Technological developments and evaluation of the radiosensitizing potential of gold nanoparticles for hadron therapy

N. Fuster-Martínez, M. J. García-Murria, M. C. Jiménez-Ramos, S. Jimeno, M.A. Cortés-Giraldo, D. Esperante, J. M. Espino, J. Fuster, B. Gimeno, L. González, D. Hermenegildo-Saucedo, P. Huertas, I. Mingarro, D. Pascual, D. Suárez-García

V Jornadas RSEF/IFIMED de Física Médica
27 October 2025

Nanoparticles as radiosensitizers

- **Several experiments *in-vitro* and *in-vivo* and Monte Carlo simulations** demonstrated the potential of high-Z NPs to enhance the efficacy of radiotherapy (**X-rays, p, and e)**

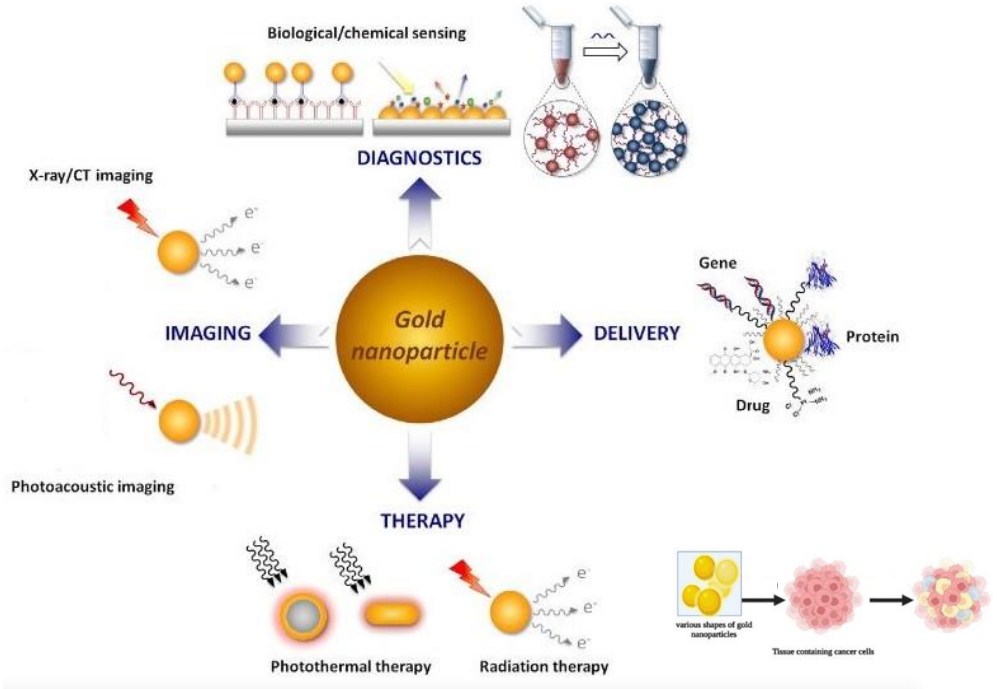
- High-Z-NPs with X-rays treatments injected into tumour cells are going through first **clinical trials in the USA** (nanobiotix)

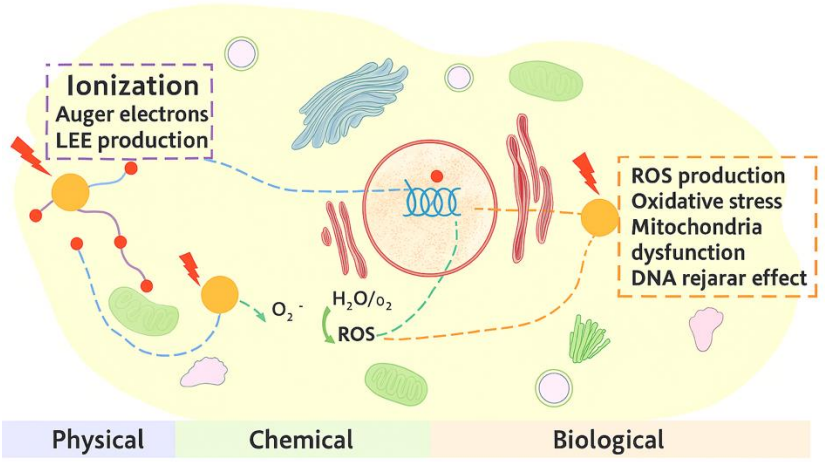
  - ✓ the efficiency depends on the **NPs, radiation and cell line properties**

  - ✓ **mechanisms behind are still controversial**

  ➡ **Non-efficient optimization procedure and application**

- **Main challenges :**
  - ✓ **Insert the NPs** only into the tumour cells
  - ✓ **Control** the concentration and biodistribution within the treatment duration
  - ✓ **Expel** of the NPs after the treatment
  - ✓ **Modelling** of the phenomenon

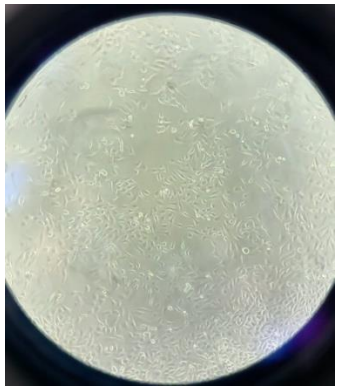




Motivation and goals

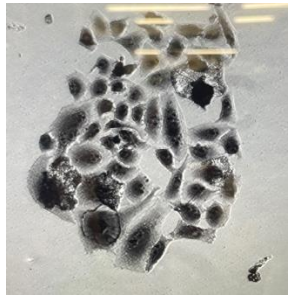
- **Radiobiology experiments** to study the radiosensitization effect of **gold NPs with protons on Hela cells at** *Centro Nacional de Aceleradores* (CNA) cyclotron external beamline

  - ✓ Evaluate and quantify the effect
  - ✓ Investigate the underlying mechanisms
  - ✓ Perform modelling studies
  - ✓ Develop and test active targeting nanoparticles

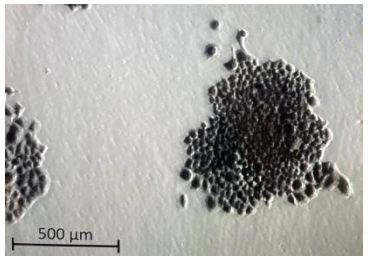


- **Develop technology to improve procedure an experimental conditions**

  - ✓ Robotic system for samples irradiations
    - Reducing exposure time to air of biological samples after irradiation
    - Faster and more systematic procedure



  - ✓ Electrostatic chopper for the CNA cyclotron external beamline
    - Short and high intense pulse delivery
    - Increasing the range of experimental parameters to be tested

