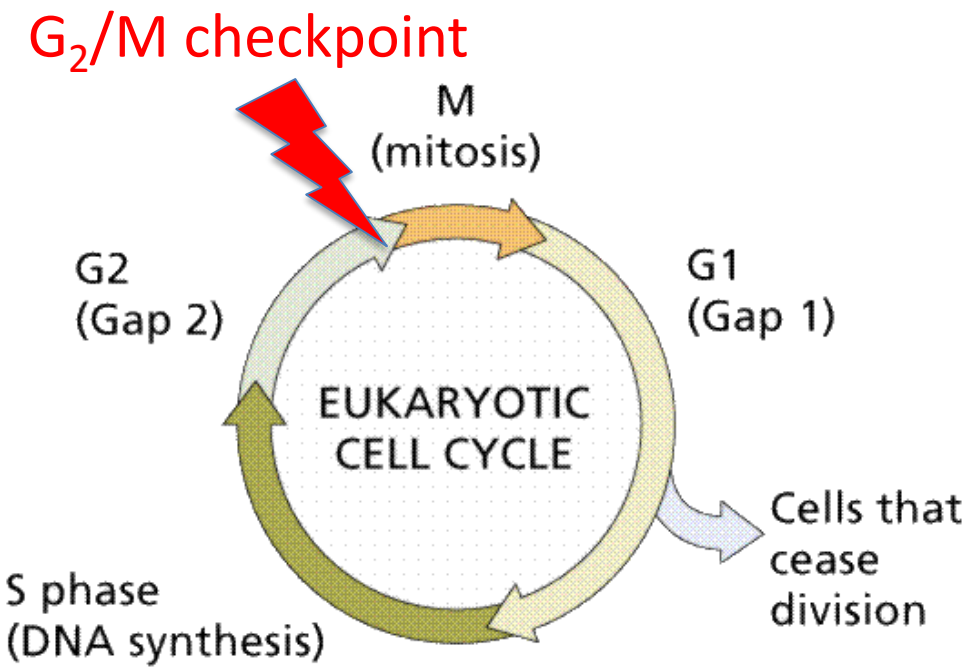


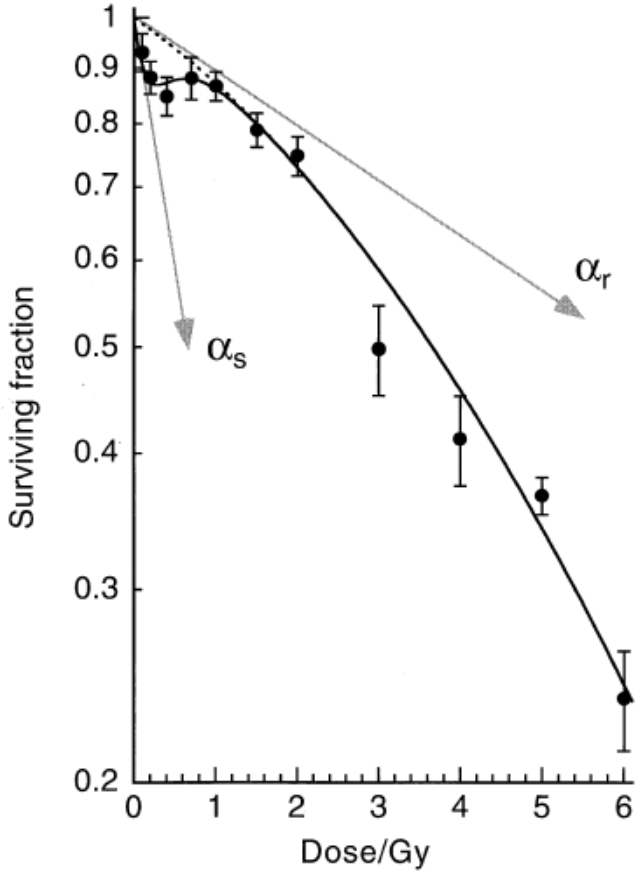

➔ Two different effects !

