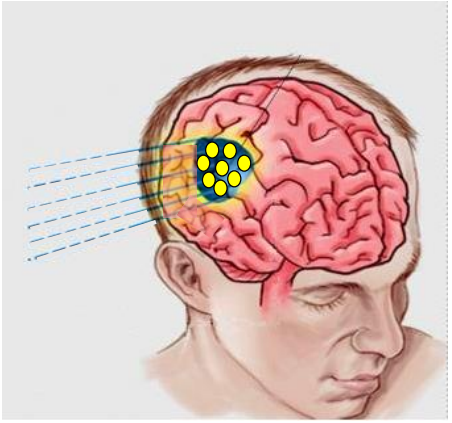
How to boost radiation therapies using nanoparticles



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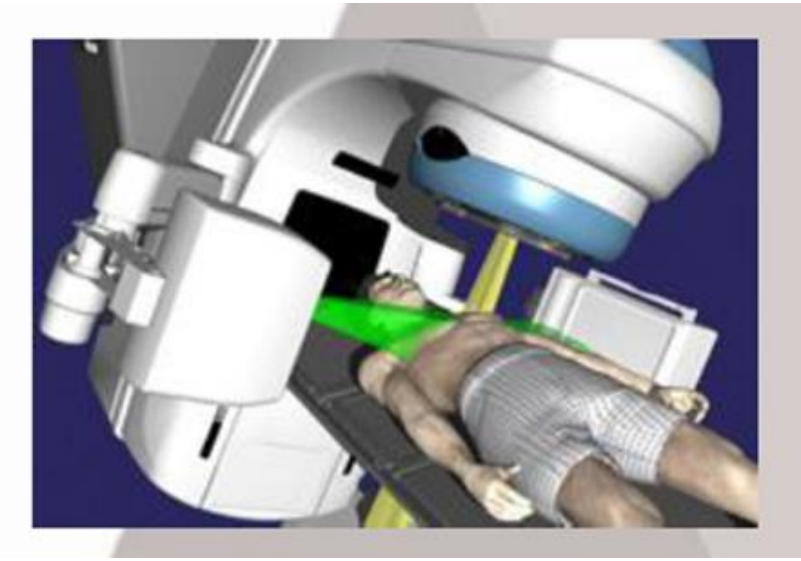
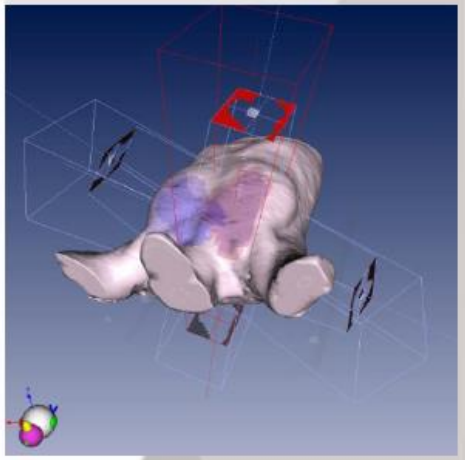
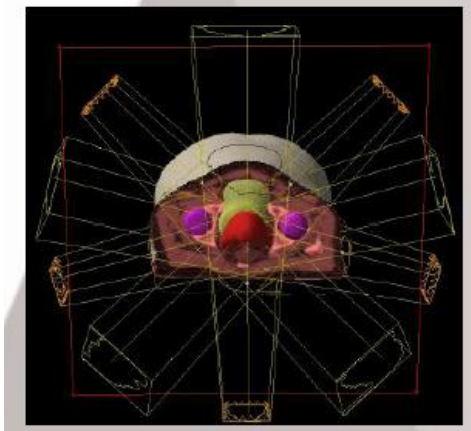
12/03/2021

Radiotherapy

Radiotherapy is a method of cancer treatment, using radiation to destroy cancer cells.







Calculation of the 3D dose in the patient, using algorithms that model the energy deposition of particles in the tissues.

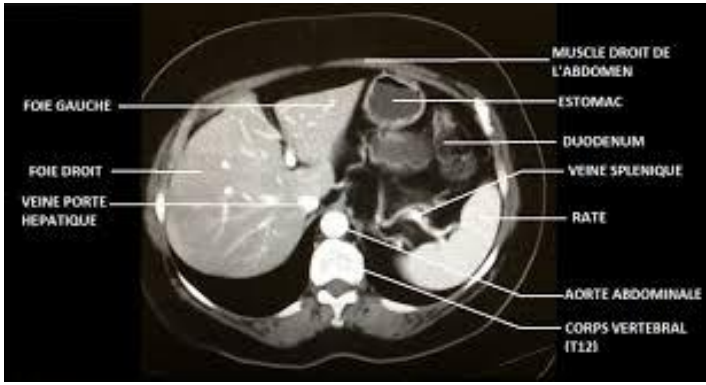
Macroscopic scale

Treatment planification system (TPS)

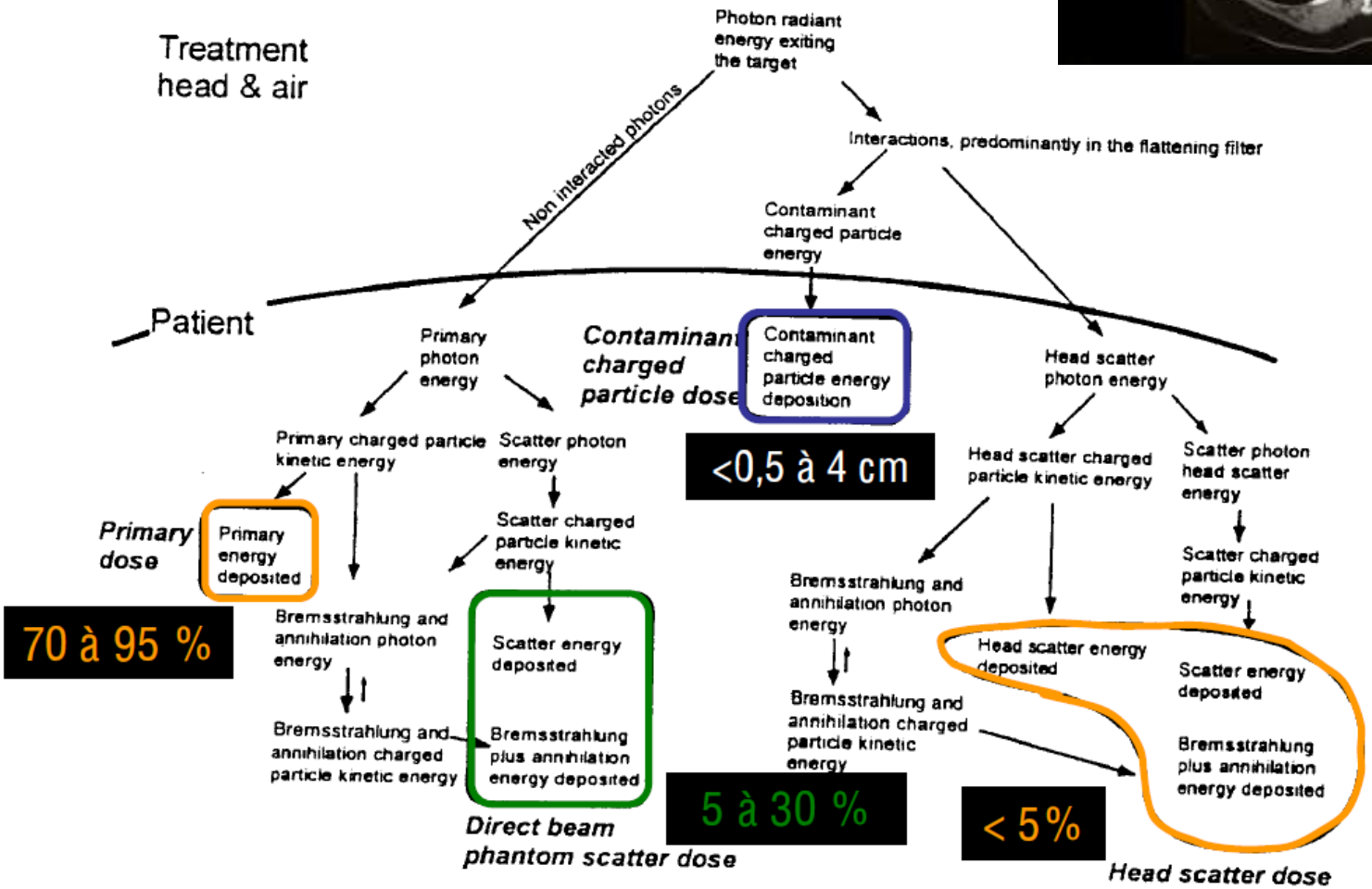
Radiotherapy

Treatment planification system (TPS)

DOSE : Gy (J/kg)



Ahnesjö and Aspradakis Phys. Med. Biol. 1999



Radiotherapy

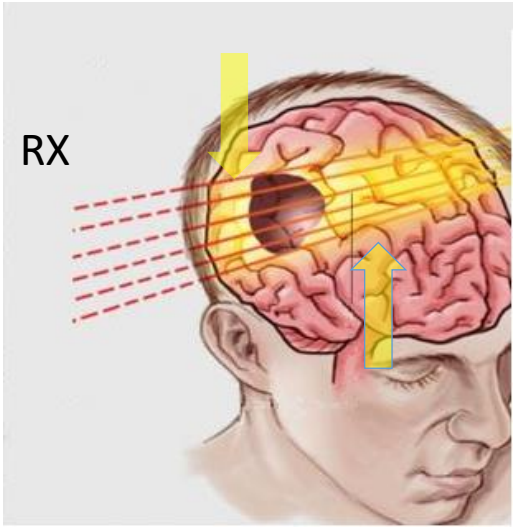
Limitation: no tissue specificity/targeting

- Severe side effects due to damage in healthy tissues
- Radioresistance

1-20 MeV

Challenge: improvement of tumor targeting

(increase of radiation effects in the tumor)

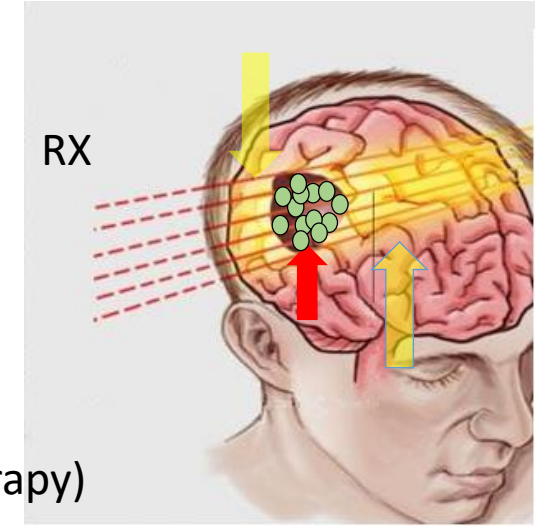


Strategy : new modalities (IMRT, microbeams, particle therapy)

Radiotherapy

Limitation: no tissue specificity/targeting

- Severe side effects due to damage in healthy tissues



Challenge: improvement of tumor targeting

1-20 MeV

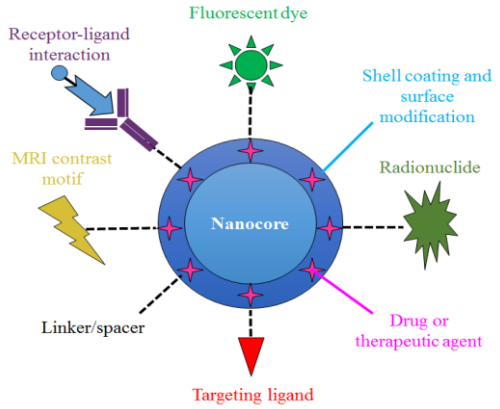
(increase of radiation effects in the tumor)

Strategy : new modalities (IMRT, microbeams, hadron/protontherapy)

Innovative strategy : ***nanotechnologies***

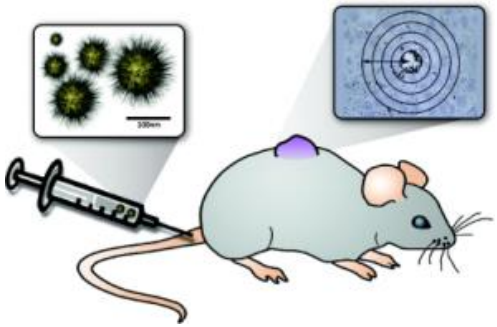
High-Z nano-agent:

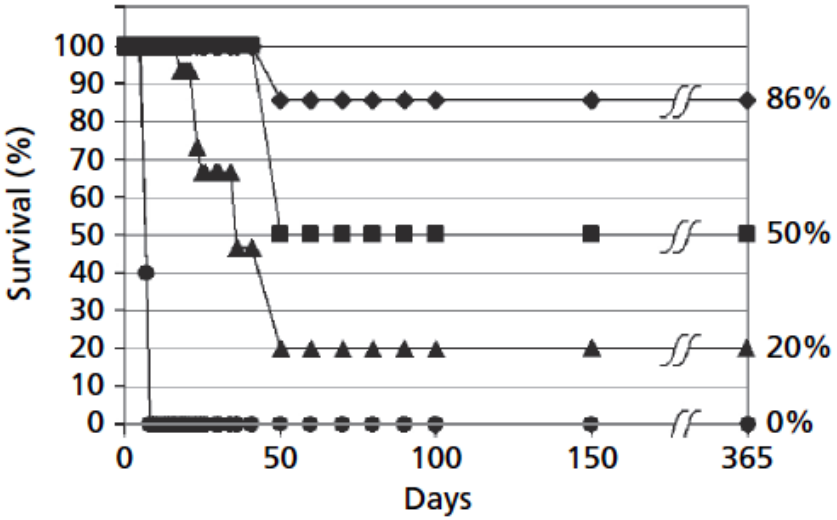
- Small (<10 nm) ….for accumulation in tumors by EPR
- Biocompatible & not toxic
- Optical, electronic , magnetic properties
- Diverse surface chemistry



Proof of concept

Hainfeld et al., Phys Med Biol (2004)





AuNPs + RX

AuNPs + RX

X- rays

AuNP

- **Higher survival of mice in the presence of AuNPs**

- Enhancement of therapeutic index of radiation (radiosensitization)
- Starting point of numerous studies with gold NPs!!!!