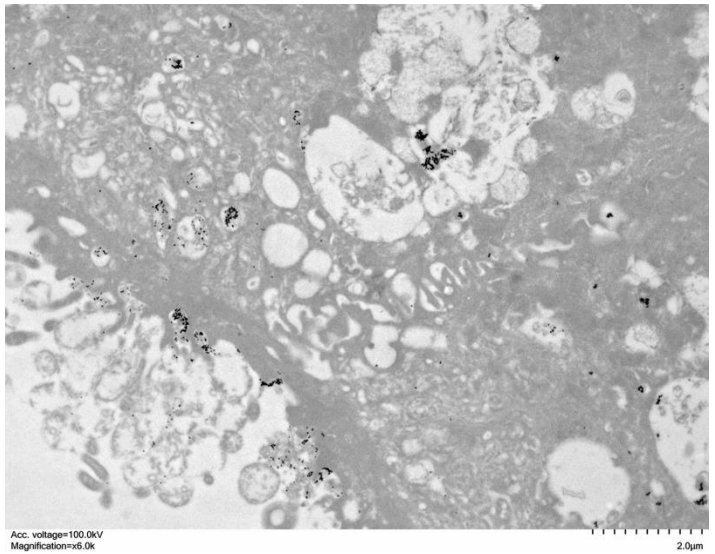


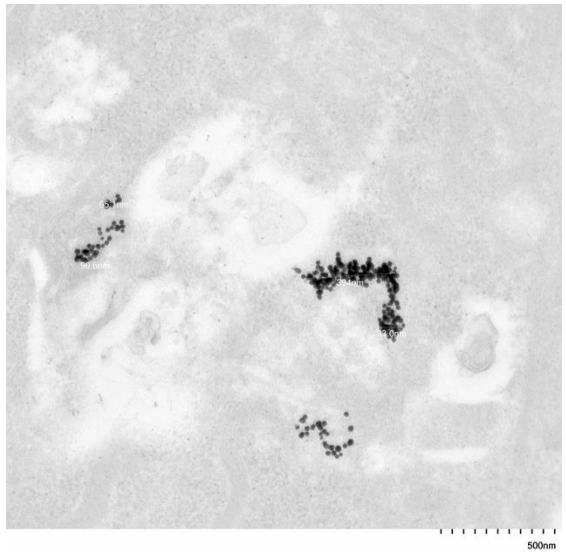
AuNPs characterization

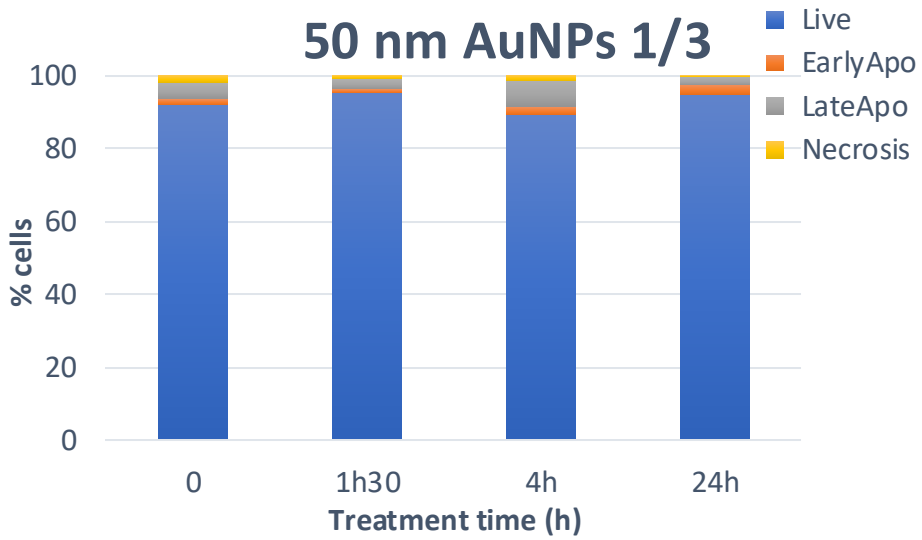
- **Cytotoxicity :**
  - ✓ Cytometry Annexin V-FITC + IP
  - ✓ CellTiter-Glo® Luminescent Cell Viability Assay
  - ✓ Clonogenic assays

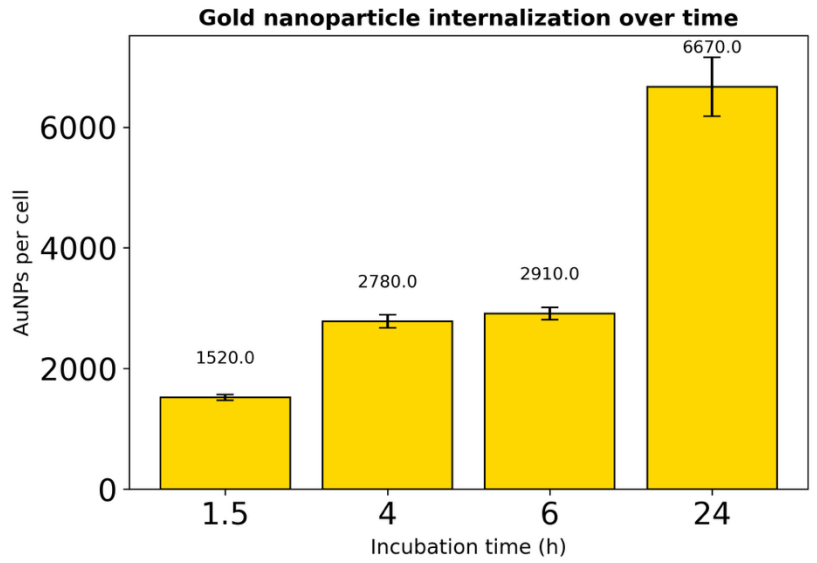
- **The viability of the cells it is not affected by the AuNPs at the concentration of 149 ug/ml**

- **Uptake of NPs** in the cells quantified using:
  - ✓ Inductively coupled plasma mass spectrometry
  - ✓ Transmission electron microscopy



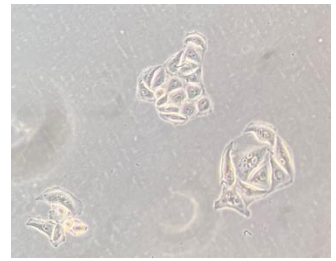




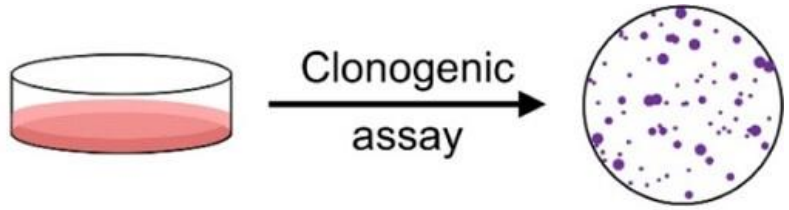


Radiobiology experiments summary

- Conventional clinical irradiation conditions: **4 Gy/min and continuous beam**
- Hela cells and gold NPs of **50 and 20 nm ⌀**



- Measurement campaigns in **2022, 2023 and 2024**
- To evaluate the effect of radiation:
  - ✓ **Clonogenic assays:** the toxicity is evaluated by studying the ability of a single cell to form a colony
  - ✓ **DNA damage assays:** analyse gH2AX and RPA markers



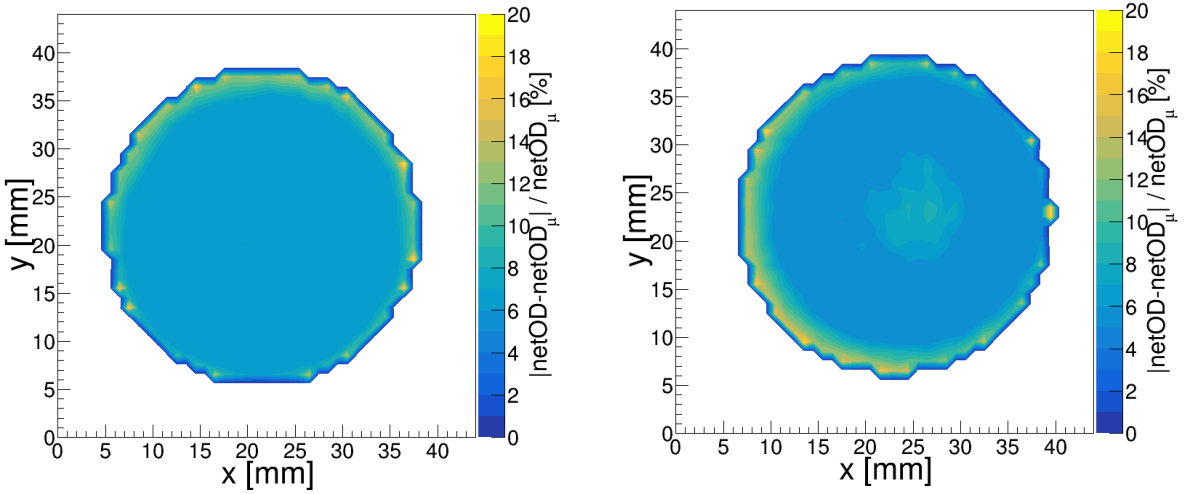
Day	Part.	I_{beam} [pA]	D_r [Gy/min]	Treatment [h]	NP ⌀ size [nm]	Doses [Gy]
2022	p	110-140	~4	~4	50	2,4,6
2023	p	110-140	~4	~4	50, 20	2,3,4,5,6
2024	p	110-140	~4	~4	50	4