- References: Heuskin, Wera, Michiels, Lucas, 2008-2015

# Low dose effects

- Low dose hypersensitivity
- Enhanced cell killing per unit dose up to 0,5 Gy of radiation
- Involves mainly $G_2$ cells[2]



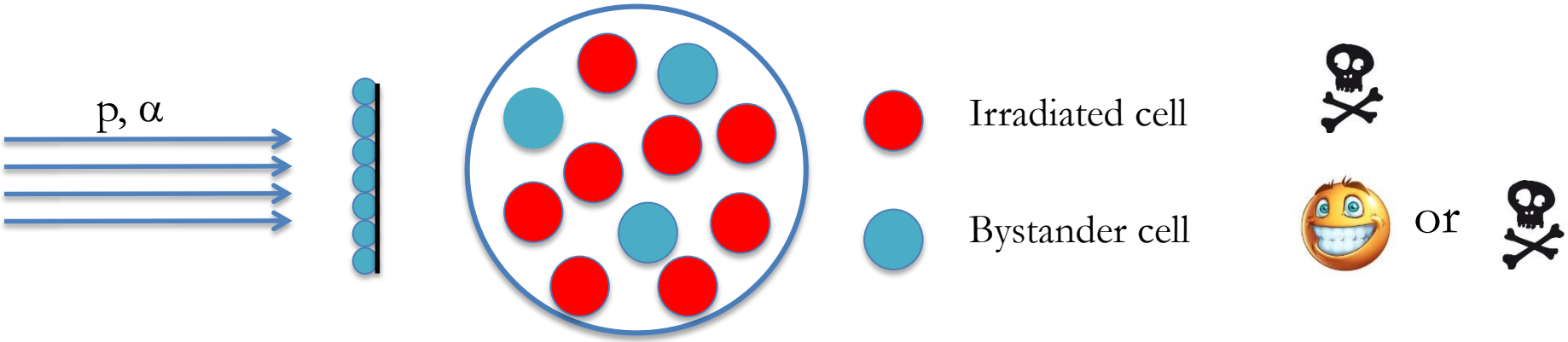




Survival of asynchronous T98G human glioma cells irradiated with 240 kVp X-rays[1].

[1] *Joiner M. et al. 2001 Int J Radiat Oncol Biol Phys*
[2] *Marples B. et al. 2003 Radiat Res*

# Low dose effects

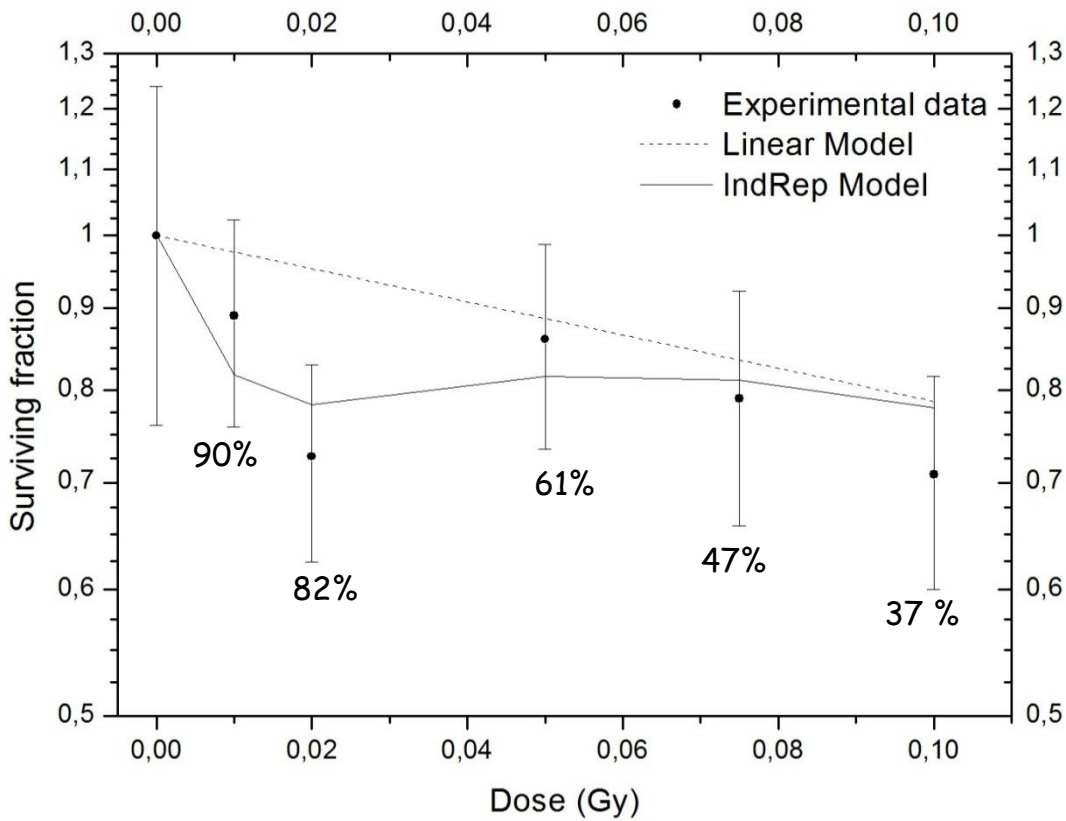
Non irradiated cells that suffer from the effects of radiation