State-of-the-art

Photons + High-Z NPs

2000-2004

Evidence of the amplification effect *in vivo* and *in vitro*

Pt complexes

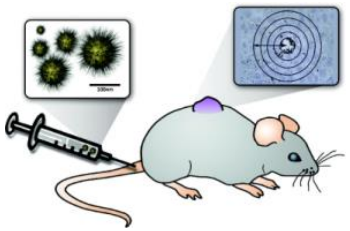
Cis-Pt and PtTC

Le Sech C et al. Radiat Res (2000)
Corde S et al. Radiat Res (2002)
Kobayashi K et al. Nucl. Instr. and Meth. in Phys. Res. B (2003)
Biston M-C et al. Cancer Res (2004)

2004

Evidence of the amplification effect *in vivo*

Au NPs



Hainfeld et al. Phys Med Biol (2004)

2005-2011

Mechanisms and amplification effect *in vitro*

Au NPs

Foley et al. Chem Comm (2005)
Debouttière et al. Adv Funct Mater (2006)
Kong et al. Small (2007)
Butterworth et al. Radiat Res (2008)
Jain et al. Int J Radiat Oncol Biol Phys. (2011)

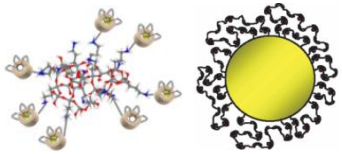
Medical Imaging

Alric et al. JACS (2008)

2011-2016

Theranostic nano-platforms *in vivo*

GdBNs and **Au NPs**



Le Duc et al. ACS nano (2011)
Mignot et al. JACS (2013)
Butterworth et al. Nanomedicine (2016)

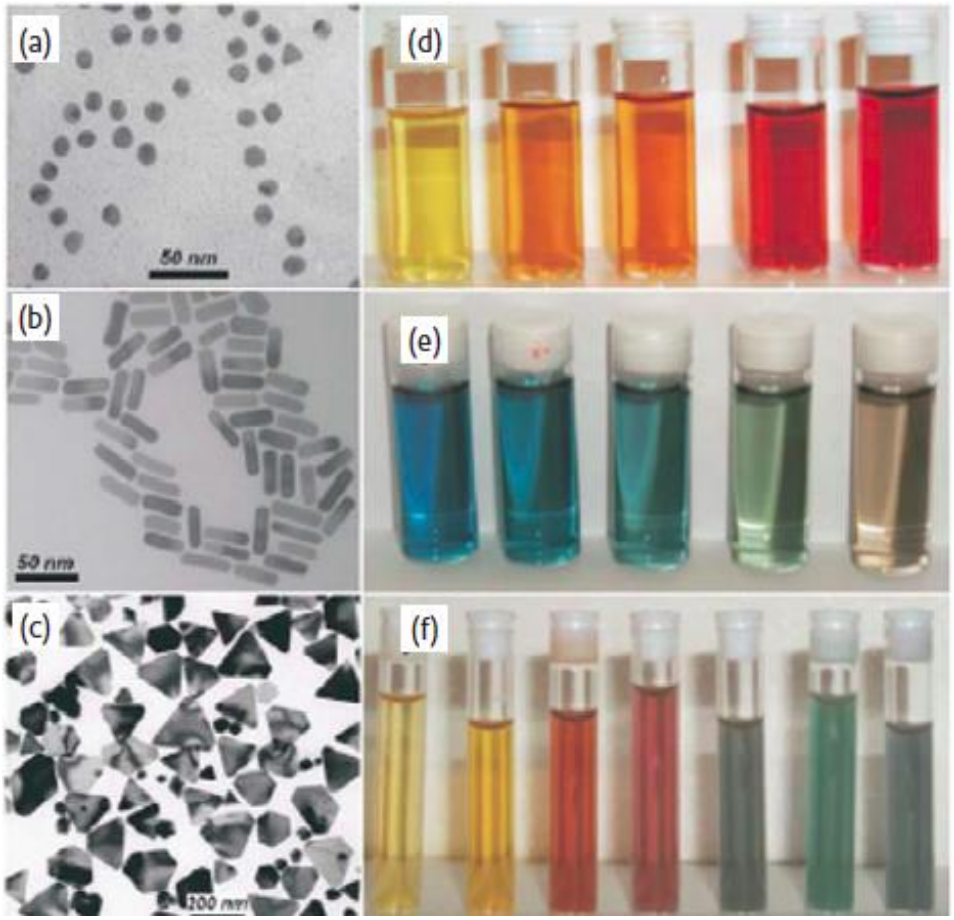
Clinical trials

NBTXR3
HfO_2

AGuIX

GdBNs

Gold Nanoparticles (AuNPs) : the favorite



Easy synthesis
Not toxic
Surface plasmon
Use for medical imaging, photothermal therapy

&

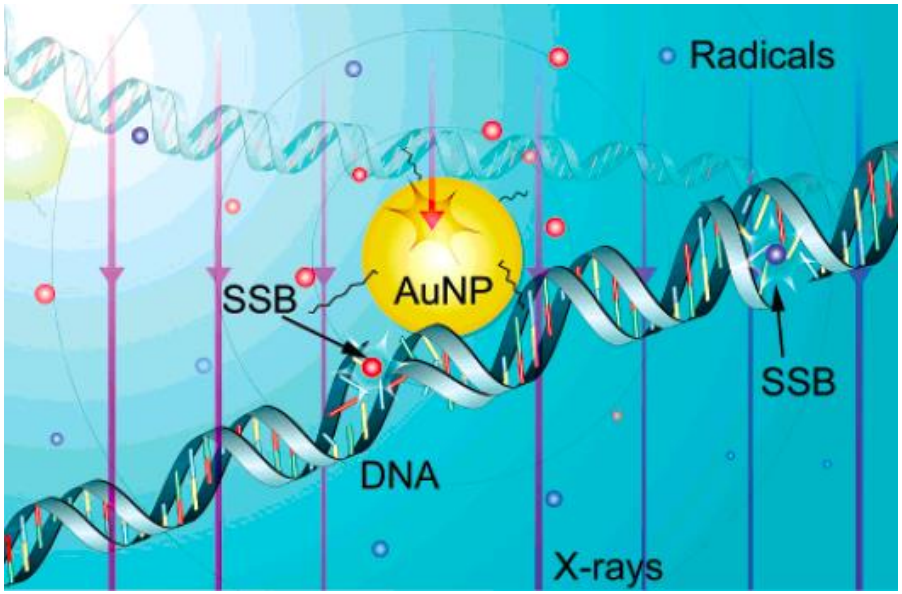
High-Z (reservoir of e-)

Jain, El Sayed et al., Nanotoday (2007)

AuNPs @ x-rays

Nanoscale impact: Studies with plasmids & simulation

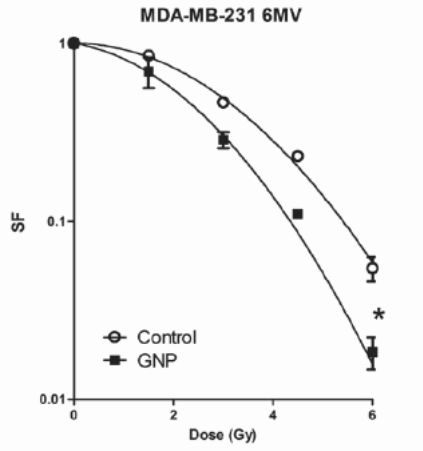
Foley, Guo et al., Chem Comm (2005) & Carter, Guo et al, Phys Med Biol (2008)
Butterworth, Prise et al. Rad Res (2008) …

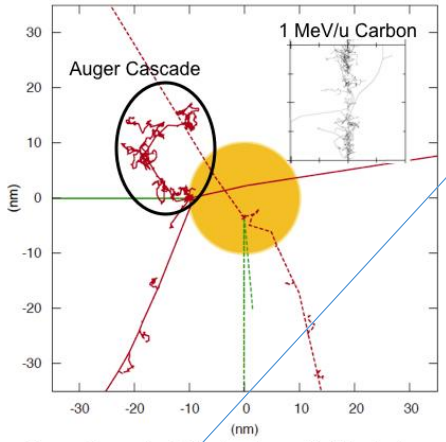


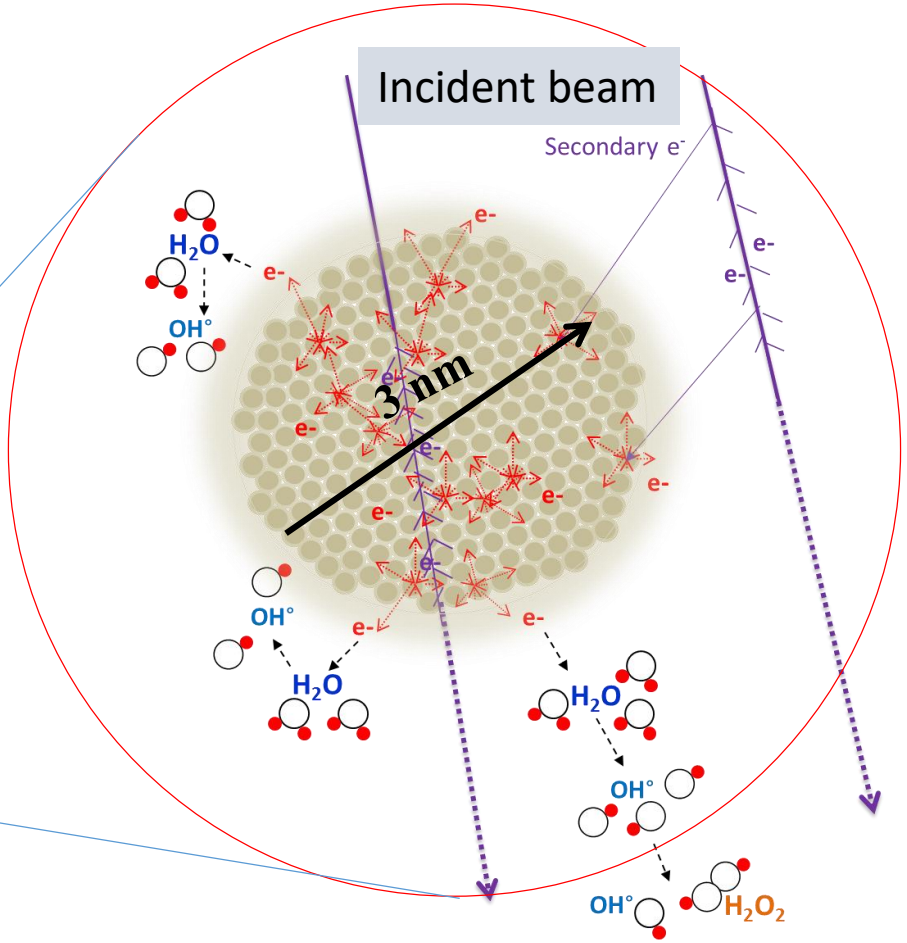
- Amplification of e- emission
- Production of radicals

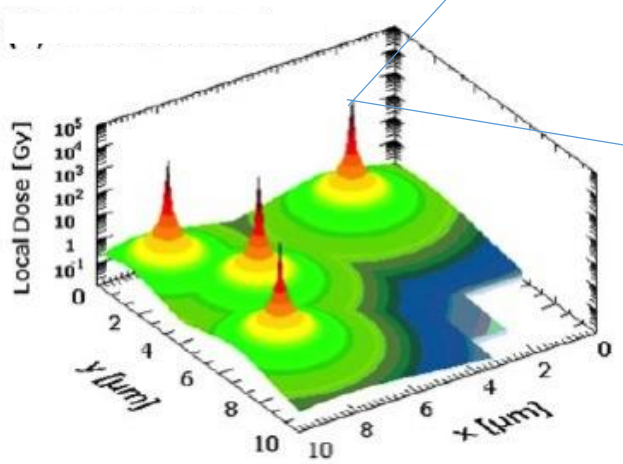
Local Effect Model

Mc Mahon et al Scientific Reports (2011)









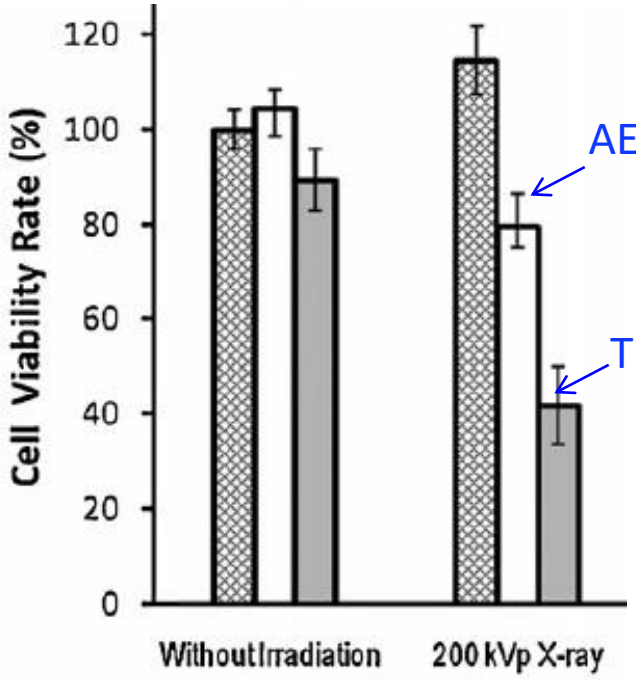
Kobayashi et al Mutation Research (2010)

- NP effect = induction of local dose « spikes »
- Confined nano-size perturbation
- Same size as biomolecules

Cellular scale effect: Influence of the NP localization (membrane or cytoplasm)

Kong et al, Small (2007)

Caped AuNPs



AET-AuNPs attached to cell membrane

Thiol-AuNPs internalized in the cell (cytoplasm)

➢ **Important role of NP localization**

The effect of AuNPs is **cell line dependent**

Butterworth et al., Nanotechnology (2010)

➢ Multiscale action :

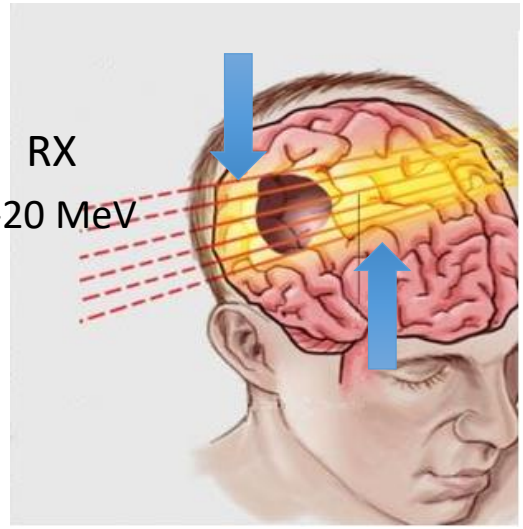
i. Early stage physics (NP activation/relaxation)

ii. Biological response

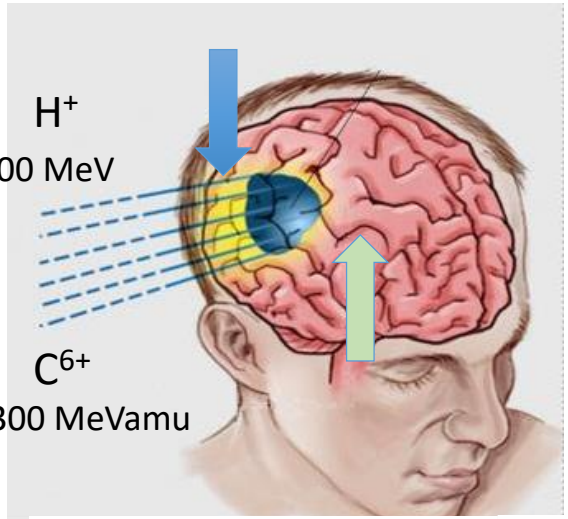
NPs with particle therapy ?