Experimental test procedure

- **Biological samples** prepared and analysed at **CABIMER**

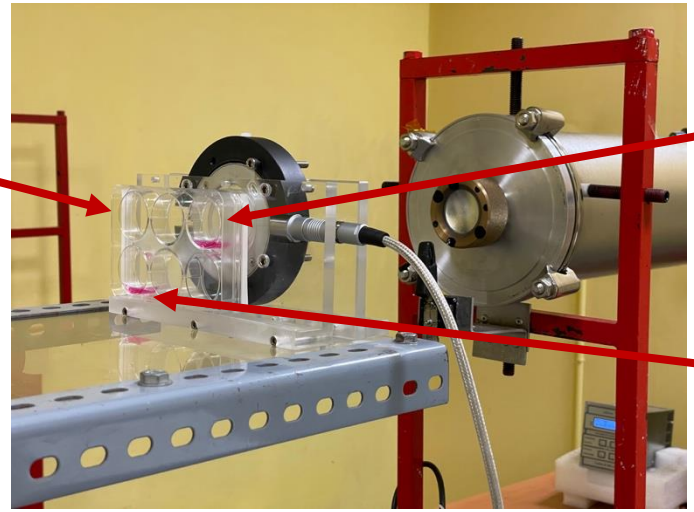
- **Cyclotron External Beam Line**
  - ✓ Optimized for 3 cm ⌀ homogeneous irradiation fields
    (A. Barattó et al, doi: 10.1016/j.ejmp.2020.04.022. Epub 2020 May)
  - ✓ Equipped with an **ionization chamber** for **on-line dose measurements**

- **Homogeneity** measurements: mean deviation ~4-5%



Ionization chamber

Radiochromic film



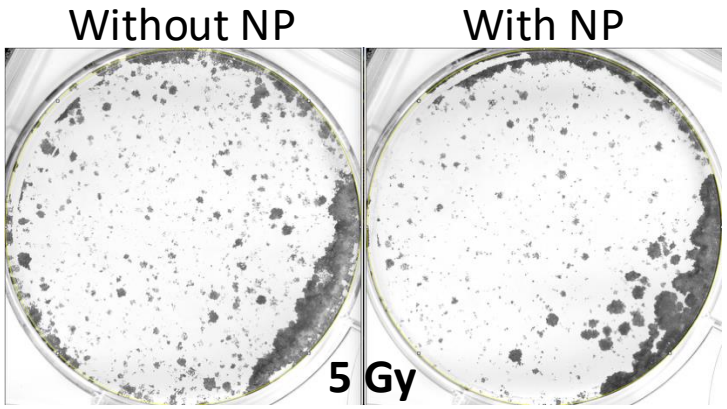
Sample to be irradiated

Control

6-well plate support

Clonogenic assays results

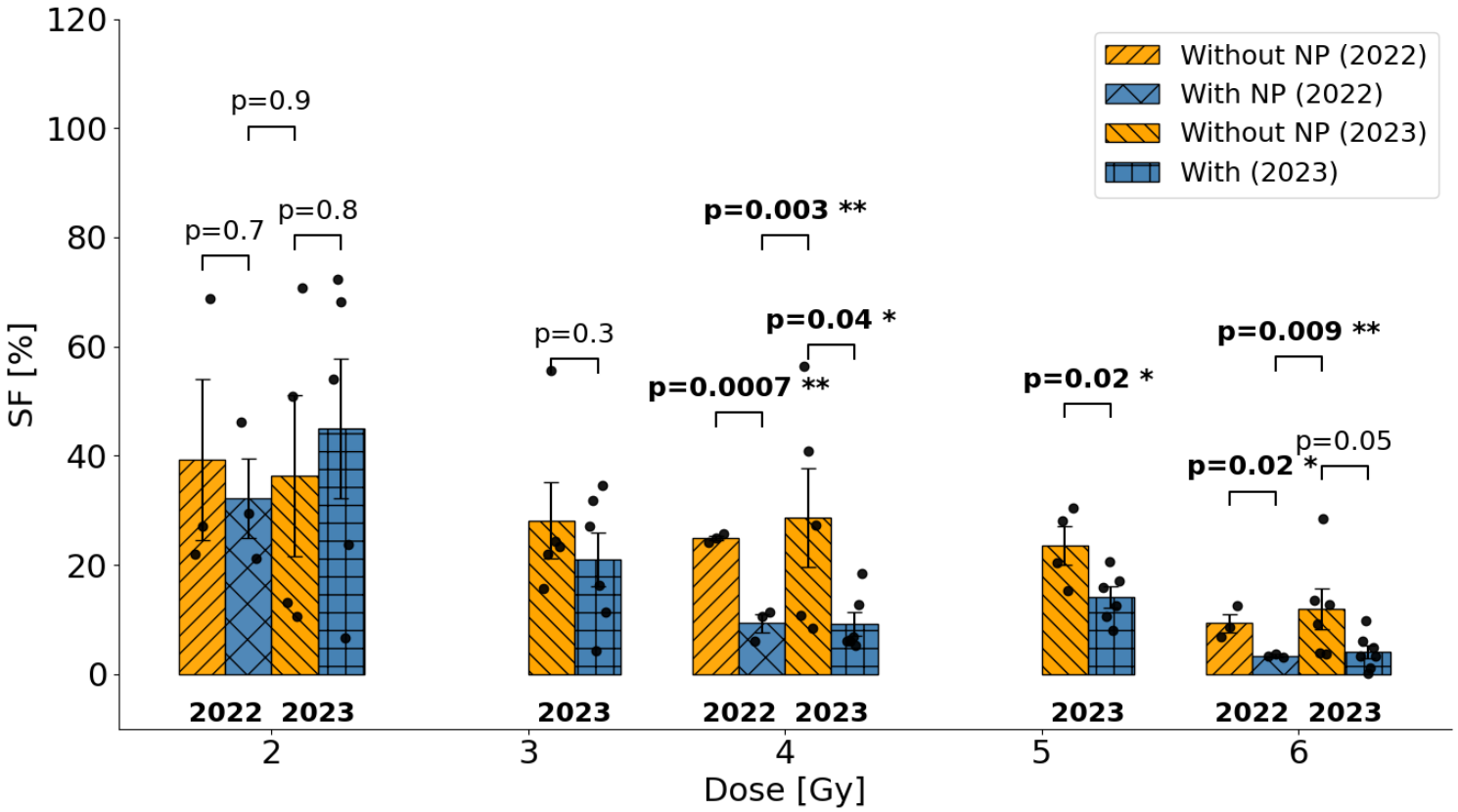
- The samples are **incubated** for 11-13 days and then the **colonies are stained and counted** using **ImageJ** software



- To quantify the radiation effect, the survival fraction is computed:

$$SF = \frac{colonies\ after\ irradiation\ at\ X\ Gy}{Seeded\ cells \times PE} \times 100$$

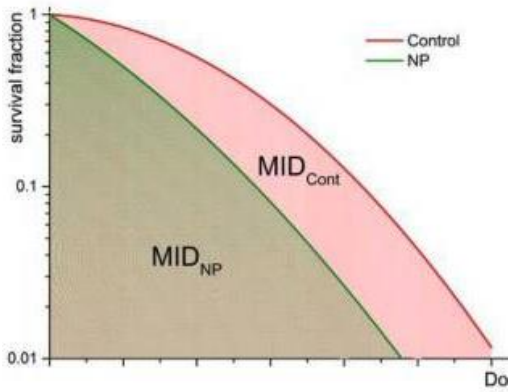
$$PE = \frac{colonies\ without\ irradiation}{Seeded\ cells} \times 100$$



*One-way ANOVA statistical tests with $p < 0.05$ showing a significant difference

Radiosensitization effect quantification

- *ICRU report: Quantitative Concepts and Dosimetry in radiobiology. Measurements International Commission on RadiationUnits. Washington DC (1979).*
  - ✓ It states that if the curves are not proportional, the entire survival curve must be considered when comparing the cases with and without nanoparticles.