- Nature of bystander signal is unclear
- Probably involves reactive oxygen species in bystander cells
- May be transmitted through culture medium or by gap junctions

# Low dose effects

100 keV/µm alpha particles




Knowing the cell size, it is possible to determine the proportion of non hit cells:

$$p(0) = \exp(-\mu)$$

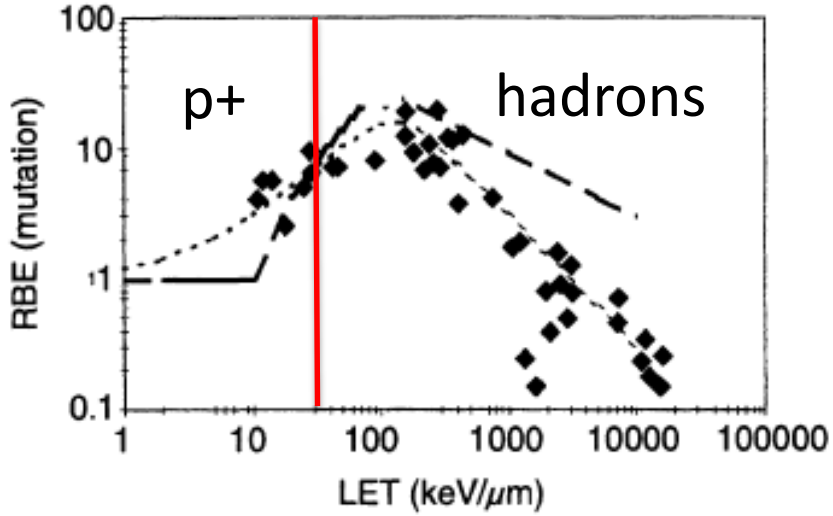
Proportion of non hit cells

Mean particle number per cell

Bystander effect

# Deal with ions unexpected effects

- Dose distribution: can be wide in a cell population when LET is high
- Low dose: hypersensitivity and bystander effect: could occur upstream of tumor (detrimental) BUT can be of advantage for some types of cancer (brain tumor)
- Mutational RBE in surviving cells ➔ secondary cancers ?