Particle therapy – advantages/limitation

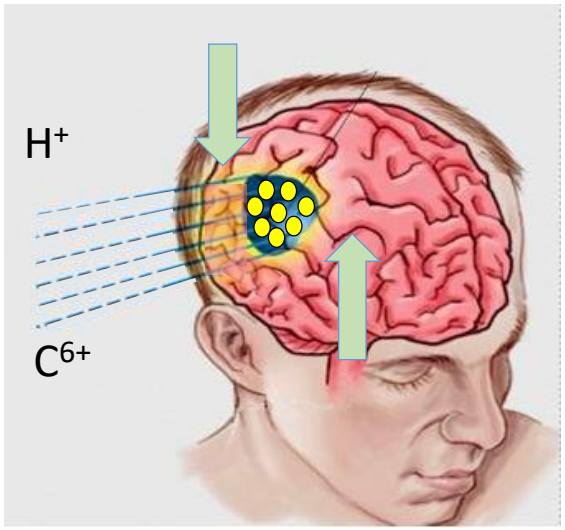
Side effects of photons

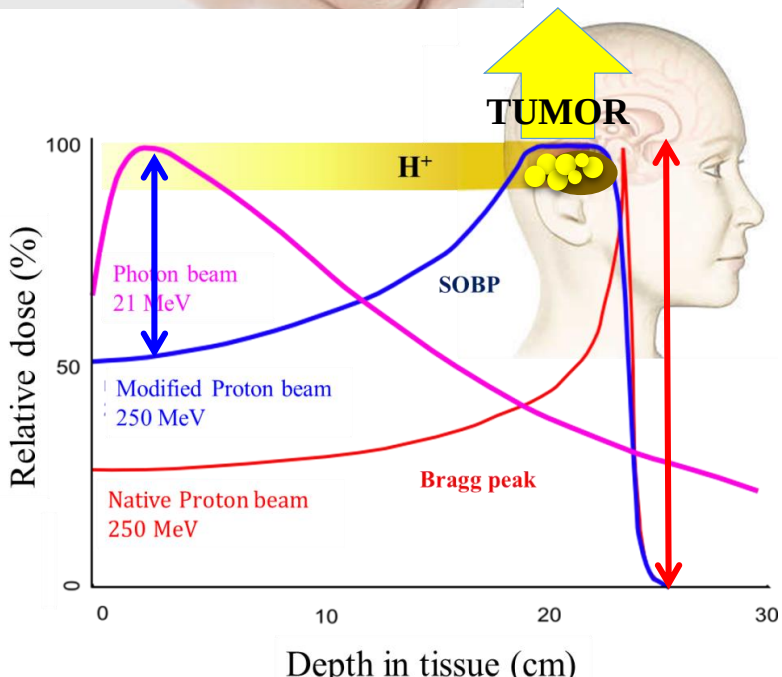


Advantage/limitation of fast ions



Strategy: addition of NPs





Aim :

Reduce the total dose

State-of-Art

- Ion Beam Radiation Therapy combined with Nanoparticles

Ions + High-Z NPs

2007-2008

Mechanisms and **amplification effect** *in vitro*

Pt complex
PtTC

Usami N et al. Int J Radiat Biol (2007)
Usami N et al. Int J Radiat Biol (2008)

2010 - 2012

Evidence of the amplification effect *in vitro* and *in vivo*

Au NPs

Pt NPs

GdBNs

Kim J-K et al. Nanotechnology (2010)
Porcel et al. Nanotechnology (2010)
Polf J et al. Appl Phys Lett (2011)
Kim J-K et al. Phys Med Biol (2012)

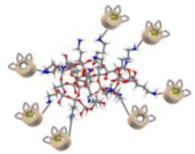
2013-...

Multiparametric amplification effect
Nanoparticle development
Theranostic perspectives
in vitro

Kaur H et al. Nucl. Instruments Methods Phys (2013)
Porcel et al. Nanomedicine (2014)
Liu Y et al. Phys Medica (2015)
Schlatholter T et al. Int J of Nanomedicine (2016)
Kuncic Z et al Phys Med Biol (2018)
Lux et al BJR (2018)
Ivosev et al nanotech (2019
Li et al ChemMedChem (2020)

⟶ Clinical trials

AGuiX**

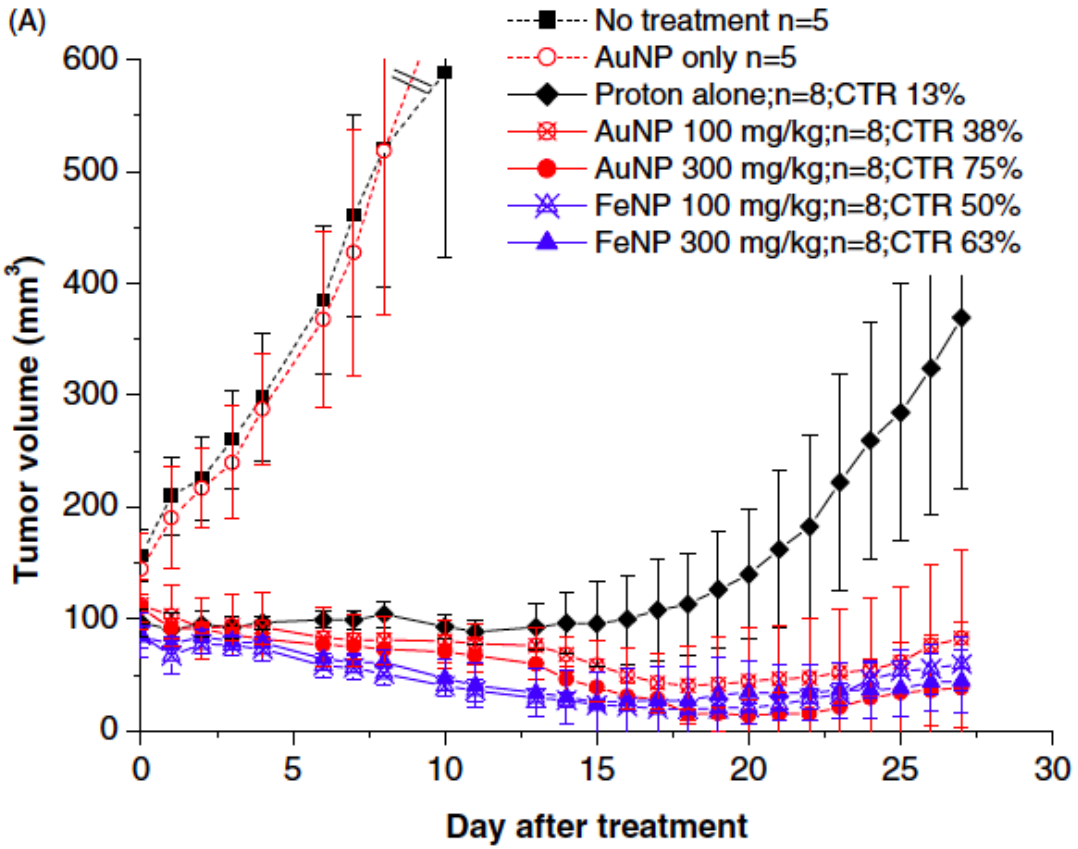


***patent : PCT/FR2008/051860, WO2009053644 A3*

AuNPs, FeNPs @ proton radiation

Proof of concept in vivo : 45 MeV proton treatment

Kim et al, Phys Med biol. 2012



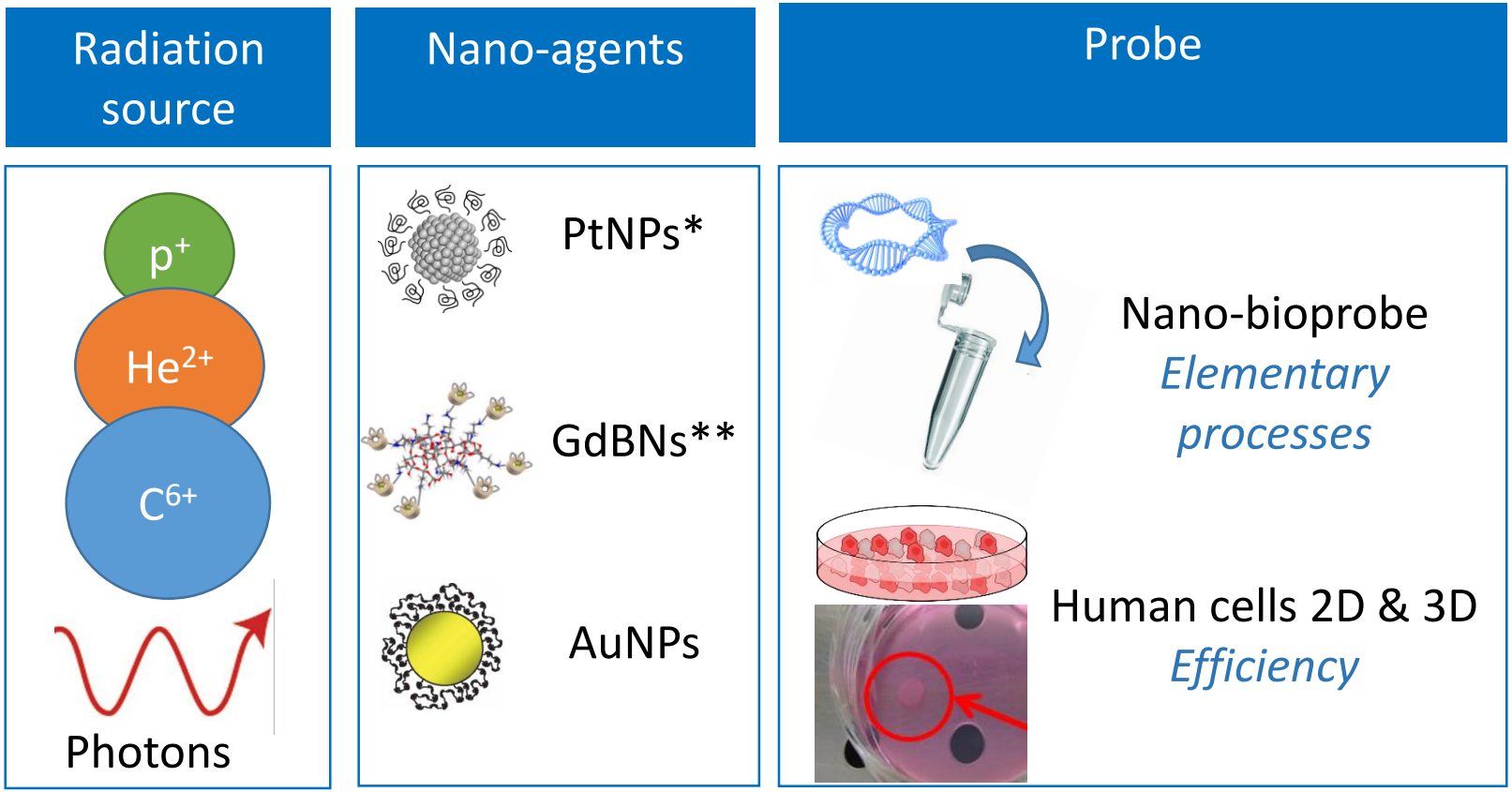
➢ **Decrease of tumor growth in the presence of NPs**

➢ Improvement of proton radiation effect with metallic NPs

Development of NPs + Understanding mechanisms

Small metallic NPs (Pt, AU, Gd…) / ions (high energy)

Multiscale approach



*French patent: FR1900008

**patent : PCT/FR2008/051860, WO2009053644 A3

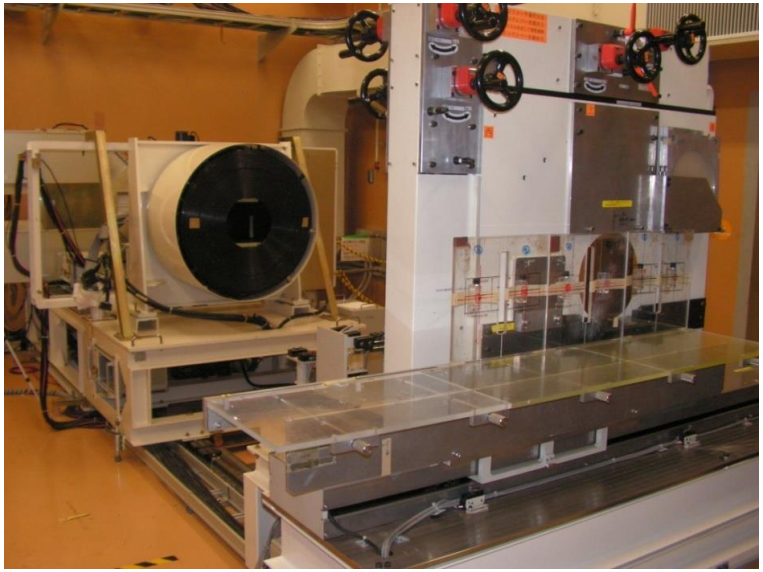
Irradiation facilities

- **Fast ion** medical beam (HIMAC, Chiba, Japon)

Broad beam (SOBP)

C $^{6+}$ (290MeV/u)

He $^{2+}$ (150MeV/u)