- Fitted to the **Linear Quadratic (LQ) model**: $SF = \exp(-\alpha D - \beta D^2)$

α and **β** describe how cells respond to a given radiation under one specific scenario

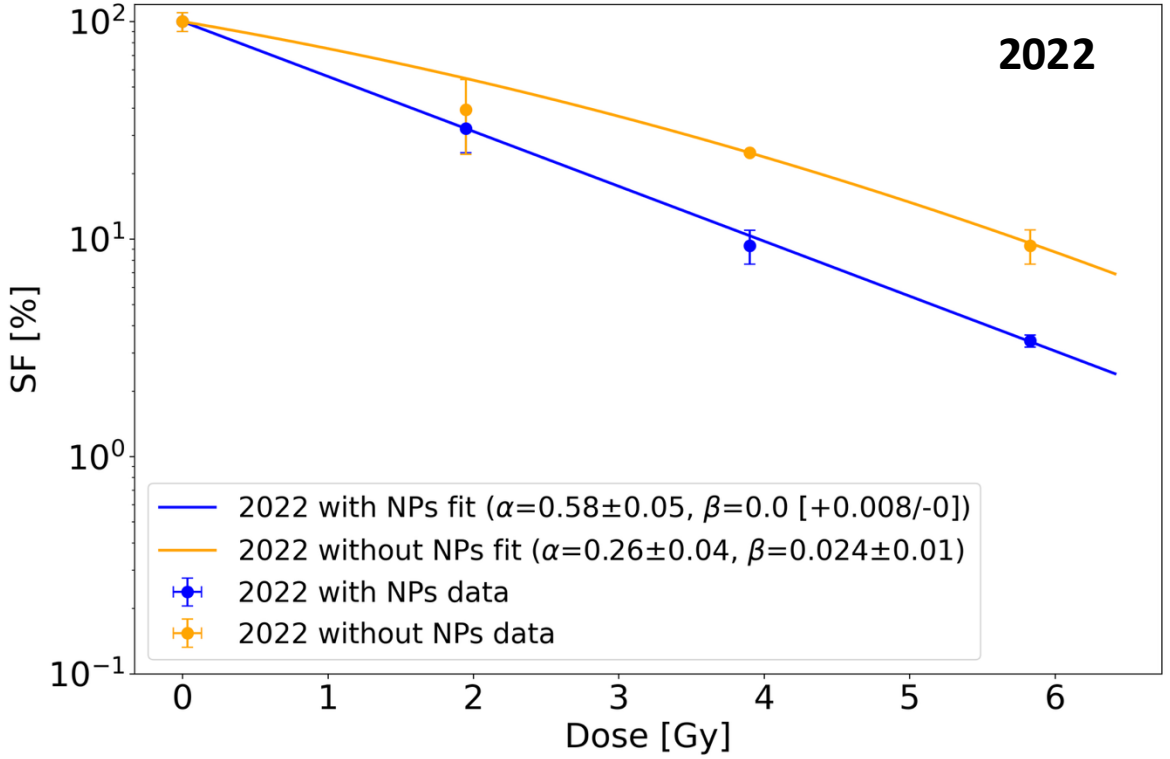
Mean inactivation dose (MID)

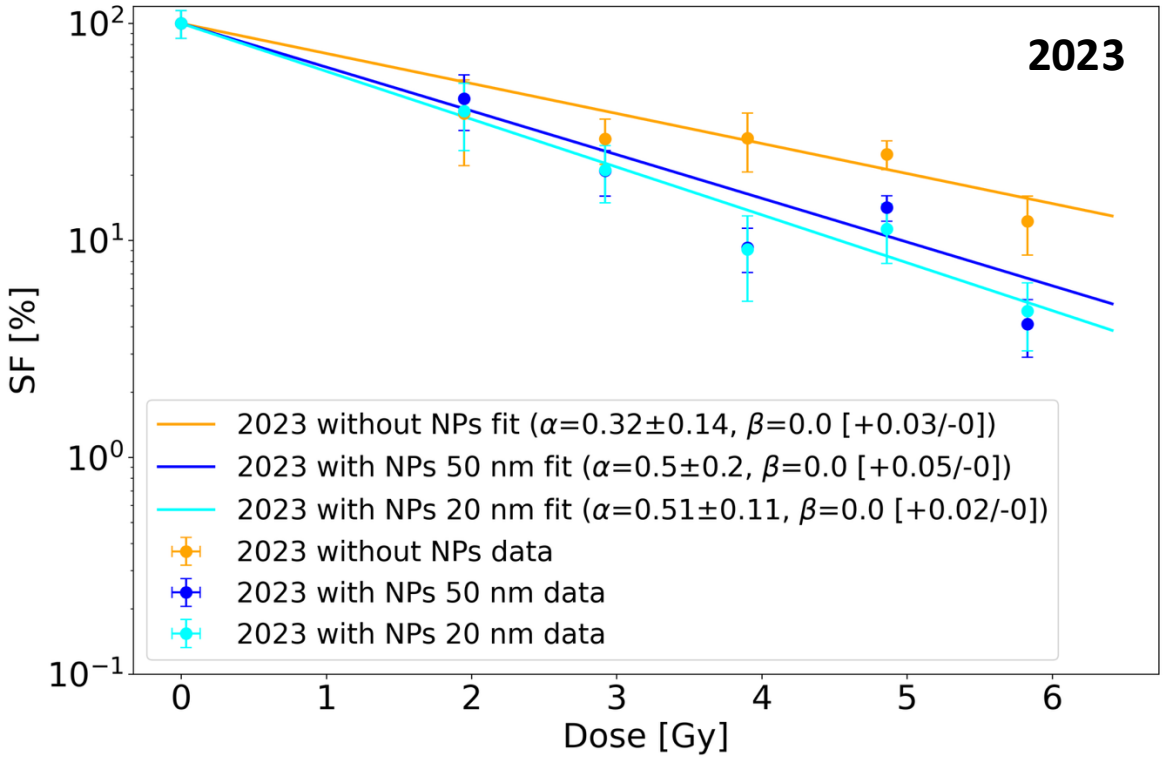
$$\mathrm{MID} = \int \mathrm{SF(D)}\ \mathrm{dD}$$