Kiefer, Radiat Res, 2001

# Nanoparticles sensitizers

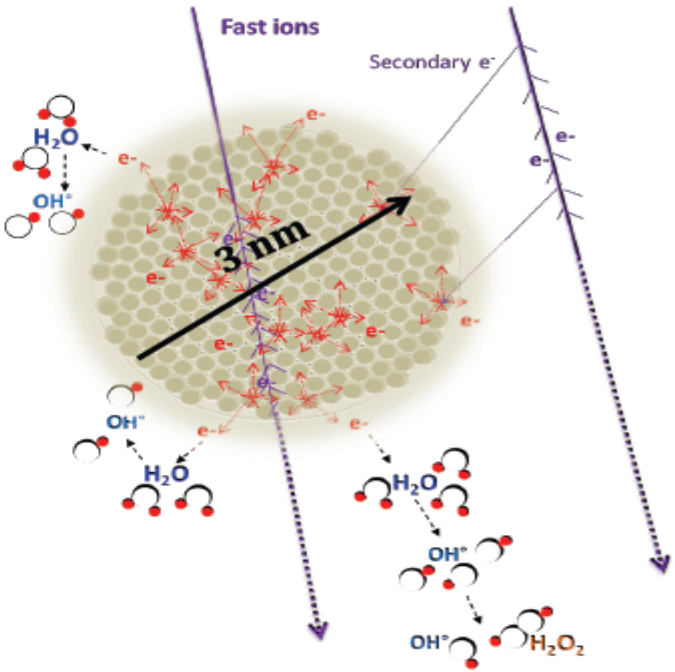
Time scale

$10^{-15}$ s — **1) Activation** *(ionization)* **of NP**

$10^{-13}$ s — **2) Relaxation of the NP:**
- *e- cascades*
- *e- bursts*
- *e- capture*

$10^{-12}$ s — **3) Production of Radicals (ROS)**
- *Nanoclusters*
- *Water radiolysis*
- *Water fragmentation*

$10^{-6}$ s — **4) Biological consequences**
Nanometric damages




**Nanoscopic perturbation**

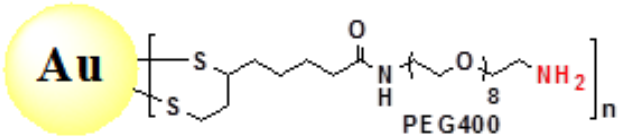
➔ Would allow to scale down the dose in tumor and thus reduce detrimental effects on upstream tissues

# Radiosensitization effect of GNPs

- GNPs used for study the radiosensitization effect

  - Commercial GNPs (*ACD,Inc*) : 5 nm and 10 nm

    GNPs functionalized by PEG and amine groups: AuNPs@DHLA-PEG400-$NH_2$




    - GNPs are lyophilized (COMs) before used for experiment *in vitro*

- Cancer cell line chosen for study the radiosensitization effect

  - Cell line: A 431 (epidermal carcinoma carry large numbers of EGF binding sites, used as a positive control for EGFR expression)

# Protocol

## I. Incubation cells (A431 – A549) with NPs

*No toxic con. (MTS test)*

1.Cells are first seeded on the mylar film for ~2 h to ensure the adherence of A-431 cells

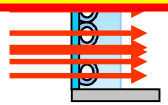
2. NPs are suspended in cell medium for a final con. of 0.05 mg/ml, this mixture is added to fill the irradiation chamber

