AGuIX®

Ultra-small Gadolinium based particles

Preclinical multimodal nanoprobes

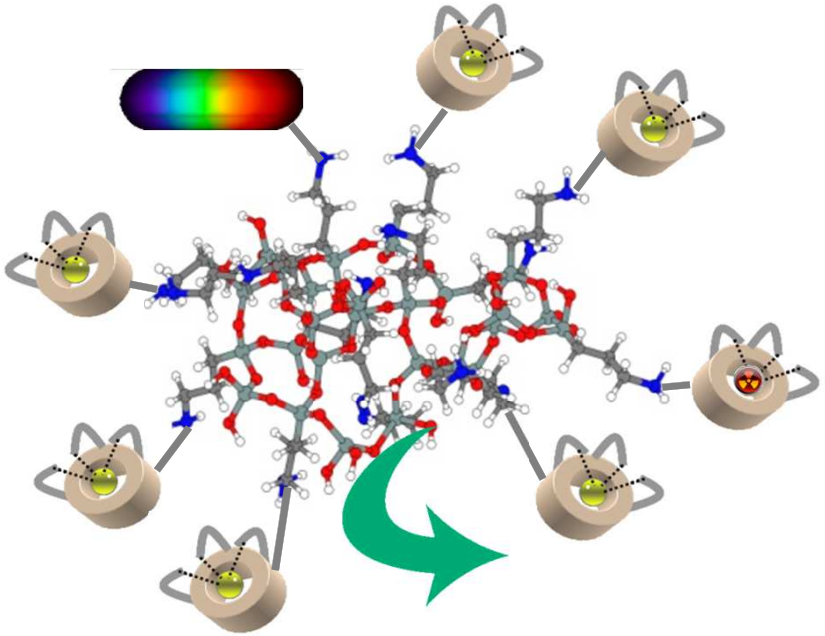
Theranostic drugs

Olivier TILLEMENT

Université Claude Bernard – Lyon 1

GDRs Lyon décembre 2012

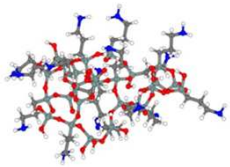
Multimodal Gadolinium-based Hybrid Nanoparticles



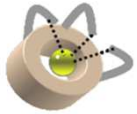
Ultra-small Size : 3-5 nm

High colloïdal stability

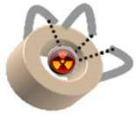
Small enough for Renal elimination



Polysiloxane network



DOTA(Gd)



DOTA(***radioactive species***)