Sensitizer Enhancement Ration (SER)

$$\mathrm{SER_{NP}} = \frac{\mathrm{MID_{Cont}}}{\mathrm{MID_{NP}}}$$

Radiosensitization effect results



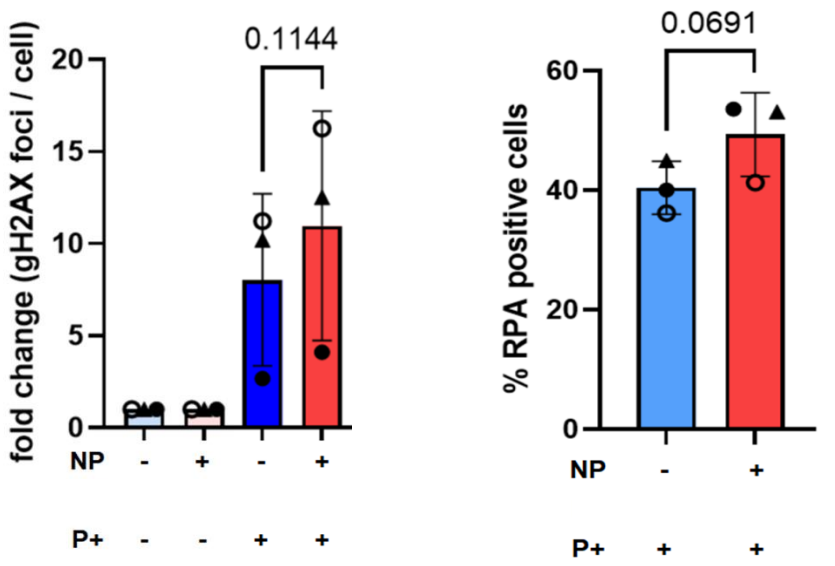


- ❑ Compatible results from 2022 and 2023 campaigns
- ❑ No significant difference between 20 and 50 nm

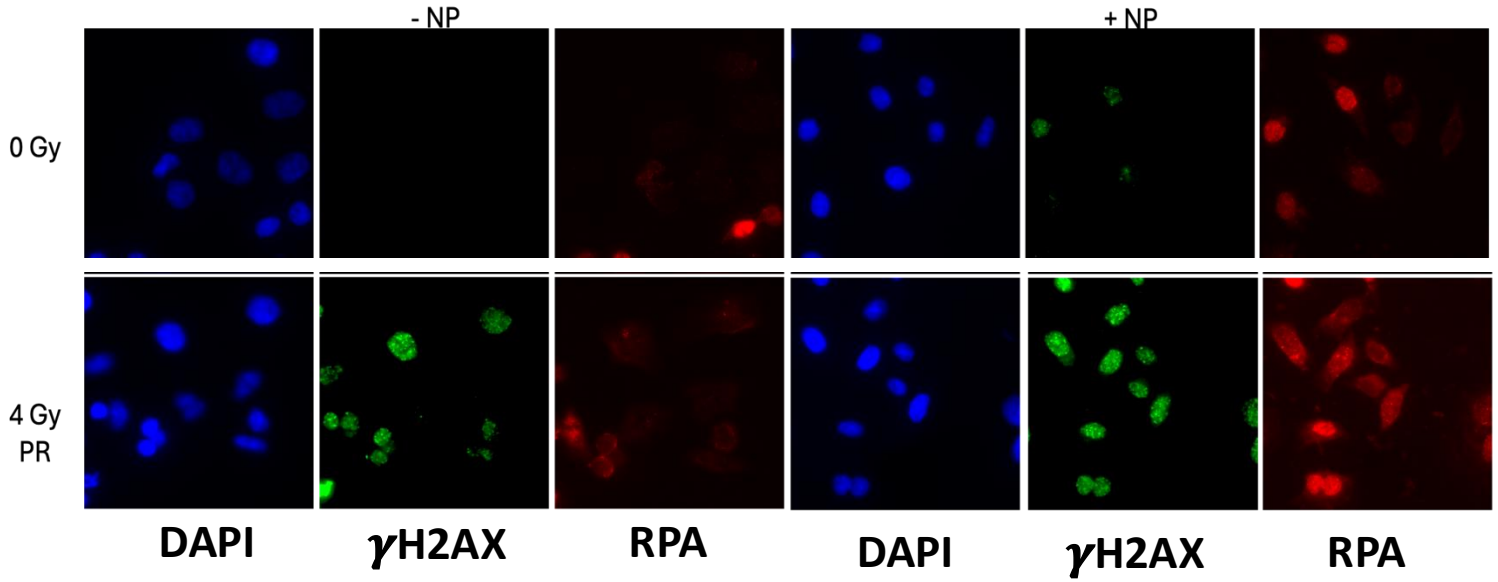
Case	SER
2022 AuNPs 50 nm	1.53 ± 0.07
2023 AuNPs 50 nm	1.3 ± 0.2
2023 AuNPs 20 nm	1.39 ± 0.18

Double strand break (DSB) assays results

- Samples are irradiated to 4 Gy and measured through microscopy techniques
- Different wavelength corresponds to a different markers
  - ✓ **DAPI (blue):** for number of cell counting
  - ✓ **γH2AX (green):** it is a marker of DSB damage happens but does not tell if it is or not being properly repair
  - ✓ **RPA (red):** the presence of RPA foci suggests that DNA repair is occurring via homologous recombination



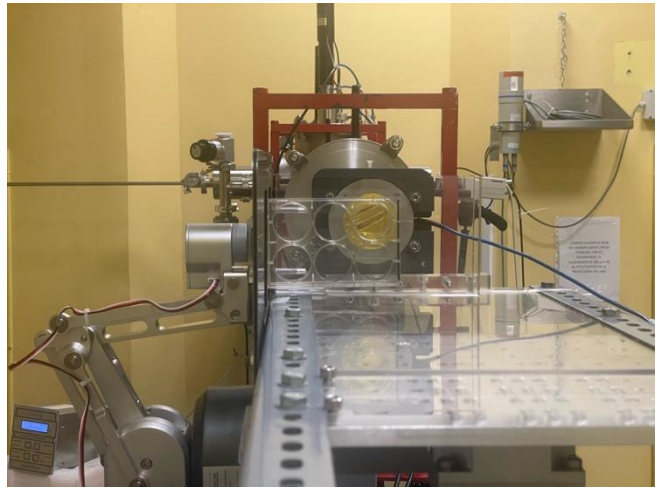
- A systematic increase of both markers is observed when we irradiate cells with AuNPs, despite not being significant statistically

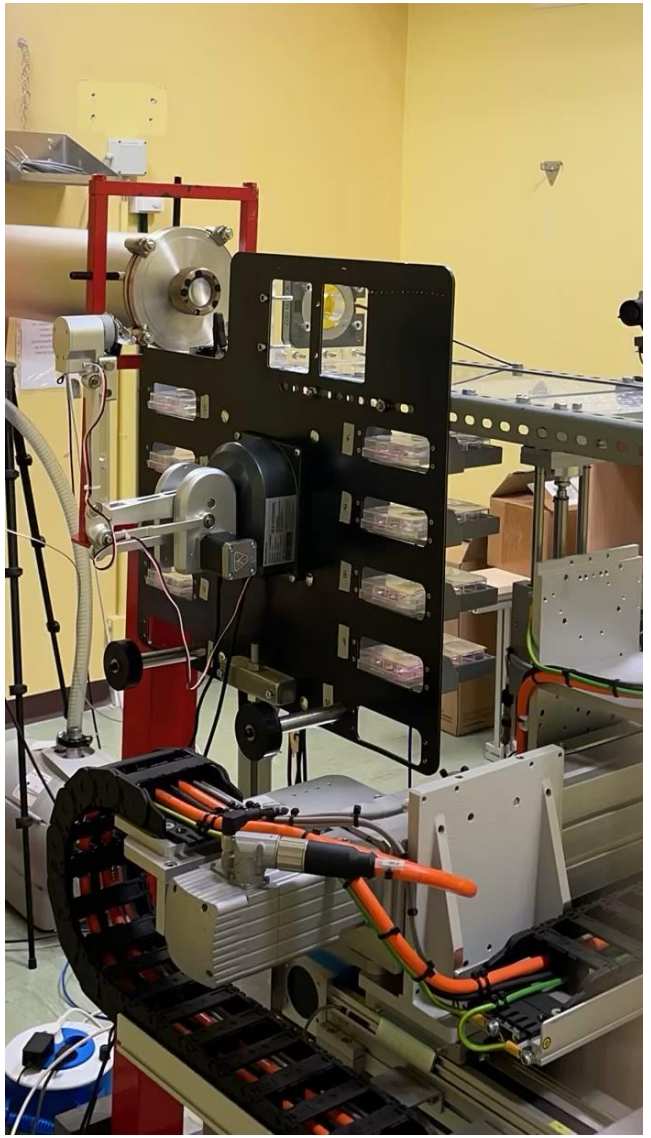


Robotic system development

Vicent Girbés, Jose Luís Sanchez
C. Blanch, N. Fuster, D. Esperante, M. Boronat
C. Jiménez, D. Hermenegildo