Cells are

**Average cell loading**

**0.5 – 1 pg Au / cell**

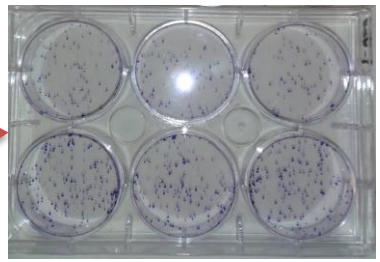
**50.000 – 100.000 NP (10 nm) /cell**



- Dose rate: 1 Gy/min
- LET :25 KeV/µm or 10 KeV/µm

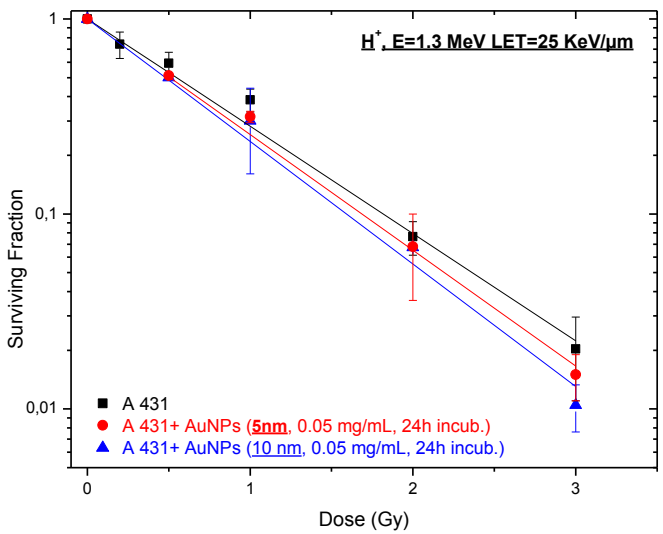
## III. Clonogenic assay

After 8 days, colonies are stained with crystal violet and then counted

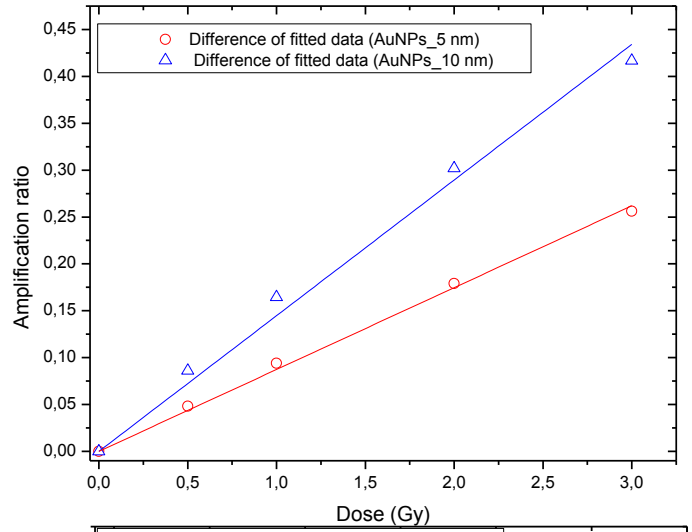


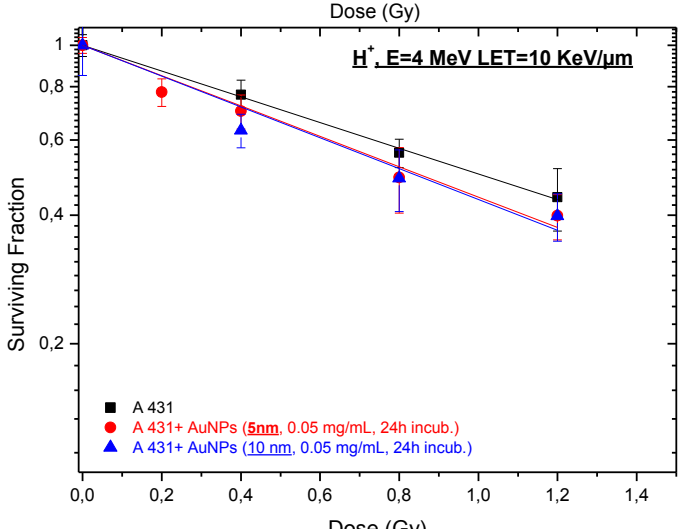
# Survival fractions

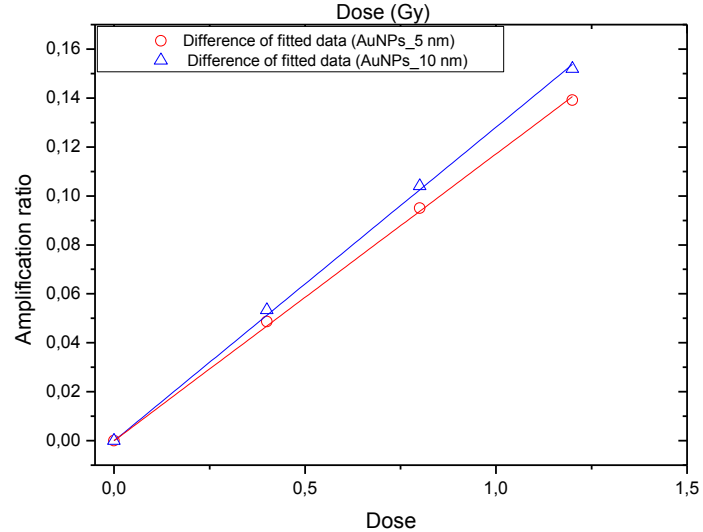
- **Proton irradiation, LET= 25 KeV/µm & 10 keV/µm**