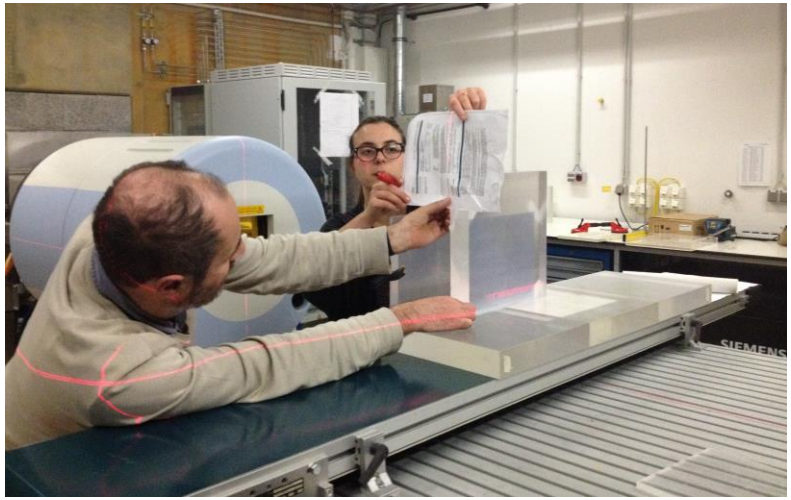


- **Fast ion** medical beam (HIT, Germany)

Pencil Beam

C $^{6+}$ (290MeV/u)

He $^{2+}$ (150MeV/u)



- **Proton** beam (KVI, The Netherlands)

Pristine peak

150 MeV

- **Gamma rays** Cs137 (Curie, Orsay, France)

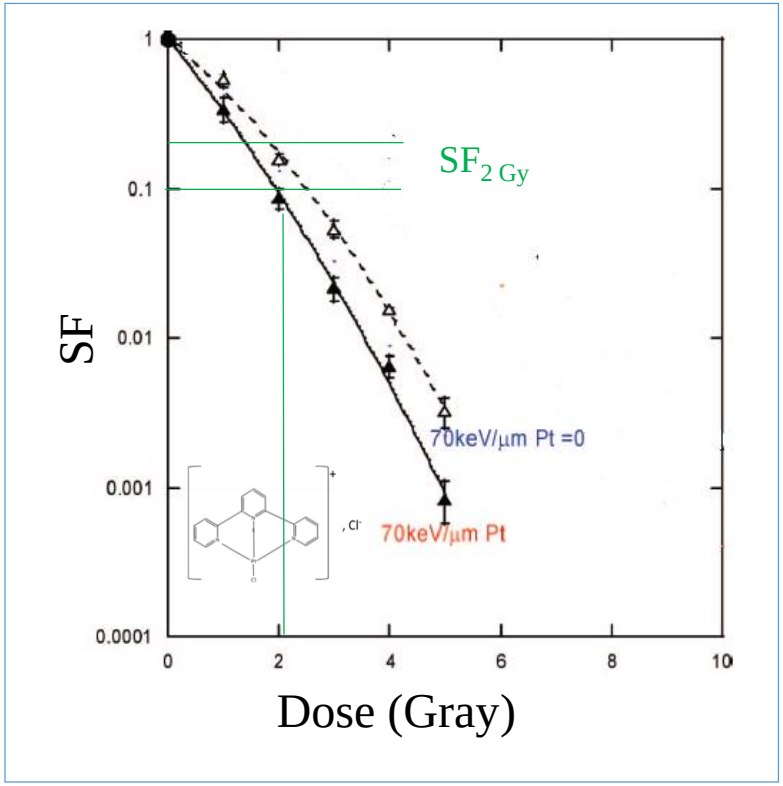
Pt complexes @ medical carbon ions (HIMAC-NIRS)

1st evidence of ion beam effect enhancement

Usami , Lacombe, LeSech et al IJRB 85 (7) (2008)

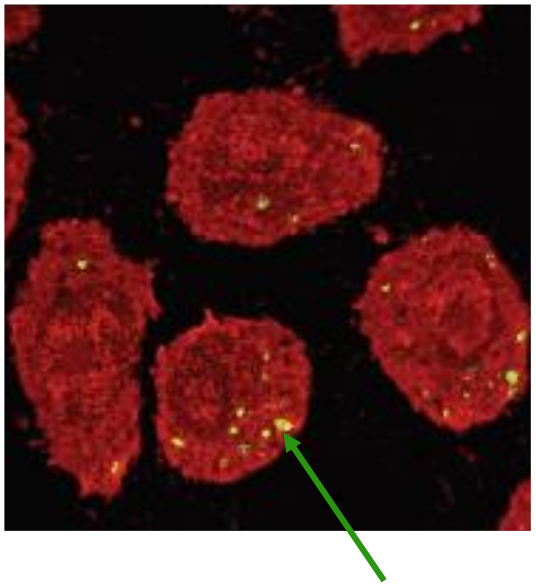
CHO + **Platinum complex** (chloro terpyridine platine) 1mM, 6h

Irradiation C^{6+} (290MeV/n)



SER (SF 2Gy)
1,5

Nano-SIMS



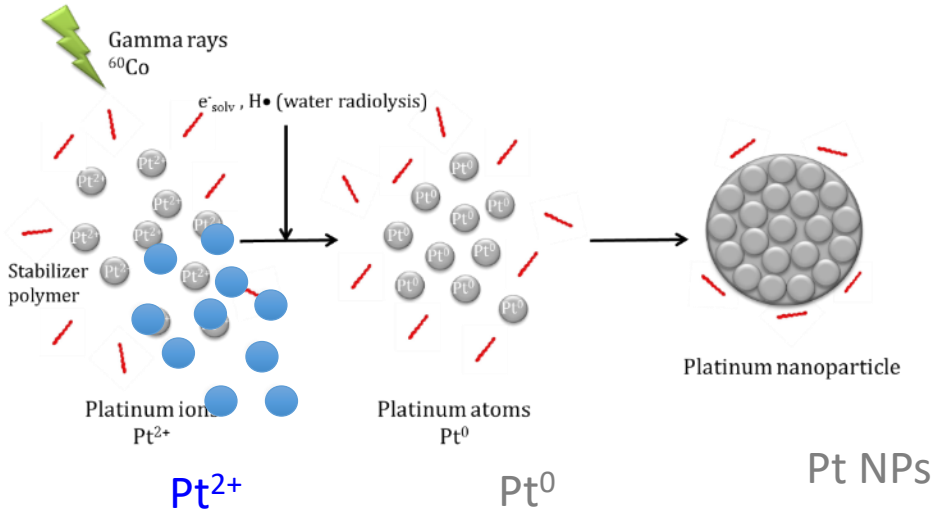
➢ **Enhancement of effects with Pt complexes located in the cytoplasm**

Limitation: Pt complexes are not tumour specific

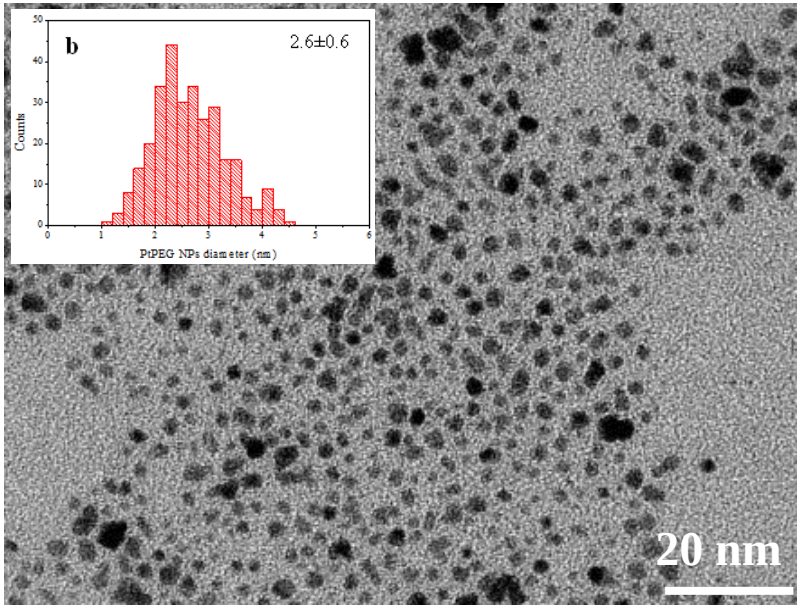
Pt NPs synthesis by radiolysis

NP synthesis by water radiolysis

- Fast (1 step)
- reproducible
- Green chemistry
- Ready -to-use sterilized solution



TEM



✓Small (3 nm) for EPR

✓Biocompatible, stable

✓Not toxic

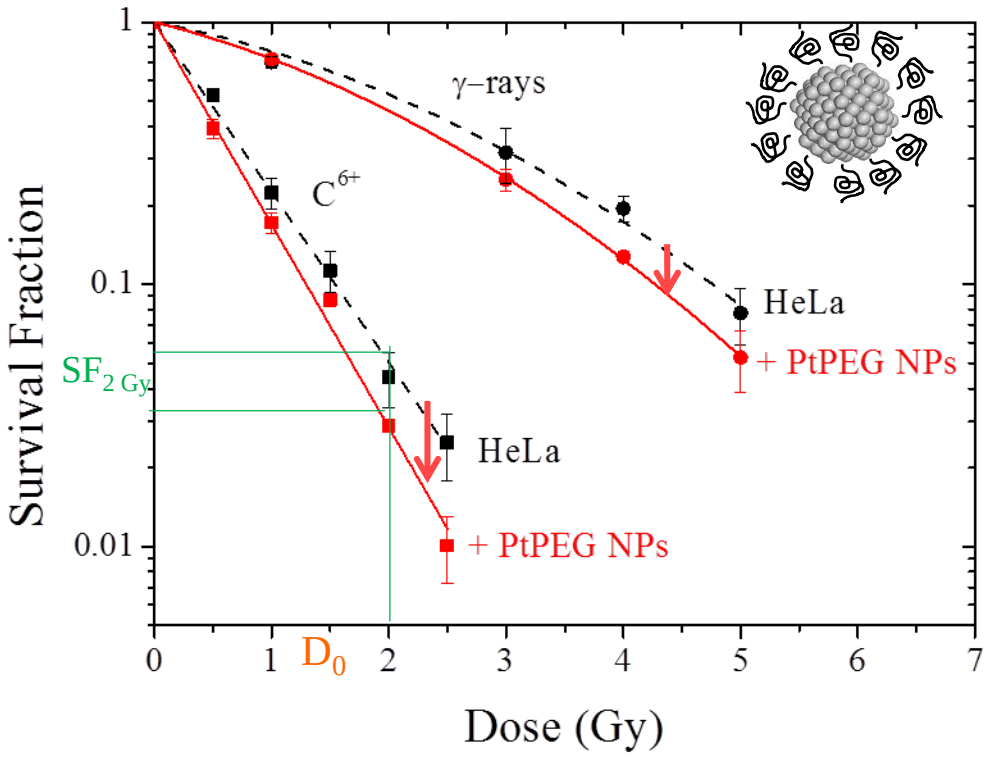
- Advantage of PtNPs: one step & « green » synthesis and sterilization method

Salado et al., Nanotechnology, Science and Applications (2020)

Effects of PtNPs

Irradiation C^{6+} (SOBP) and gamma rays /**HeLa** cells
Incubation : 0.5 mM Pt, duration= 6h

$$SF = \exp -(\alpha D + \beta D^2)$$



Source	Sample	α (Gy^{-1})	β (Gy^{-2})
γ-rays	Control	0,20±0,06	0,06±0,01
	PtPEG NPs	0,26±0,01	0,07±0,01
C^{6+}	Control	1,5±0,02	-
	PtPEG NPs	1,78±0,03	-

Increase of **directly lethal damages**

	SER (SF 2Gy)
γ-rays	14%
C^{6+} (SOBP)	**42%**

➢ **Enhancement of carbon ion efficiency**

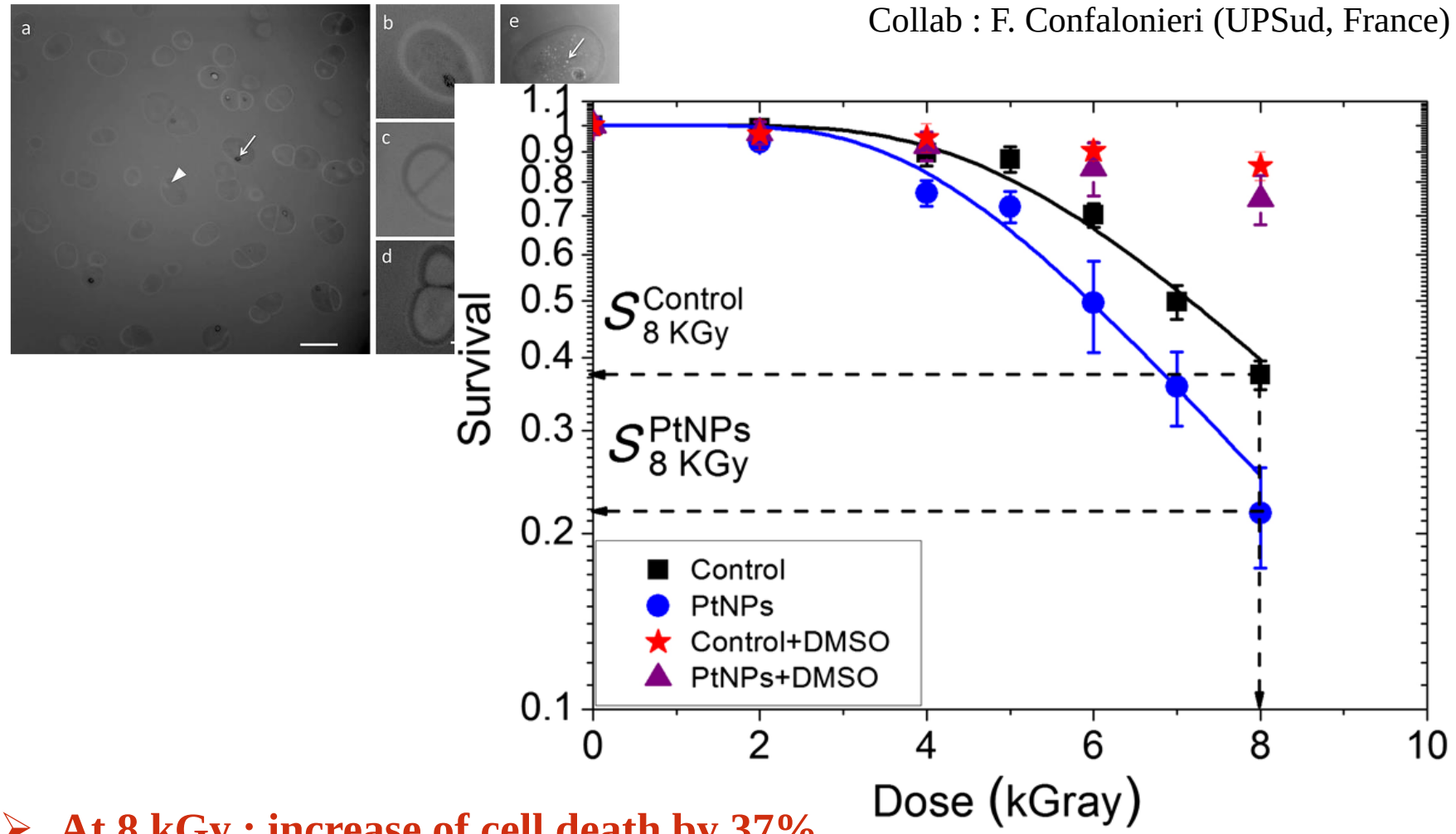
Salado et al., Nanotechnology, Science and Applications (2020)

PtNP : radioresistance overcoming?