- Installed and first tests performed in September 2025
- 6 irradiation plates in 20 min vs 90 min
- On-going work on improving the stability and the attachment of the plates





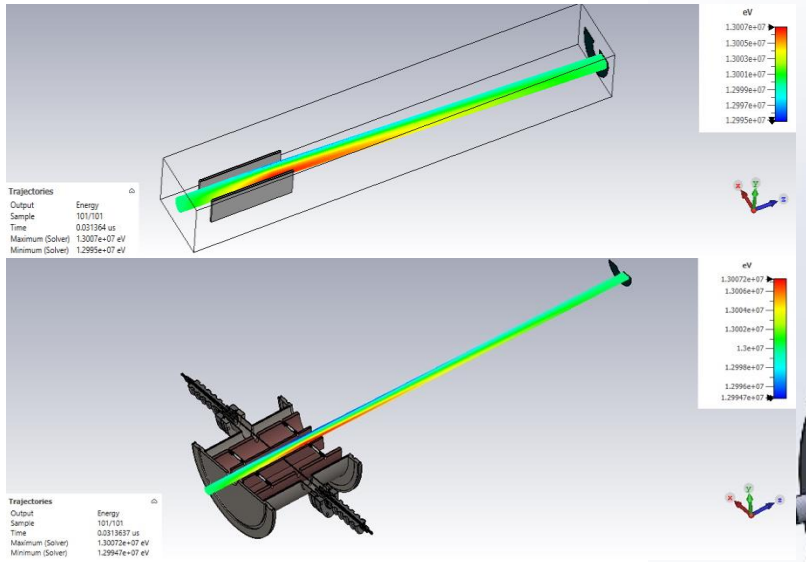
Electrostatic chopper

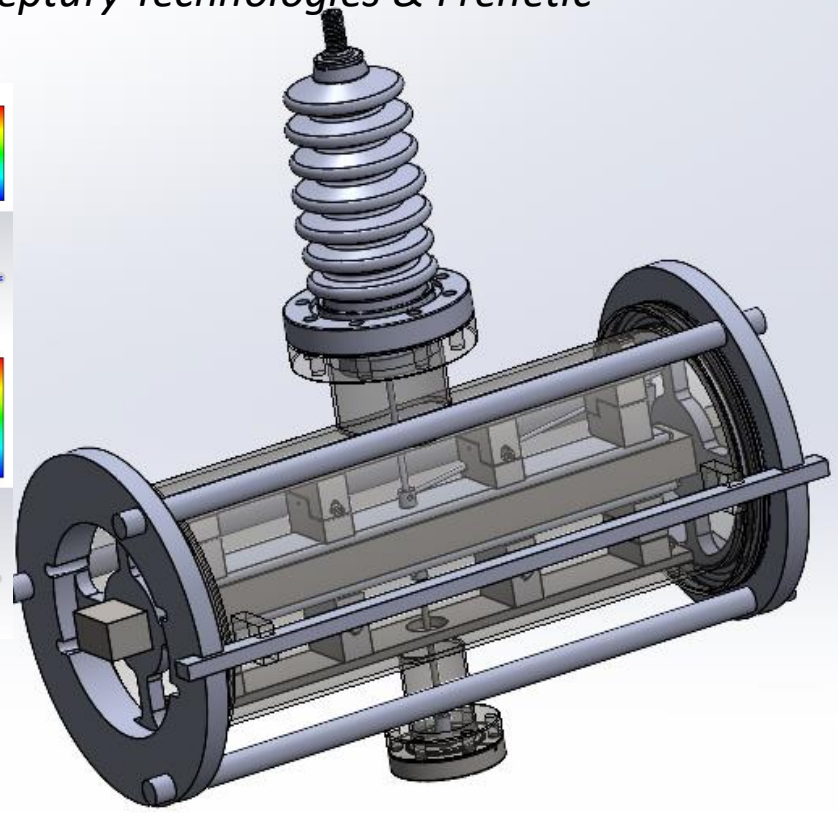
- Goal: achieve high-pulsed irradiation conditions: **pulsed beams with average dose rates higher than 40 Gy/s**

- How: ion source current set close to maximum values and beam focused & **Pulsing system**

C. Blanch, N. Fuster-Martínez, D. Esperante
C. Jiménez, J. García, D. Hermenegildo
Neptury Technologies & Frenetic

Pulse amplitude	24-27 kV
Rise/fall time	1 us
Pulse Rep. Rate	1 kHz
Chopping pulse width	10 - 50 us
Beam energy	13-18 MeV
Absorber location	1.3-1.6 m
Absorber aperture radius a [m]	0,010-0,015





- Currently under construction and working on the beamline optimization and collimator design

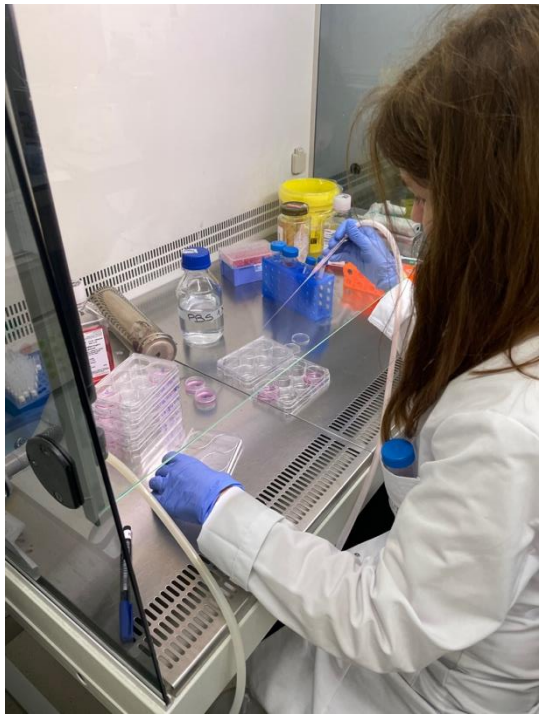
Summary

- A significant radiosensitizing effect has been observed by **AuNPs** on **Hela cells** irradiated with **12-13 MeV protons** beams at doses from 4 Gy

Case	SER
2022 AuNPs 50 nm	1.53 ± 0.07
2023 AuNPs 50 nm	1.3 ± 0.2
2023 AuNPs 20 nm	1.39 ± 0.18

- Double-strand break assays were performed at 4 Gy to quantify DNA damage
  - ✓ A **systematic increase** of both markers is observed when we irradiate cells with AuNPs
- **Working on data interpretation**
- **A robotic system** has been developed and first tested in 2025 showing **promising performance** to improve the radiation condition and increase daily irradiations in future experiments
- **An electrostatic chopper** has been **designed**, and it is **under construction** to be installed at the **external beam line of the CNA cyclotron** for high intensity pulse generation

Thank you very much for your attention!