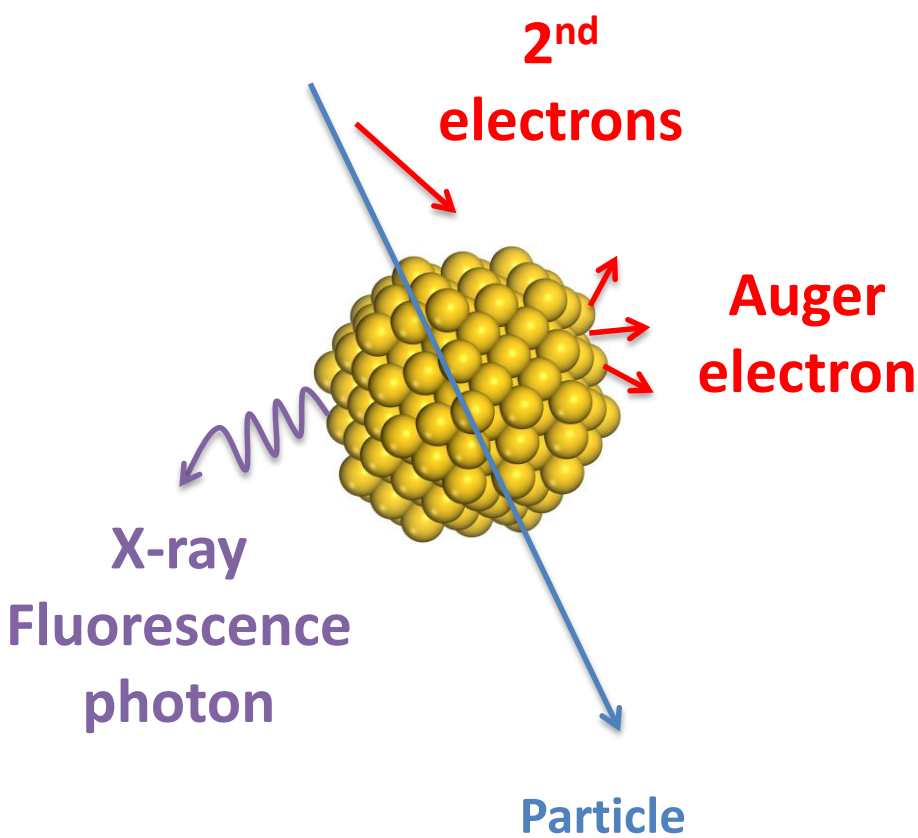

$$\frac{Sf^{Ctrl}_{Dose\ X} - Sf^{NP}_{Dose\ X}}{Sf^{Ctrl}_{Dose\ X}}$$