NIR fluorophore

Functionalization

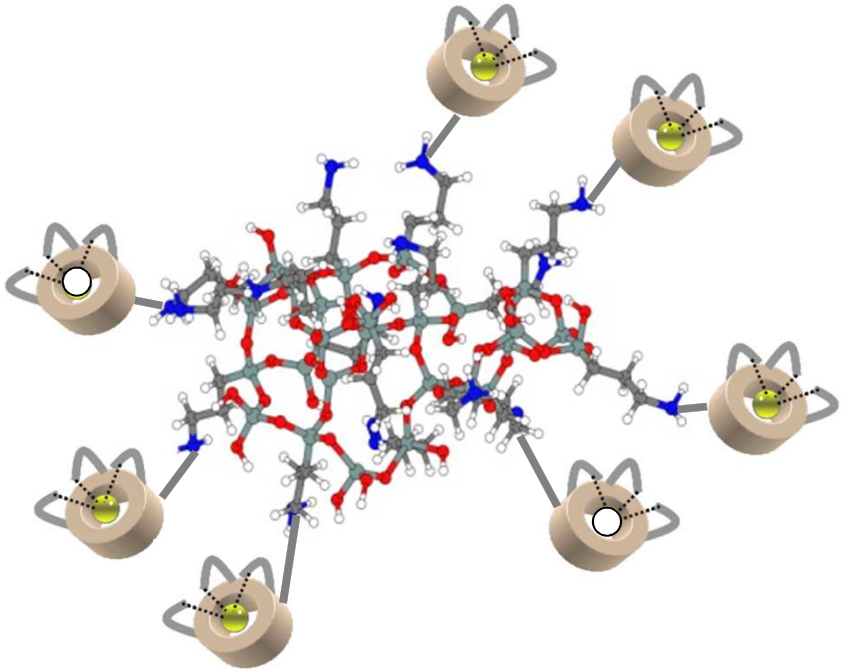
by targeting ligands

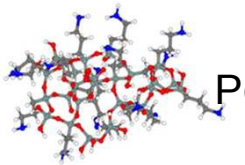
Patent : 1053389 & F. Lux et al., Angew. Chem. Int. Ed., ***2011,*** *50, 12299*

Principle of AGuIX®

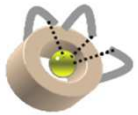
Polysiloxane Skeleton (with amino functions)
grafted with high chelating species (DOTAGA / DOTA / DTPA / NODA…)
including some gadolinium ions and some empty sites

Average simplified formula : **$Si_{50}O_xDOTA_{10}$ (Gd)$DOTA_5$(*empty*)**





Polysiloxane network (APTES/TEOS)



DOTA(Gd)

DOTA(*empty site*)

Size **: 3-5 nm – 5/10 kDa**

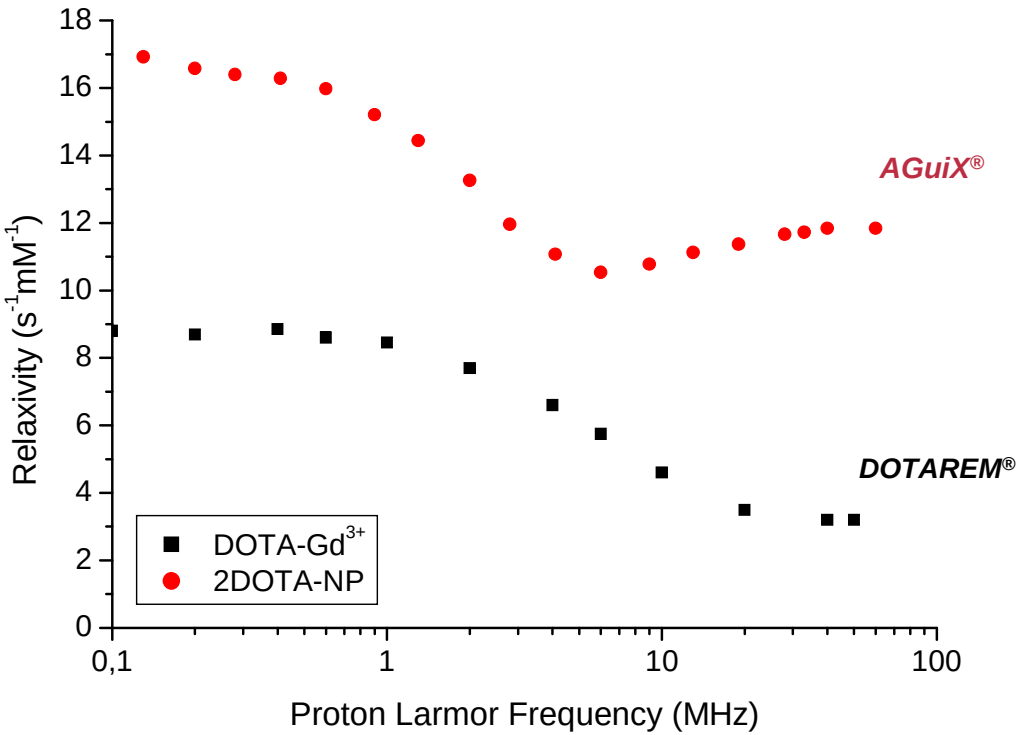
High colloidal stability and freeze drying ability