Acknowledgements

This work was supported by the Generalitat Valenciana through grant CDEIGENT 2021/012, by the R+D+I grant PID2022-140603N funded by MICIU/AEI/10.13039/501100011033/FEDER/UE to S.J., by the *Ideas Semilla* Project IDEAS222767JIME from the Spanish Association Against Cancer (AECC), and by the Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033, PID2020-119111GB-I00, PID2023-152568NB-I00) and CIPROM/2022/062 from la Generalitat Valenciana. Additional support was provided by the European Union–NextGenerationEU and the Regional Ministry of University, Research and Innovation of the Junta de Andalucía through the Recovery, Transformation and Resilience Plan (PRTR) and the Complementary Plan in *Astrophysics* (subproject C17.I01.P01.S17, public grant ASTRO21/1.4/4). Furtheremore, funded by the Spanish Ministerio de Ciencia e Innovación under grant PID2021-098117-B-C21, funded by MCIN/AEI/10.13039/50110001103/ERDF, EU.

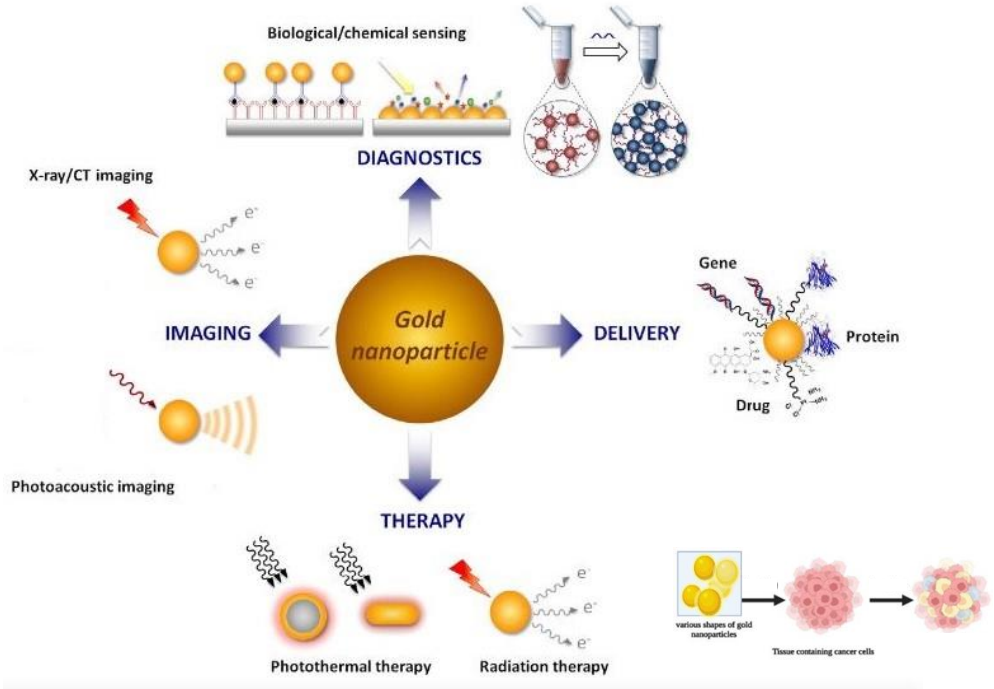
Back up slides

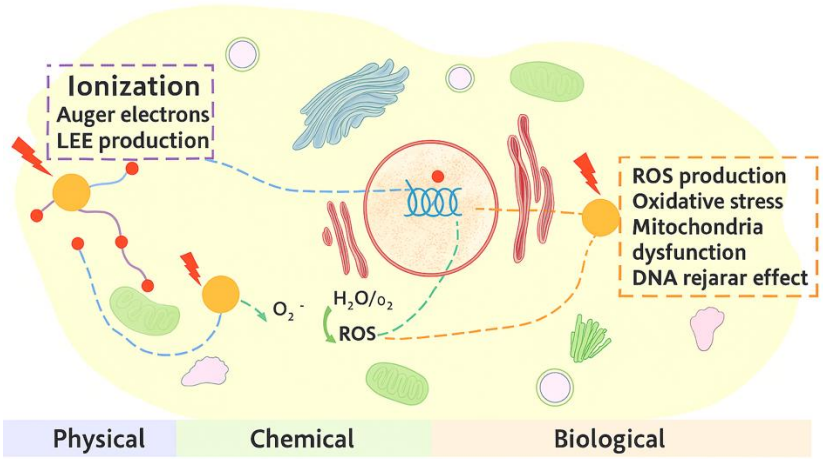
Outline

- ❑ Introduction
- ❑ Motivation and goals
- ❑ Characterization of gold nanoparticles
- ❑ Radiobiology experiments results
- ❑ Technological developments
- ❑ Summary

Nanoparticles as radiosensitizers

- **Several experiments *in-vitro* and *in-vivo* and Monte Carlo simulations** demonstrated the potential of high-Z NPs to enhance the efficacy of radiotherapy (**X-rays, p, and e)**
  - ✓ the efficiency depends on the **NPs properties, radiation characteristics and cell line**
  - ✓ **mechanisms behind are still controversial**
- **Main challenges :**
  - ✓ **Insert the NPs** only into the tumour cells
  - ✓ **Control** the concentration and biodistribution within the treatment duration
  - ✓ **Expel** of the NPs after the treatment
  - ✓ **Modelling** of the phenomenon
- High-Z-NPs with X-rays treatments are going through first **clinical trials in the USA** showing promising results (https://nanobiotix.com/media-publications/)





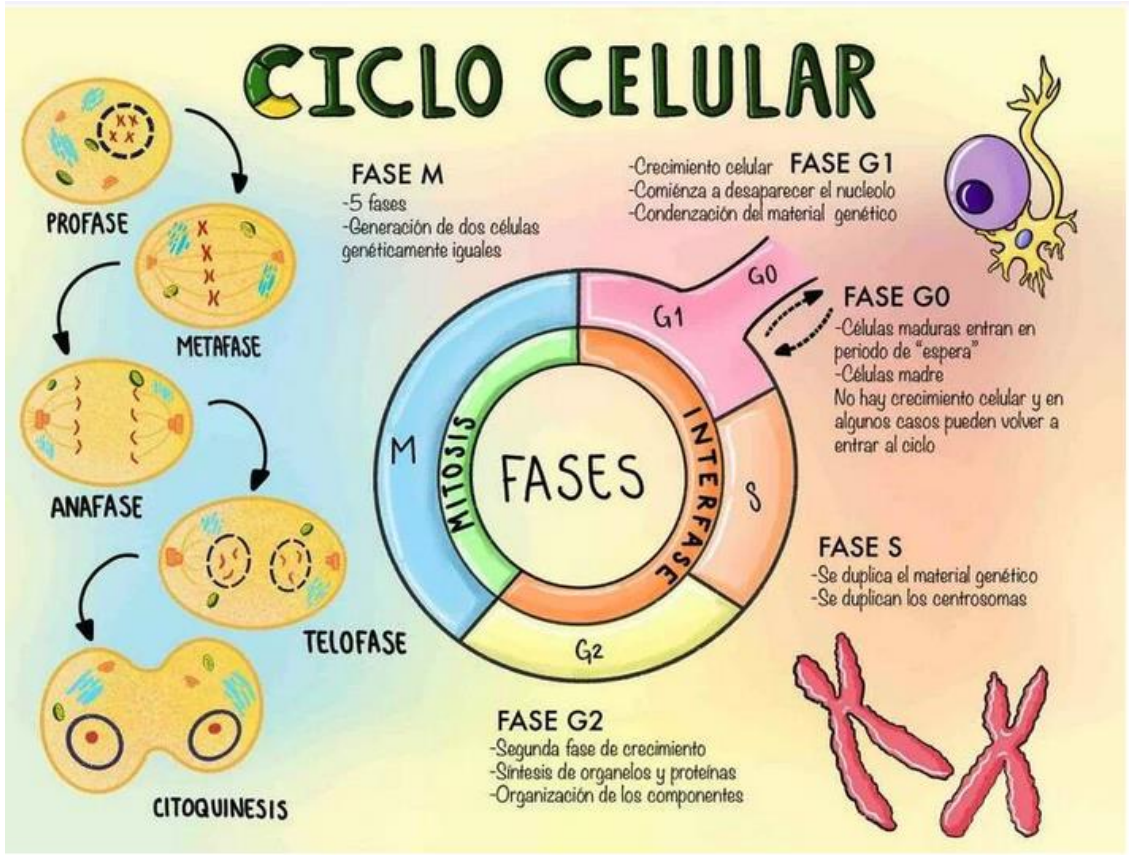
Ciclo celular (unas pinceladas)

- Las células se duplican cada~16-25h (depende del tipo celular).