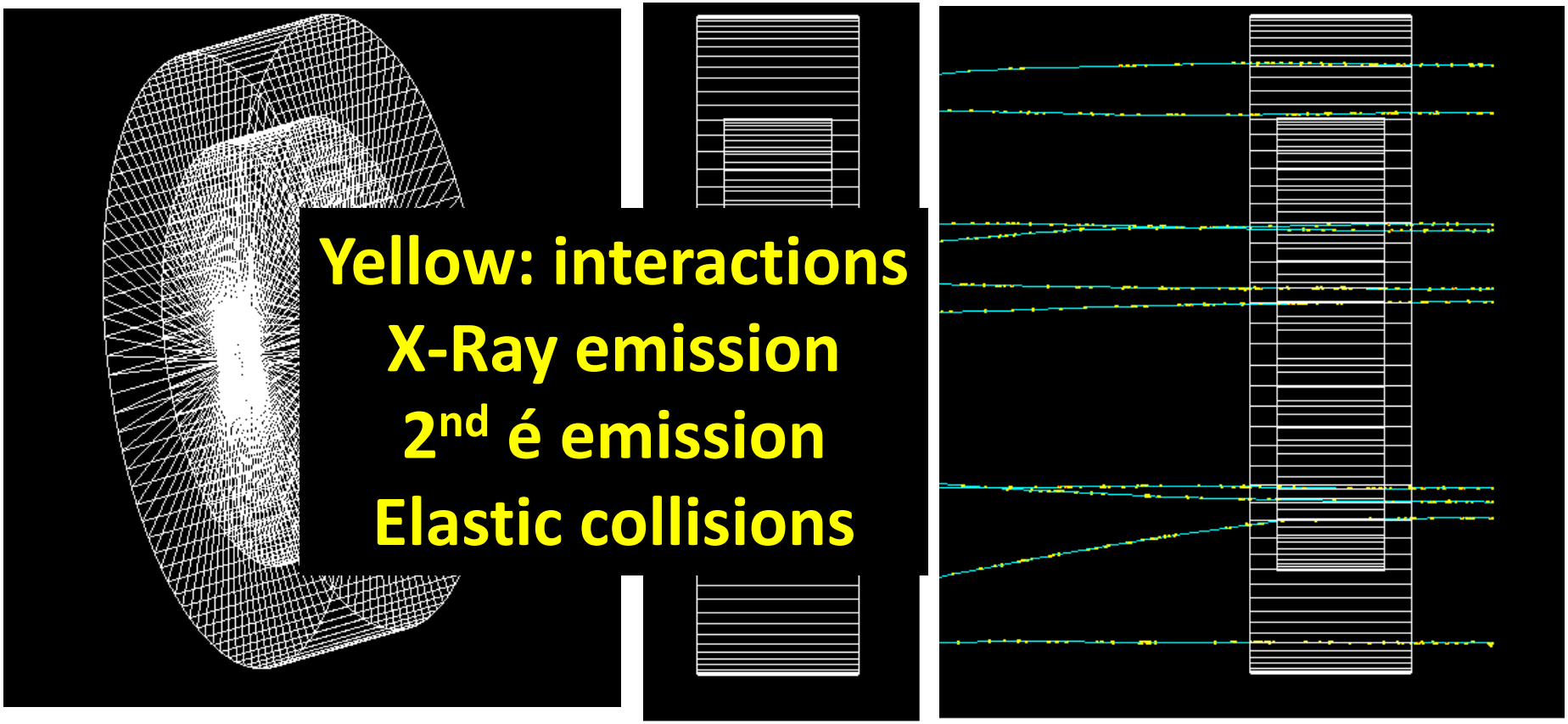



# What are the mechanisms ?



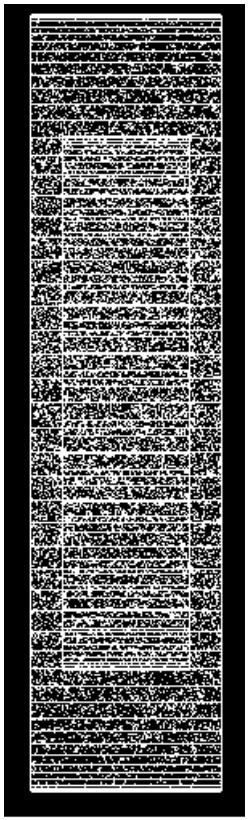

Reactive Oxygen Species (ROS)