Effects of PtNPs

Irradiation gamma rays / **Radioresistant organism : D Radiodurans**

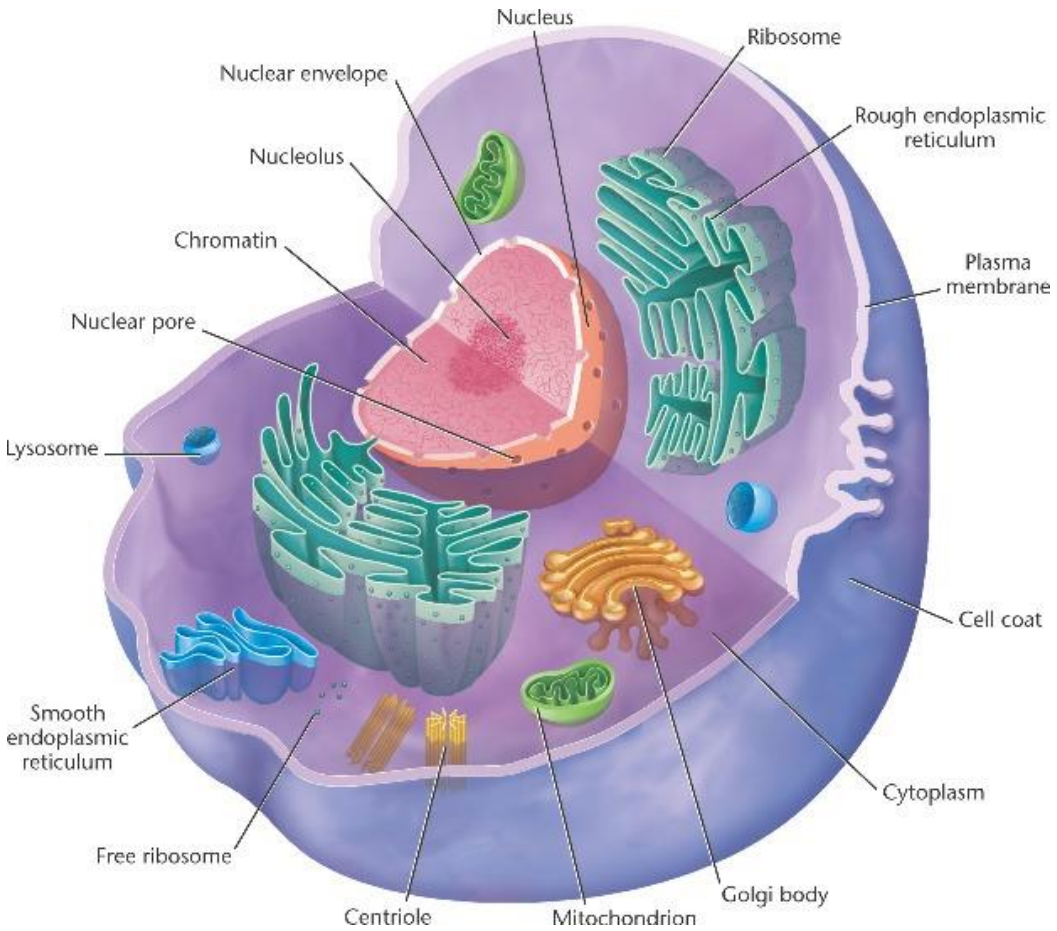
Collab : F. Confalonieri (UPSud, France)



- **At 8 kGy : increase of cell death by 37%**
- **Scavenging effect : major role of radicals**

Li et al Cancer Nanotechnology (2017)

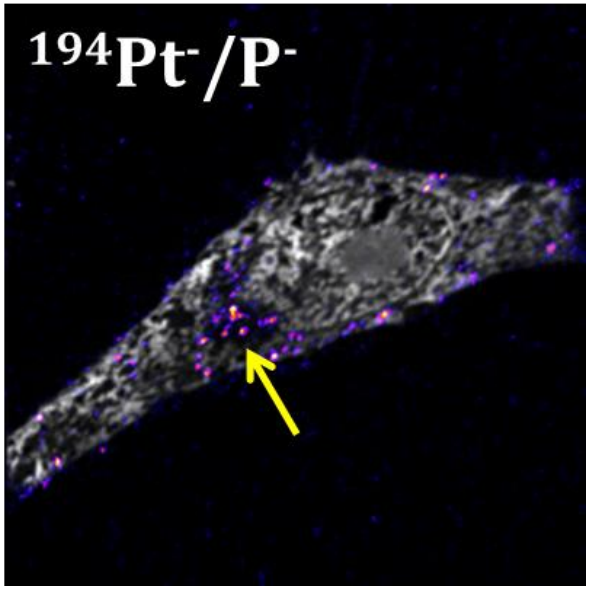
Action sites in the cell ?



PtNP –action sites ?

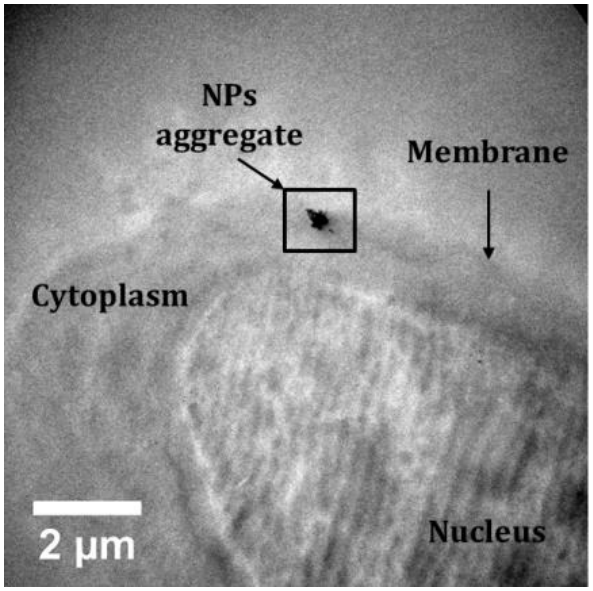
Intracellular distribution

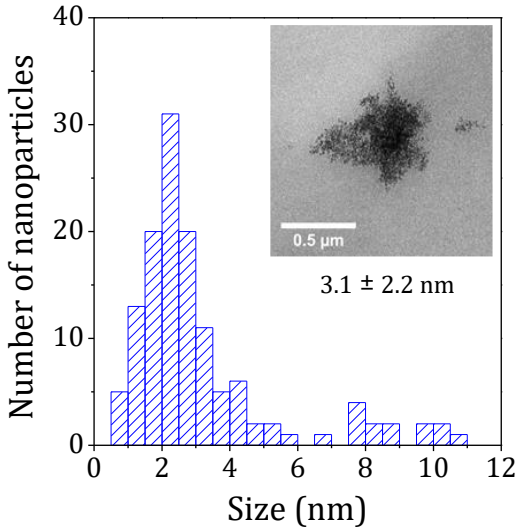
Quantification by ICP MS : 5x10^5 NPs per cell



1st characterization of intracellular PtNPs

NanoSIMS





TEM

Higher resolution (atomic)

➢ **PtNP** are localized exclusively in the **cytoplasm**

Salado et al., Nanotechnology, Science and Applications (accepted)

PtNP –action sites ?

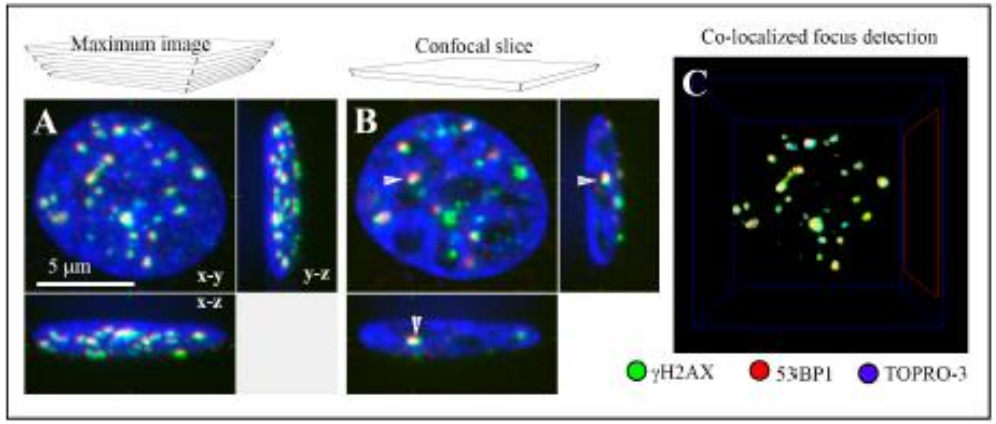
Effects of PtNPs

French patent: FR1900008

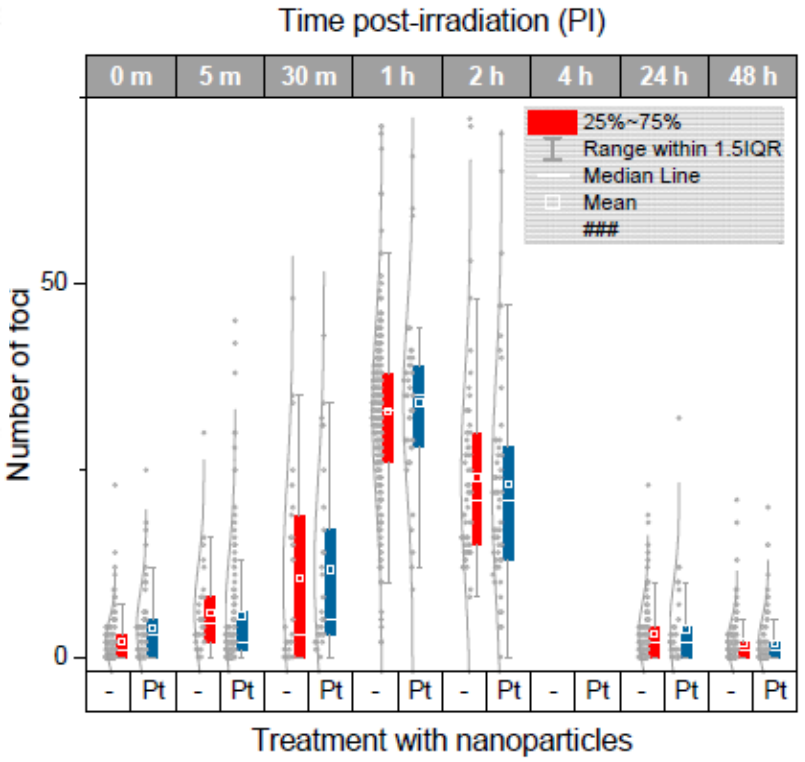
DSBs induction and repair: DSB foci

Irradiation gamma rays /**HeLa** cells
Incubation : 0.5 mM Pt, duration= 6h

Collab : M. Falk (IBP, Brno, Czech Republic)



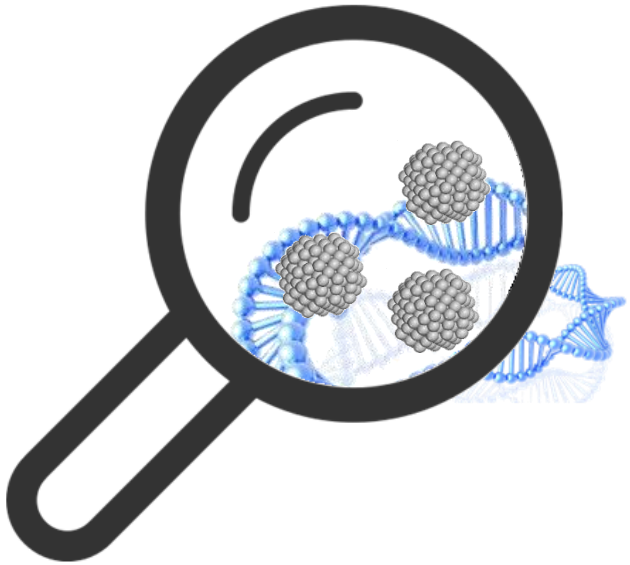
γ-H2AX 53BP1 TOPRO 3



➢ **No increase of DSB foci with PtNPs**

Pagacova et al IJMS (2019)

Molecular level effects ?