- El ciclo celular se divide en distintas fases:
  - G0: periodo de "espera"
  - G1: crecimiento y preparación (6-12h)
  - S: replicación de ADN (6-8h)
  - G2: crecimiento y preparación final (3-4h)
  - M: división celular (1h)

- Las células sanas *in vitro* después de un cierto número de divisiones acaban muriendo.

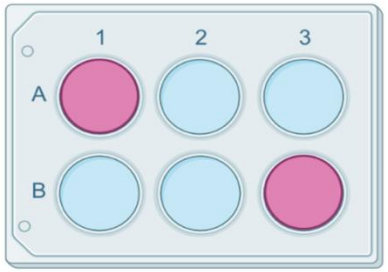
- Las células cancerígenas proliferan indefinidamente si tienes las condiciones necesarias para vivir.



Experimental procedure

CABIMER

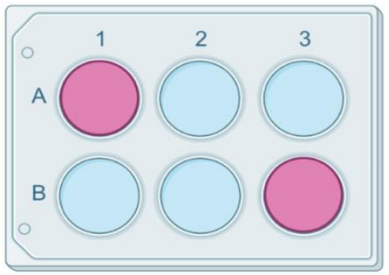
16-24h before irradiation:
cell monolayer seeding



16-24h

Au NPs treatment

AuNPs



4h

Cleaning and
medium change +
adding a mylar film

CNA-cyclotron-~13 MeV p

Moving to
CNA
~5 min walk

Moving back
to CABIMER
~5 min walk

45min-1h out of the incubator

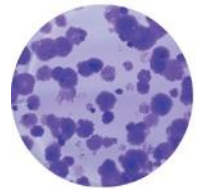
IFIC/UV

Data analysis

CABIMER

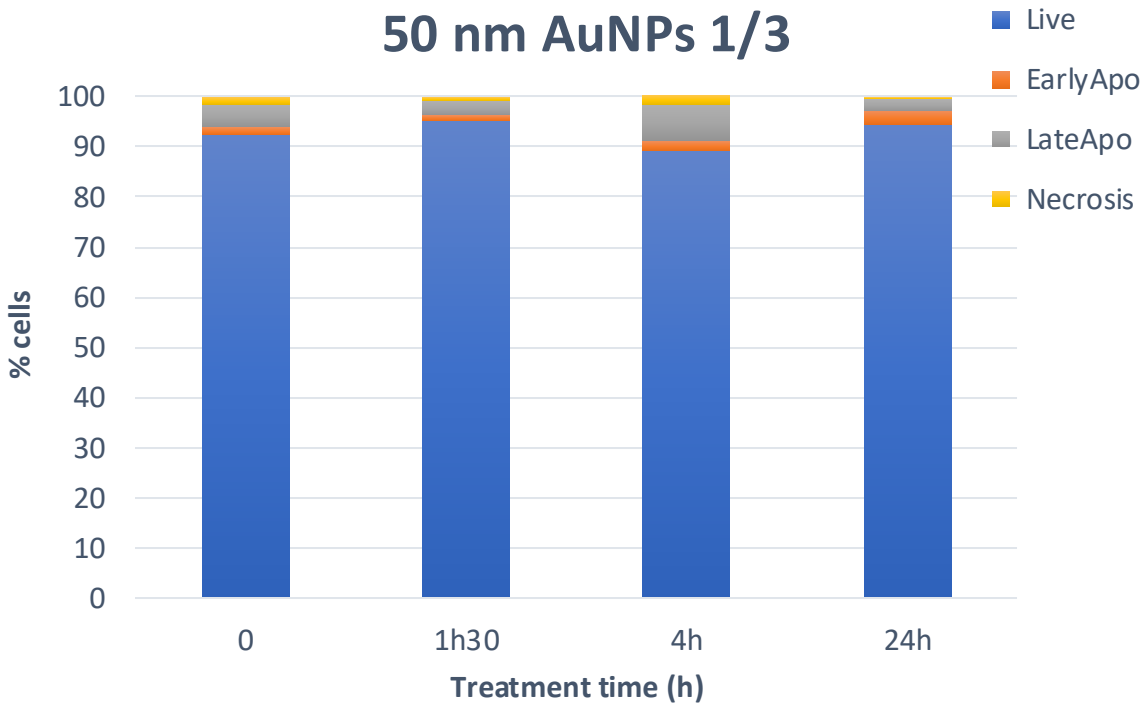
Remove mylar film and
change the medium

For clonogenic assays
place into the incubator
for 11-13 days

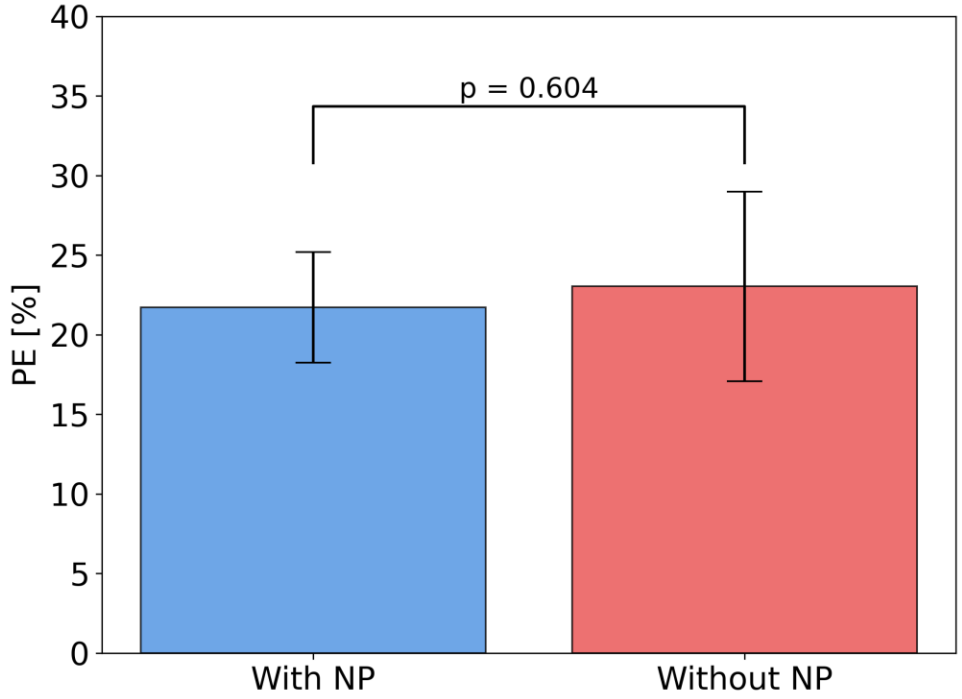


AuNPs charcaterization

- **Cytotoxicity :**
  - ✓ **Cytometry Annexin V-FITC + IP**
  - ✓ **CellTiter-Glo® Luminescent** Cell Viability Assay
  - ✓ Clonogenic assays



- **After 13 days:**
  - ✓ **Clonogenic assays:** viability tested after 13 days



- **The viability of the cells it is not affected** by the AuNPs at the concentration **of 149 ug/ml**

Uptake of gold NPs

- **Uptake** of NPs in the cells quantified using **inductively coupled plasma mass spectrometry** and **Transmission electron microscopy**
- NPs cell uptake by endocytosis
- NPs aggregate and they are placed in the cytoplasm