# GEANT4 single cell irradiation




Proton, 25 keV/µm

# GEANT4 single cell irradiation



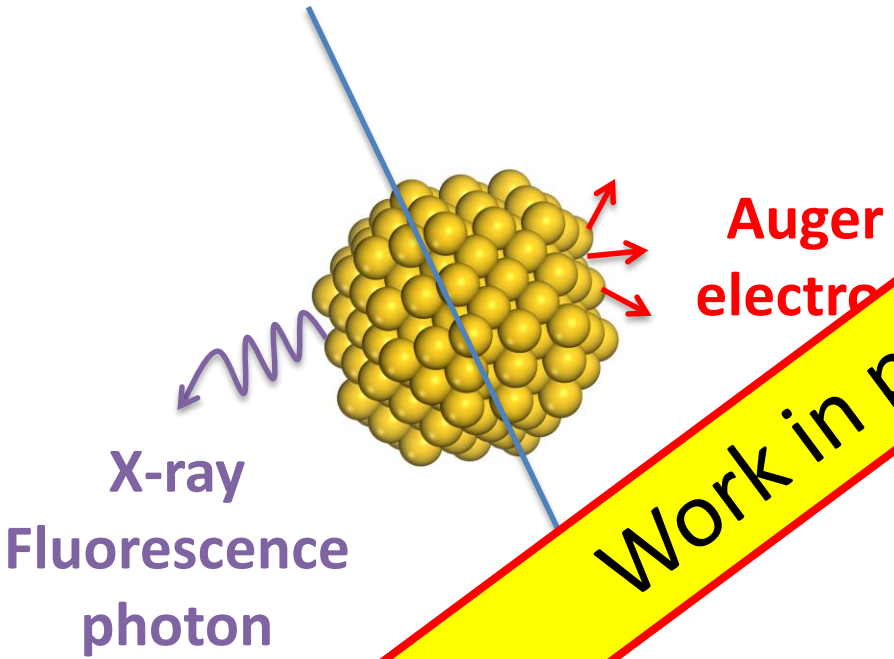
25 keV/um

| Dose | Geant4 p25 Mean | sd | Geant4 p25 + GNP 10 nm Mean | sd |
|---|---|---|---|---|
| 0.5 | 0.499667 | 0.005437 | 0.501667 | 0.007506 |
| 1 | 1.012667 | 0.010371 | 1.023333 | 0.005774 |
| 2 | 2.023333 | 0.004714 | 2.033333 | 0.025166 |
| 3 | 3.03 | 0.014142 | 3.053333 | 0.015275 |

- 0.5 pg/cell → 49 500 GNP 10 nm (white random spots)
- Total surface area = 3.89 µm² (vs 322 um² for the cell)
- 3Gy: 240 protons, and only 2.85 protons hit GNP

1 % proton in GNP for a 50 % increase of cell death ?

# What are the mechanisms ?




Work in progress

3 Gy: 3-6 protons hit 50 000 GNP
Marginal dose increase by 2nd é

e is an effect
ze, material, LET)

Main route is ROS amplification

Marginal increase of 2nd electrons by direct proton impact

? ROS due to NP + delta e ?

# In vitro radiobiological research

- Dose response (SF, Radiosensitivity, ...) of any type of cells

***Low energy accelerators may help***

- Targeted & non-targeted effects
- I
- I
- C
- A
- R
- .

***As said yesterday, there is a strong evidence showing that radiobiology is of prime importance for clinical radiotherapy.***

***and***

***Low energy accelerators are very valuable tools to study radiobiology (in vitro)***

# Conclusions

- Low energy particle accelerator is a convenient tool
- Bottom up approach that supports clinical science.
- Helps to understand and evaluate new clinical procotols
- Our radiobiology platform is available for experiments.

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# Thank you for your attention

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