DNA plasmids = molecular probe

- Objective: quantification of nano-size damages

  pBr322 **plasmid = Nano-biodosimeter** (probe)

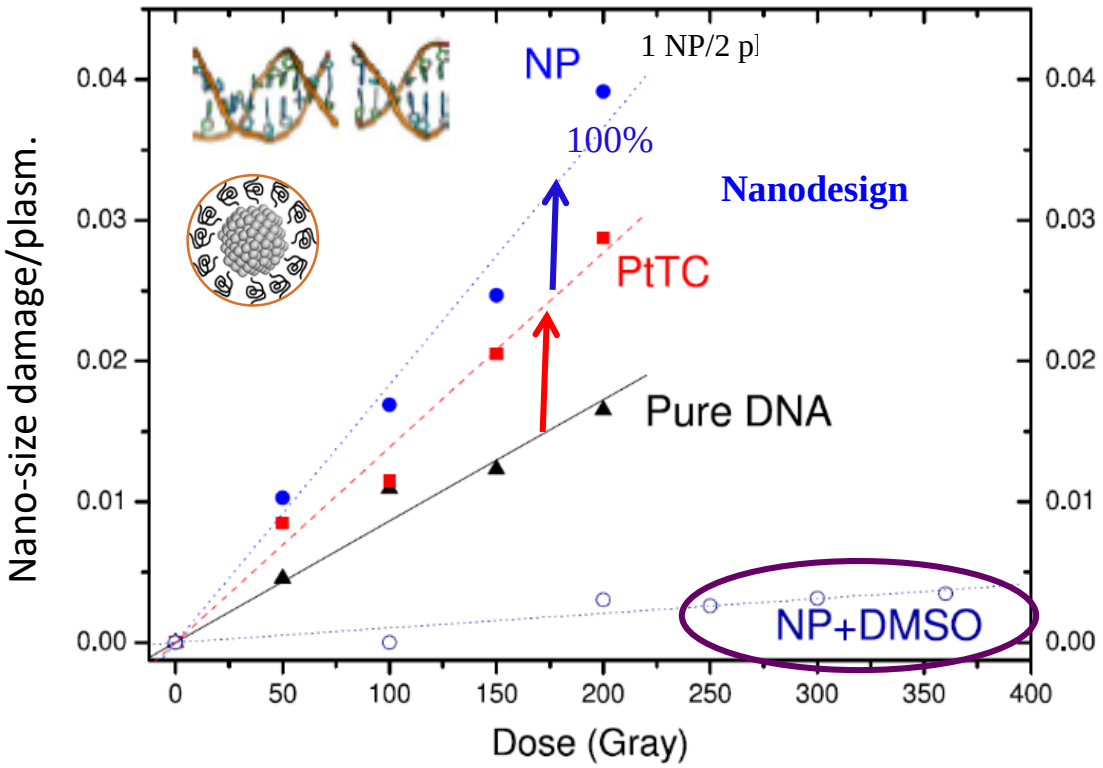
Double strand break (DSB)

> 2 nm damage

Nano-bio effects

Induction of nano-size biodamage

Irradiation C^{6+}



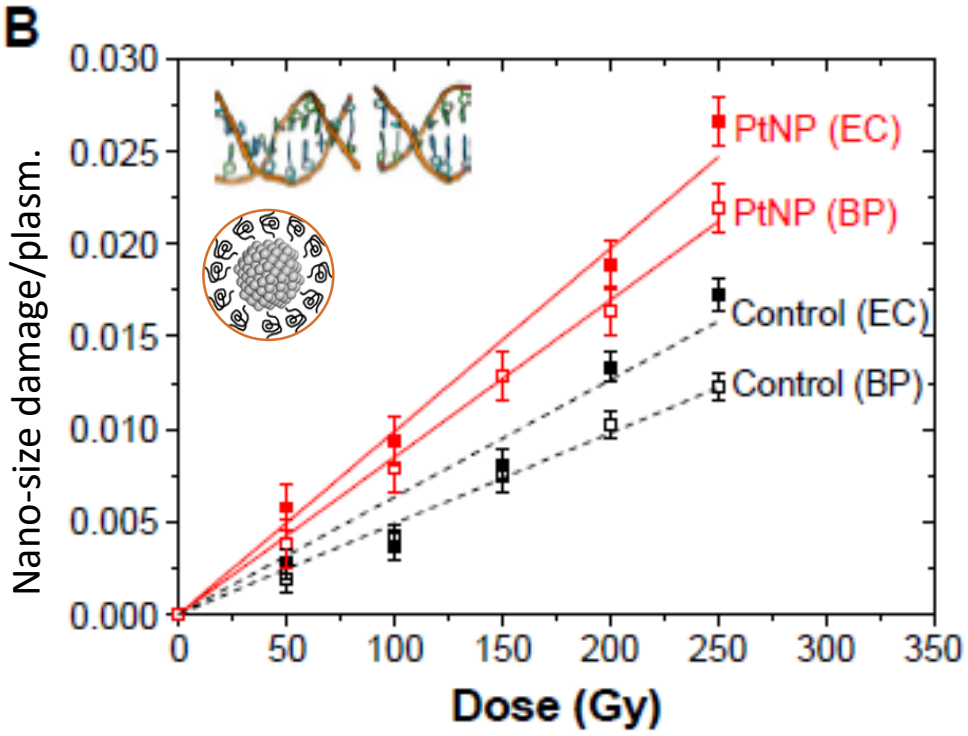
- Enhancement of radiation effect by NPs
- Effect of atom confinement (nano-design)
- Scavenging effect : major role of radicals

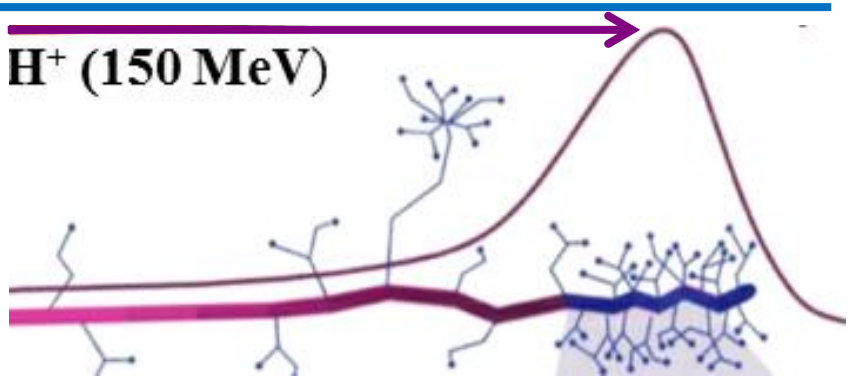
Porcel et al Nanotechnology 21, 85103 (2010)

Nano-bio effects

Enhancement effect along a proton track

Irradiation H^+





Amplifying factors

Entrance : 1.6

Bragg Peak : **1.9**

- Amplification effect **stronger at the end** of the track
- NPs activated by the **secondary e-** of the track

Schlathölter et al. International Journal of Nanomedicine 11, 1549-1556 (2016)

Nanoscale mechanisms ?

Elementary mechanisms

Time scale

10^{-15} s — **NP electronic activation**

10^{-13} s — **NP relaxation**

- *e^- emission (out)*
- *e- capture to neutralize the NP (in)*

10^{-12} s

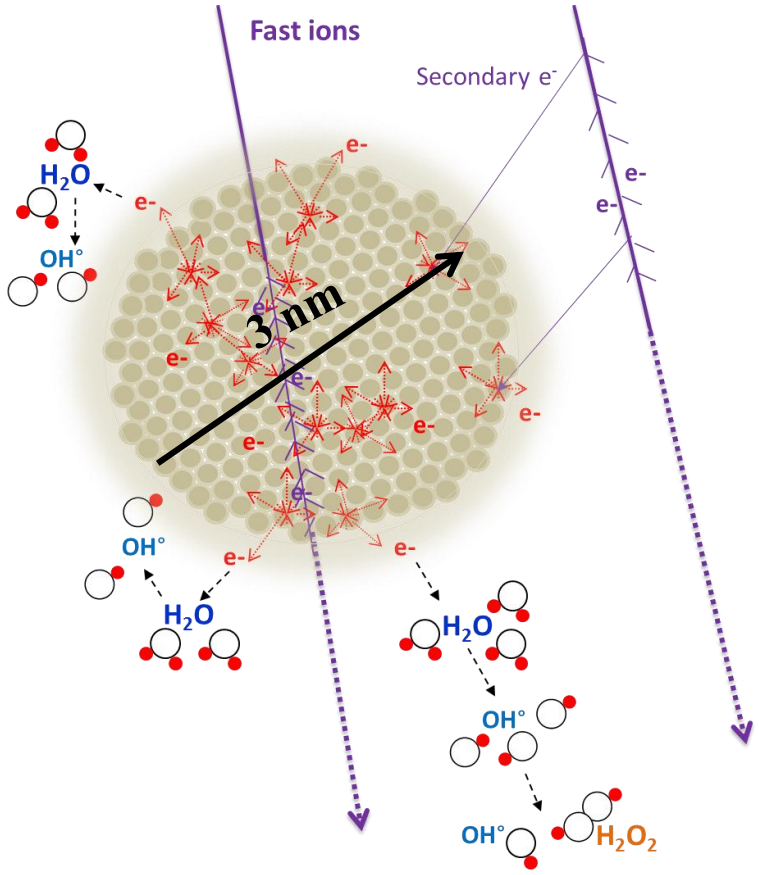
10^{-6} s — **Radicals clusters (ROS)**

10^{0} s

➢ *Reactive **nano**-clusters*

➔**Nanosize bio-damages**

➔**Cell death**



Porcel et al Nanotechnology 21, 85103 (2010)
Kobayashi et al Mutation Research 704, 123 (2010)

Nanoscale mechanisms ?

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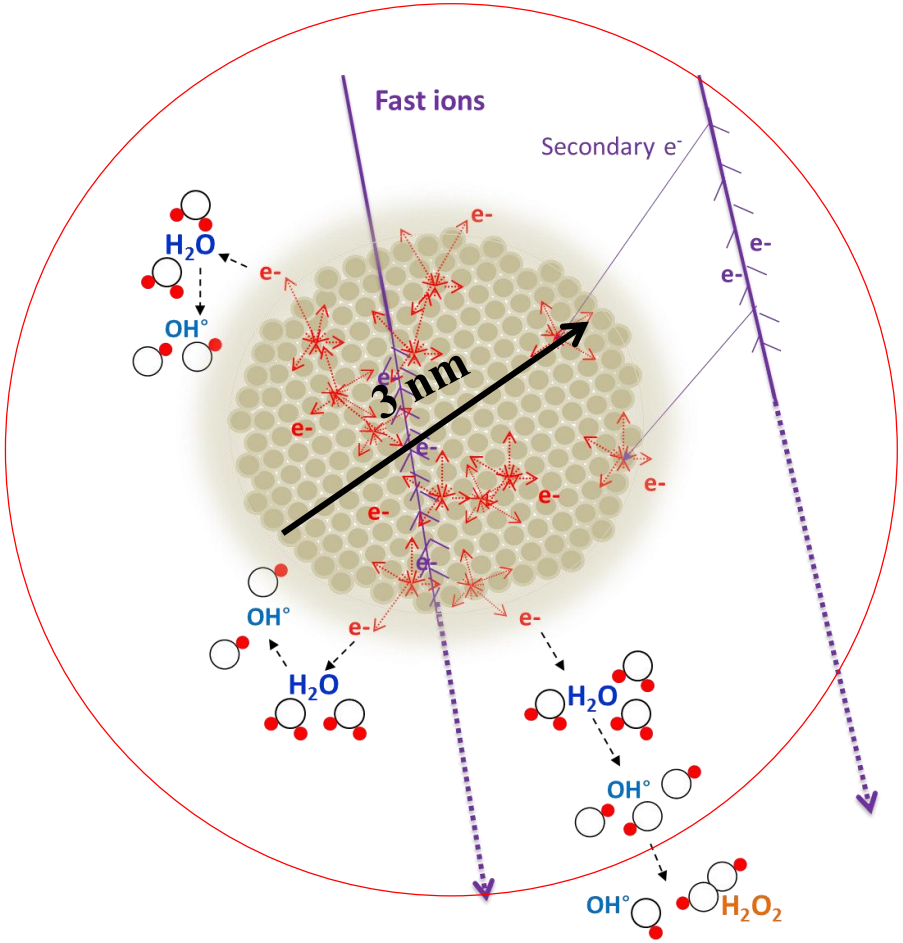
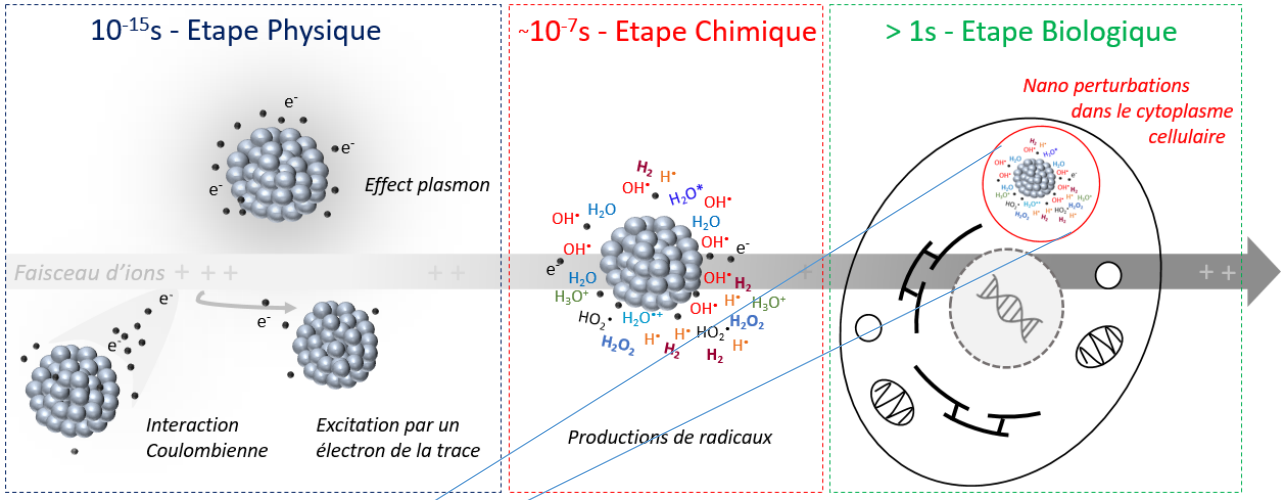
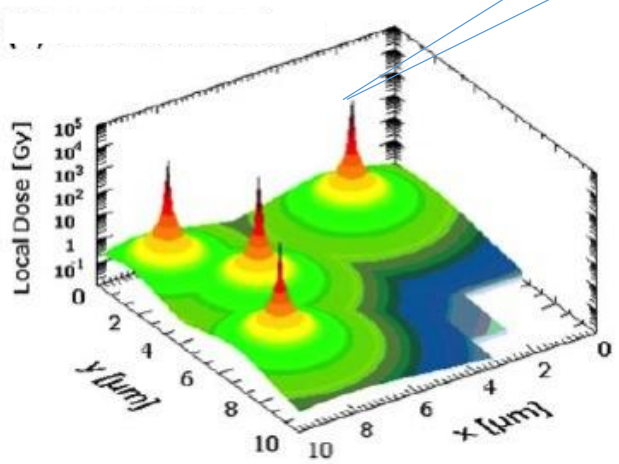


Confinement :
Nano-size perturbation

Porcel et al Nanotechnology 21, 85103 (2010)
Kobayashi et al Mutation Research 704, 123 (2010)