AGuIX® Average simplified formula : **$Si_{50}O_xDOTA_{10}$ (Gd)$DOTA_5$(*empty*)**

Gadolinium based particles

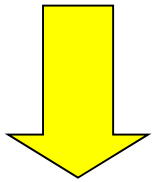
MRI probes

relaxivity r_1/Gd = 11,4 s^{-1}.$mmol^{-1}$ (60 MHz)



MRI particles and…

$Si_{50}O_xDOTA_{10}$ (Gd)$DOTA_5$(*empty*)



Different utilizations of the empty chelating sites

Increase the Gd content by particles
(high doping in Gd: **20% < Gd/Si < 40%)**

Chelation of a radioactive species
(☢ lanthanides, transition metals, Ga,…)

$Si_{50}O_xDOTA_{10}$ (Gd)$DOTA_n$(☢)

Multimodality
Increase the chelating ability and efficiency (several available chelating species by particles)
Increase the number of active ions by particles (targeting biomolecules)
Induce “direct Auger radiosensitizing” near the active radioactive ions (increase the local efficiency)

AGuIX® radioactive particles

$Si_{50}O_xDOTA_{5-15}$ (Gd)$DOTA_n$(☢)

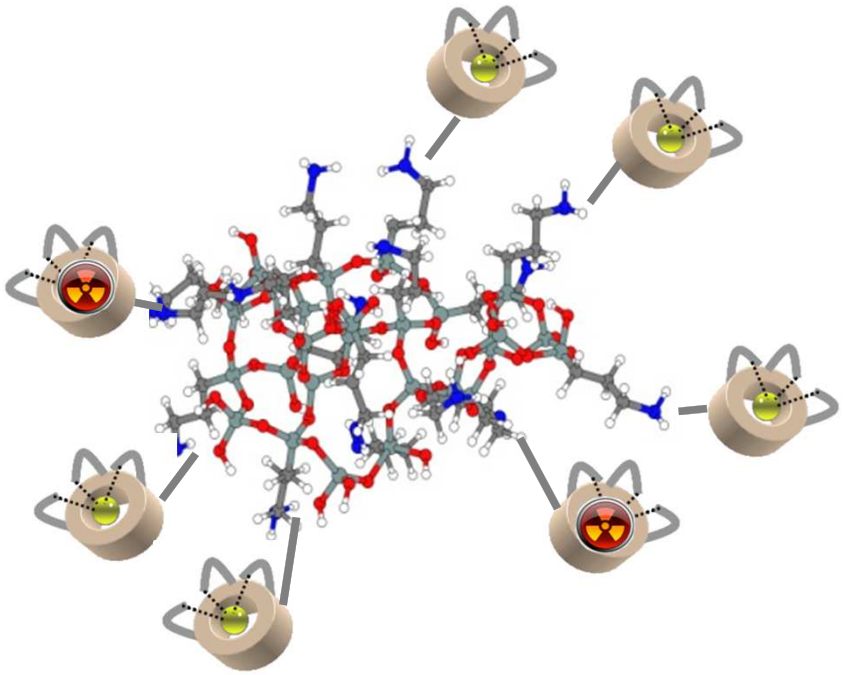
Multimodal imaging

MRI with Scintigraphy : SPECT/PET (In^{3+}, Ga^{3+}, Cu^{2+}, …)

Therapy

brachytherapy (Lu^{3+}, Ho^{3+},Y^{3+}, Ga^{3+}, …)

Theragnostic particles



DOTA(Gd)

DOTA(☢)