Nanoscale mechanisms





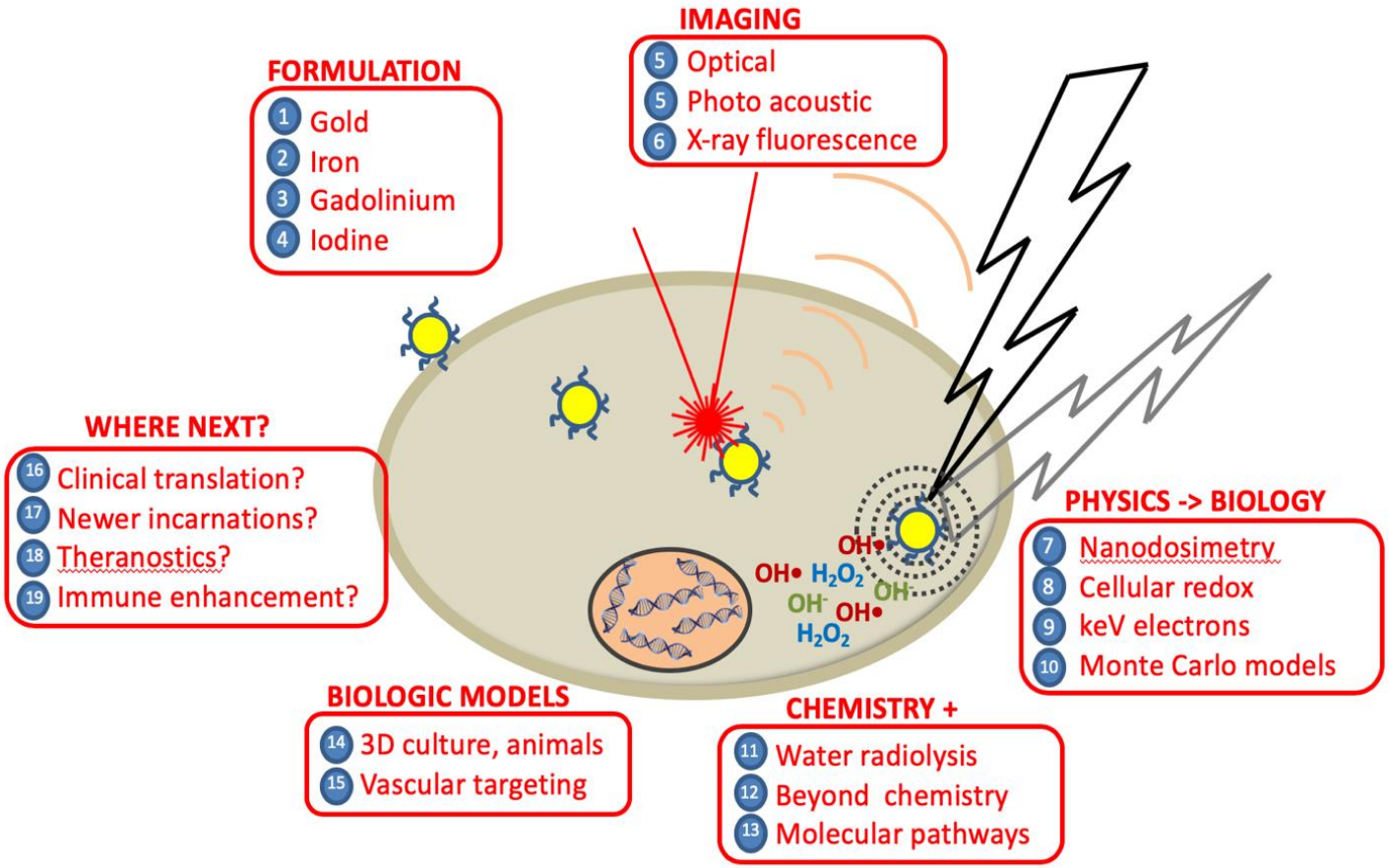
- NP effect = induction of local dose « spikes »
  - Confined nano-size perturbation
  - Same size as biomolecules

Local Effect Model

Mc Mahon et al Scientific Reports (2011)

SUMMARY

Multiparametric Effect



… prediction ?

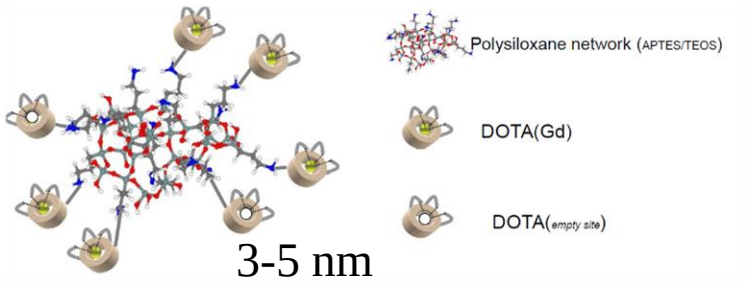
Schuemann et al. Phys Med Biol (2020)

NPs for radiotherapy / particle therapy ?

…towards prediction

GdBN @ medical carbon ions (HIMAC-NIRS)

Gadolinium-based NPs (GdBN)

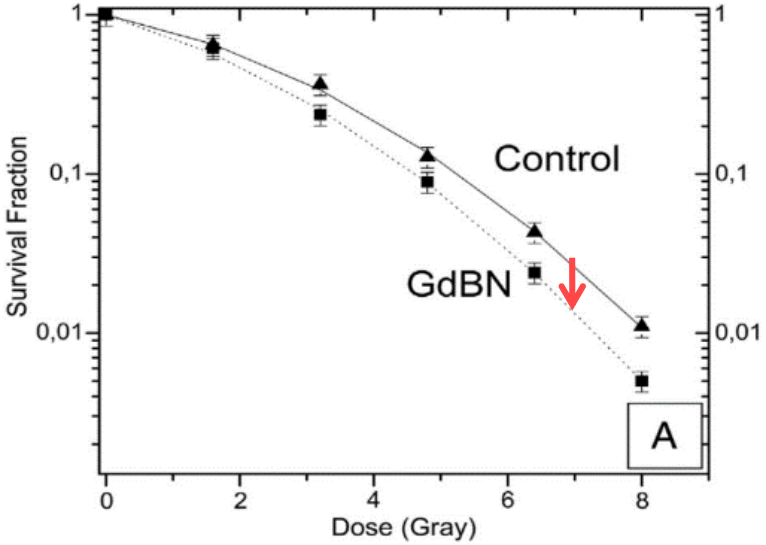


- ✓ MRI active
- ✓ High colloidal stability and freeze drying ability
- ✓ *In vivo*: rapid elimination by the kidneys, no evidence of toxicity

Coll. O. TILLEMENT
F.LUX (Lyon 1)
http://nhtheraguix.com/

	SER(SF 2Gy)
C $^{6+}$ (SOBP)	17%

Irradiation C^{6+} (entrance)/ CHO cells
Incubation : 1 mM Gd, 6h



Source	Sample	α	β
C $^{6+}$ (plateau)	Control	0.19	0.047
	NPs	0.27	0.049

- ➢ Effect (even low) with GdBNs
- ➢ Towards **theranostic** with carbon ion irradiation

Increase of directly lethal damages

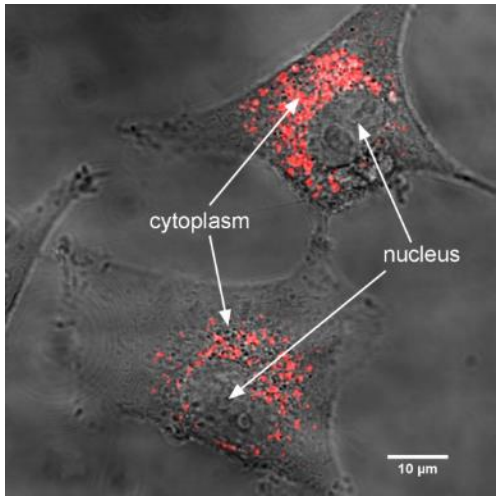
Porcel et al, Nanomedicine NMB 10:8 (2014)
Sancey et al BJR 87:1041 (2014)

Localization of NPs in the cell

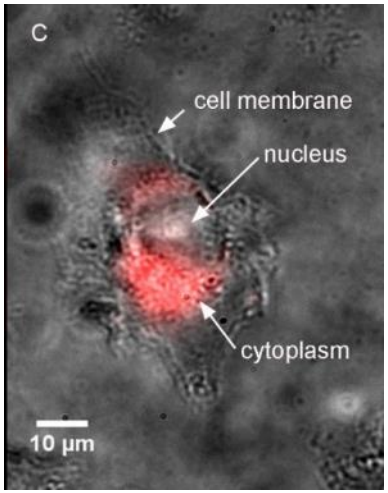
GdBN in U87 cells

Confocal microscopy

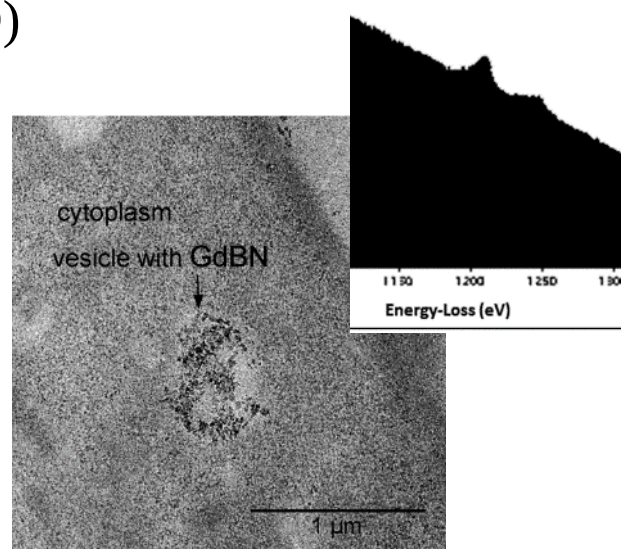
Cy5.5



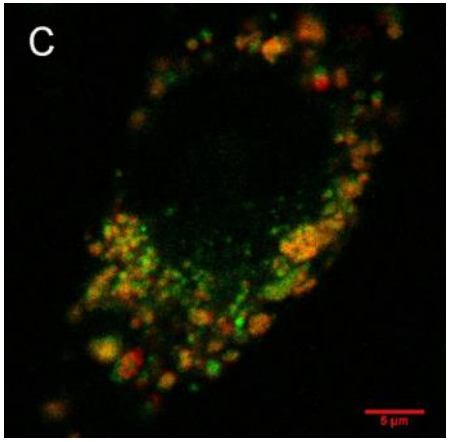
Native GdBN: SR-Deep UV microscopy (SOLEIL-DISCO)



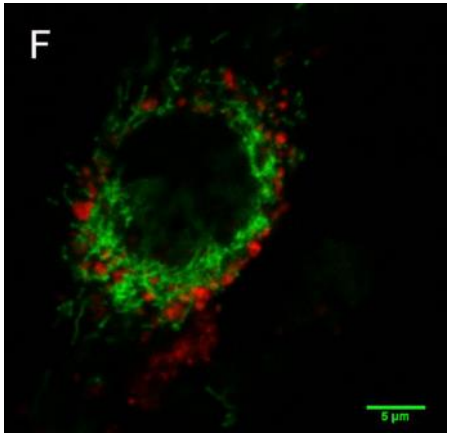
TEM/EELS



➢ Where in the cytoplasm ?



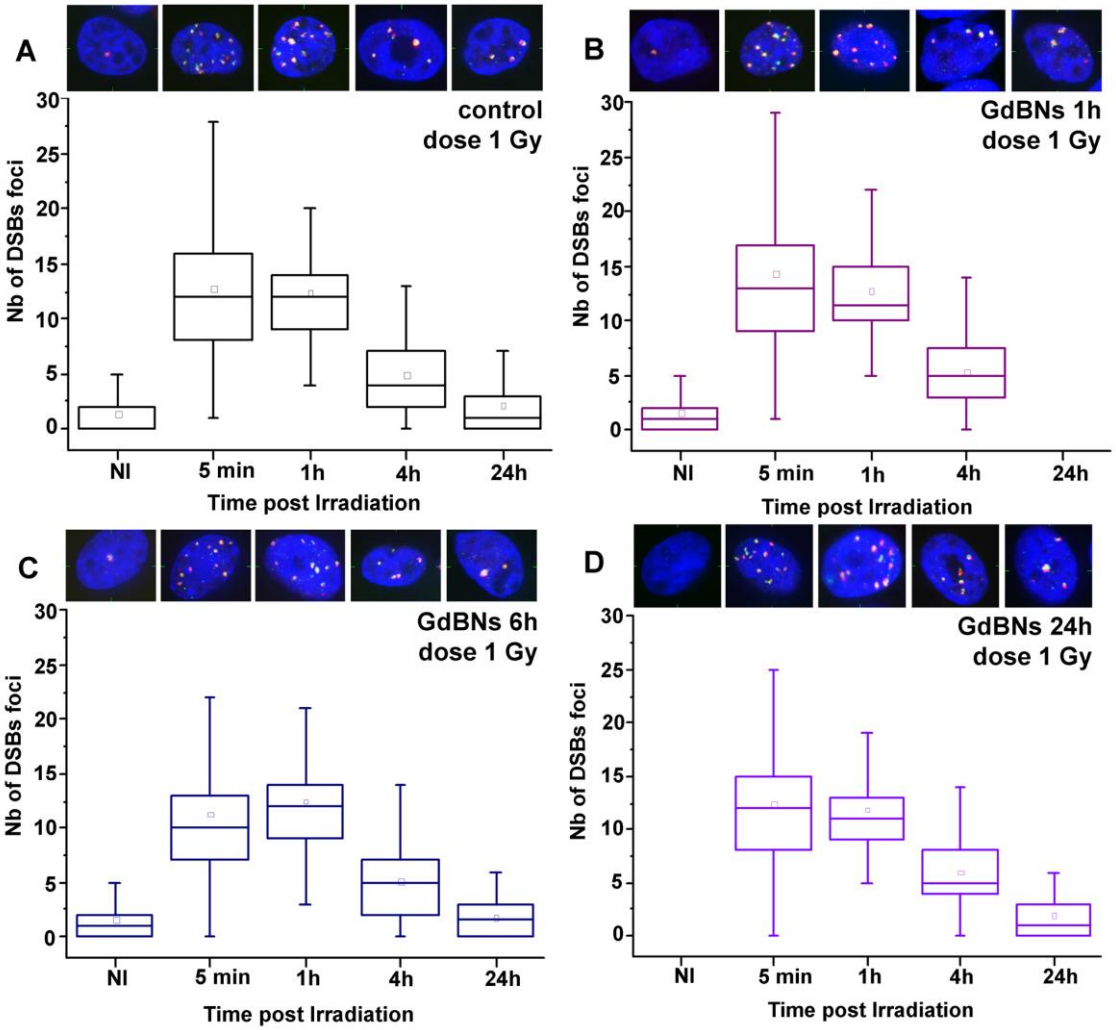
Co-localized with lysosomes

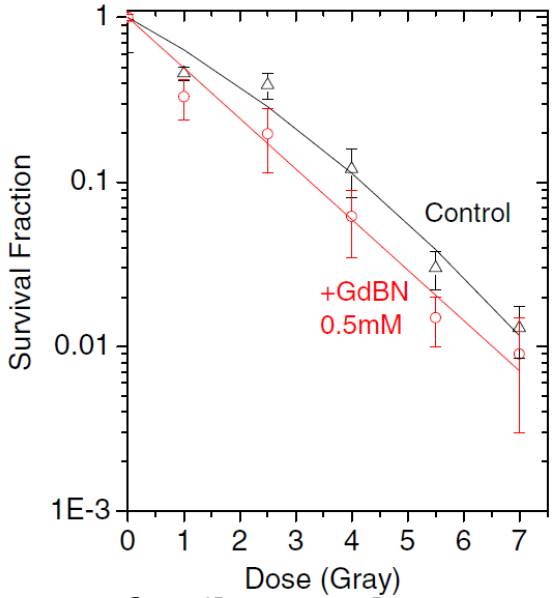


Not Co-localized with mitochondria

Stefancikova et al., Cancer Nanotechnology, 2015, 5:6

GdBN in U87 cells – γH2AX / 53BP1 Assay



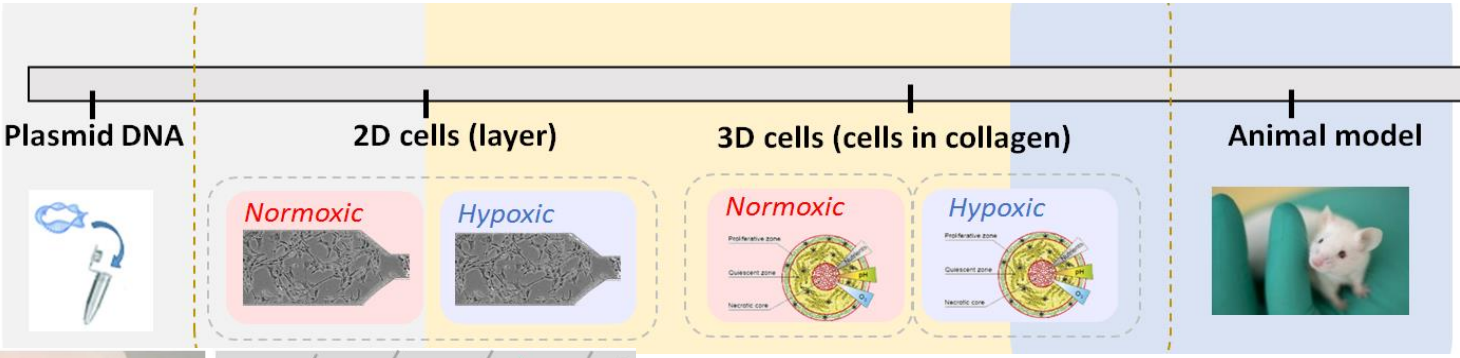


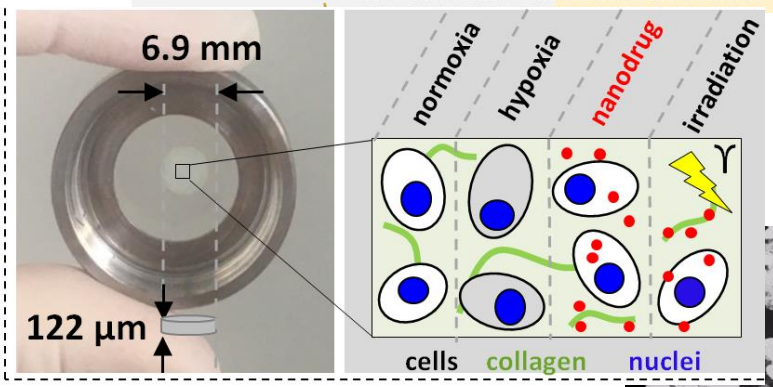
Stefancikova et al., Cancer Nanotechnology, 2015

- No evidence of nuclear damage
- Amplification of radiation effects due to other bio pathways

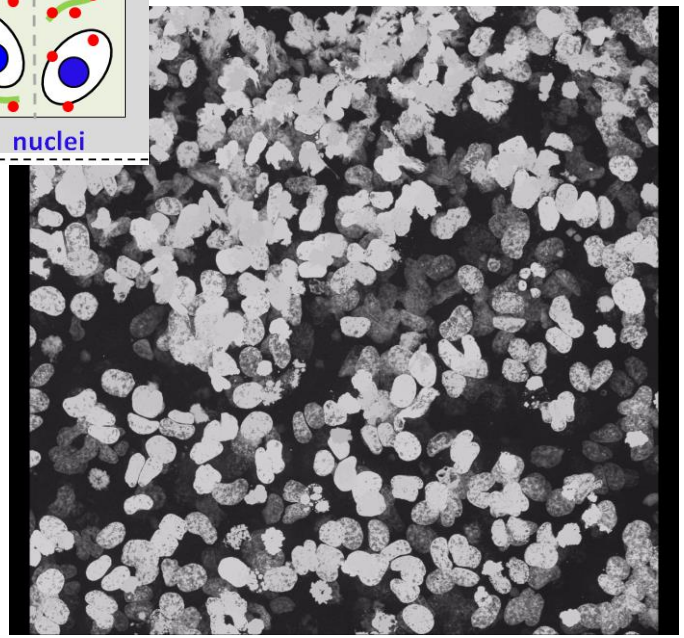
Stefancikova et al., J.Nanobiotechnology, 2016 in press

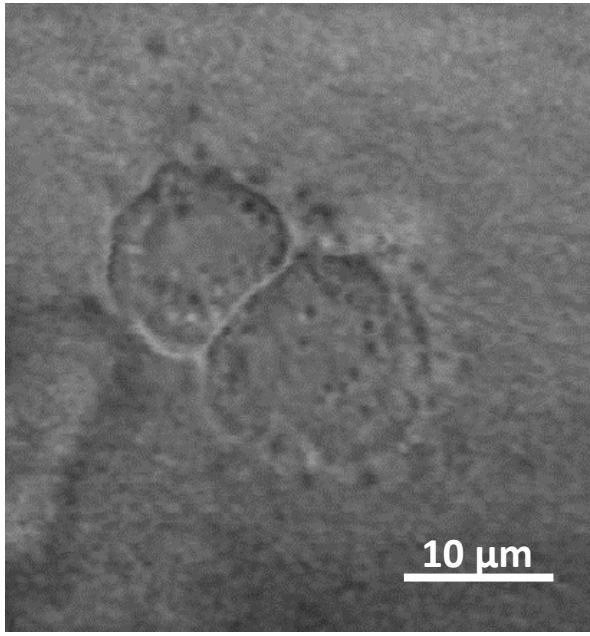
3D models





Maury P et al. *Evaluation of novel therapeutic strategies using a physiologically relevant 3D collagen-based model*. Frontiers in Bioengineering and Biotechnology





MICROSCOPY : Do the NPs penetrate into cell ?

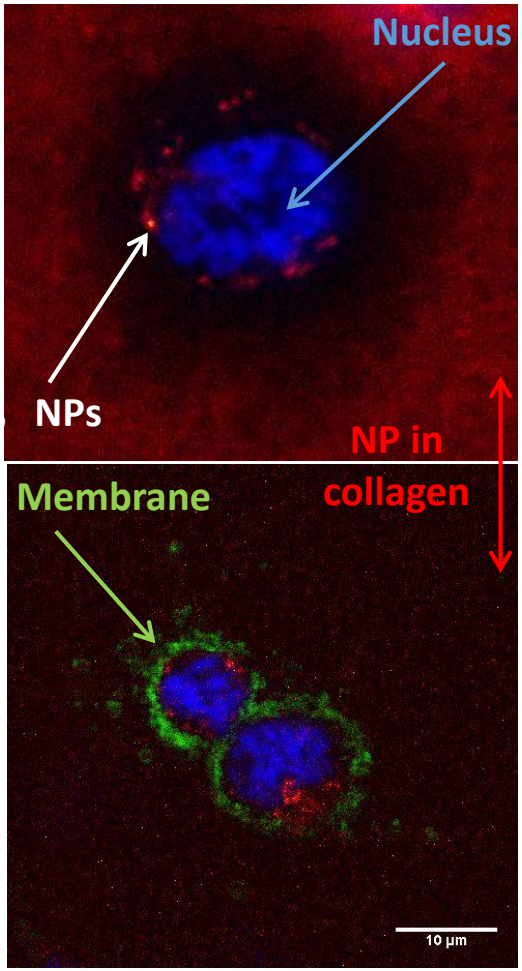
Ex: Gadolinium nanoparticles concentration: 0,5mmol/L - incubation time : 18h

Centre de Photonique Biomédicale

Confocal microscopy

UNIVERSITÉ PARIS SUD

- Conventional technic
- Limited anisotropic resolution

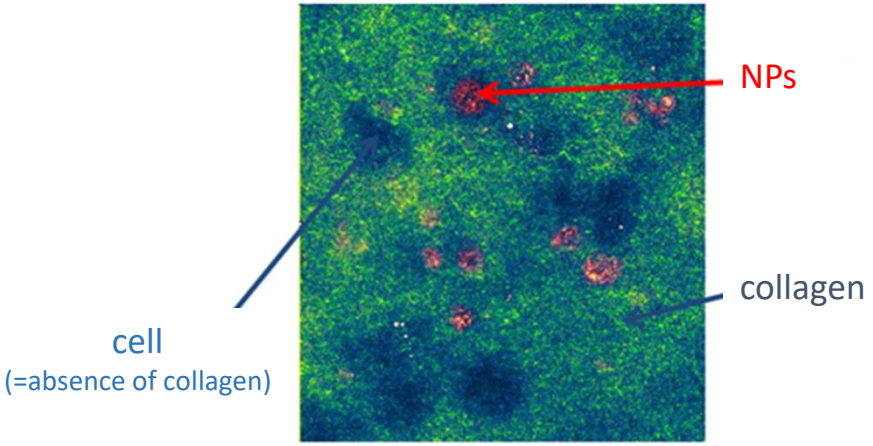


ÉCOLE POLYTECHNIQUE UNIVERSITÉ PARIS-SACLAY

Second Harmonic Generation Two-photons microscopy

LABORATOIRE D'OPTIQUE ET BIOSCIENCES

- Enable to image the collagen without any labelling and with a better resolution (isotropic)
- Better contrast collagen/cells

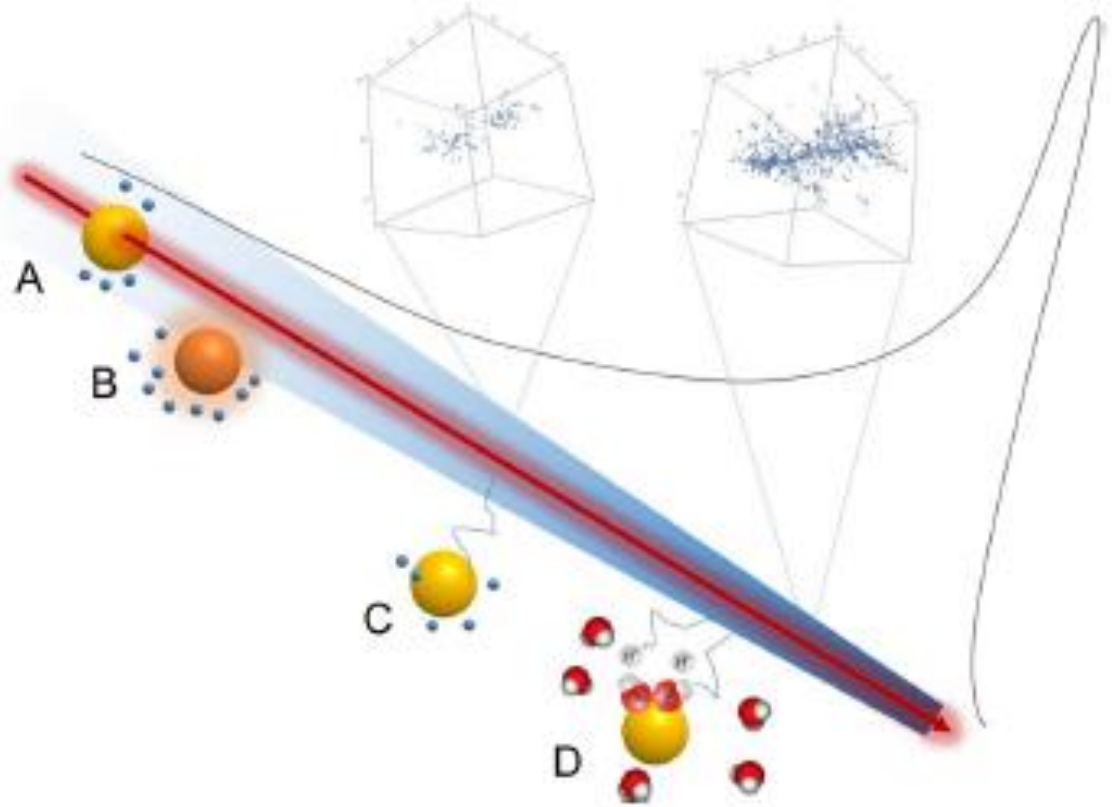


- NPs in the collagen and inside the cytoplasm of the cells
- Penetration through collagen in the whole sample
- Heterogeneous distribution

SUMMARY

NPs amplify the effects of fast ions

- Nano-size e- bursts and ·OH clusters in the cytoplasm
- C^{6+} + NPs > gamma + NPs
- RBE increased at the bragg peak



Lacombe et al. Cancer Nano (2017)
Kuncic & Lacombe 2018 Phys. Med. Biol. 63

NP development

Platinum NPs : Preclinical studies

New particles

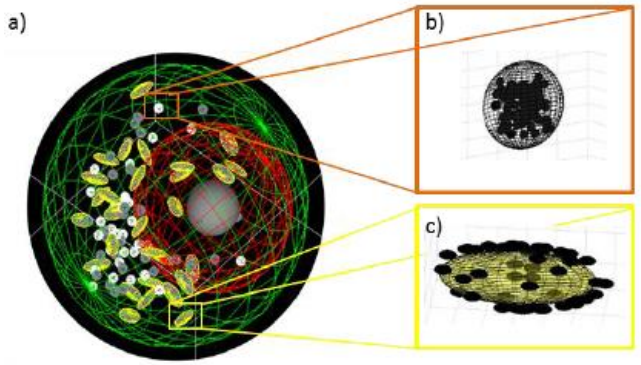
3D models…towards prediction

Hypoxia studies

Prediction …. IA

Pauline MAURY
Charles BOSSON

- Realistic cell geometry to better understand particle interactions leading to the microscopic dose enhancement and possible induced damages



- INTERNALIZATION
- LOCALISATION OF NPS
- QUANTIFICATION OF NPS

Aknowledgments

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