☢ ***Radioactive ions …***

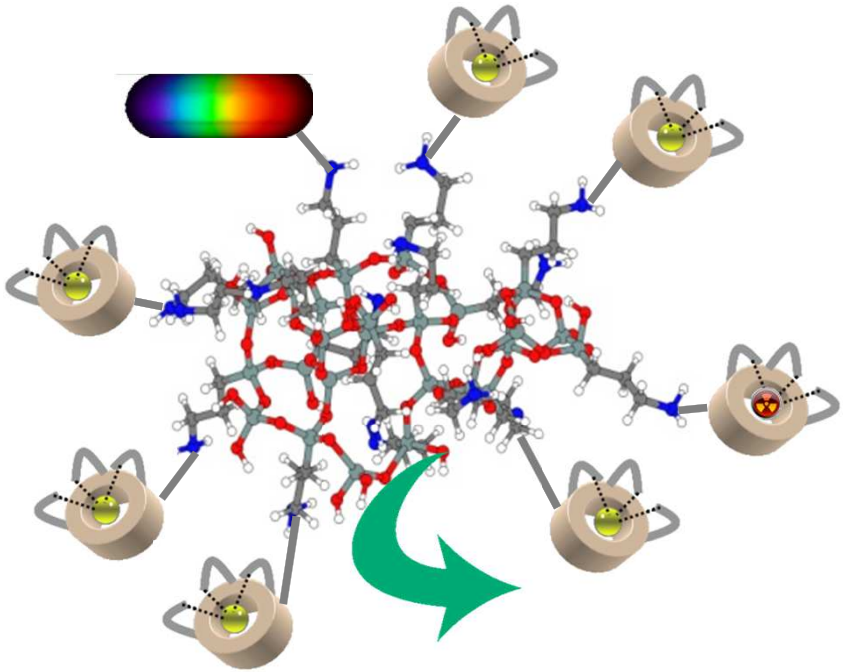
Further-Functionalizations

Polysiloxane network via NH2 groups

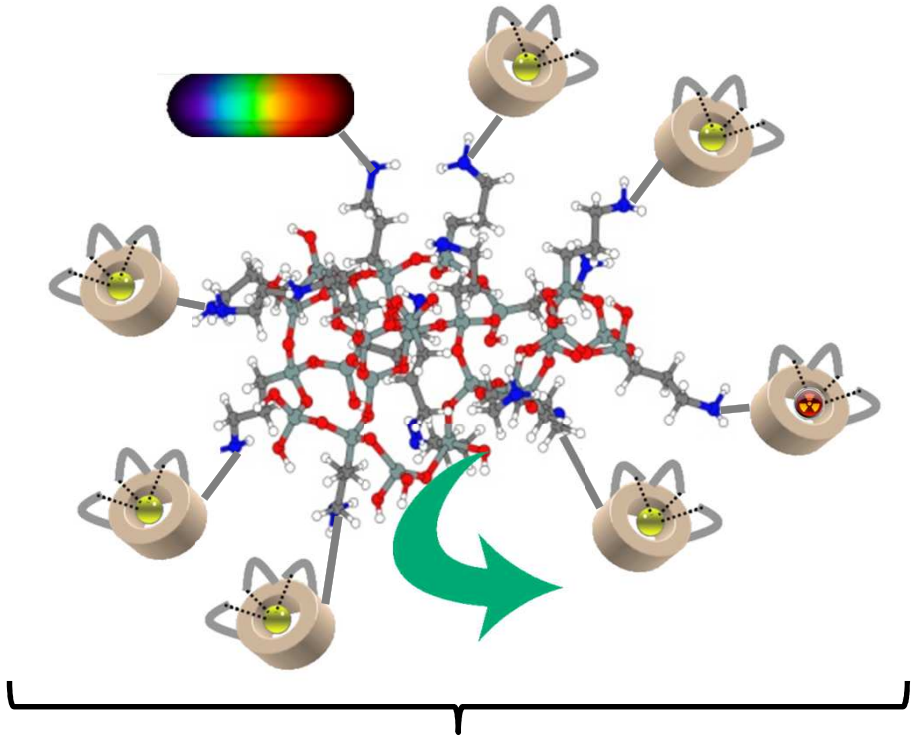
Chelating molecules (remaining empty) via COOH groups

Organic Dye : NIR fluorescent (Cy 5, Alexa), FITC, …

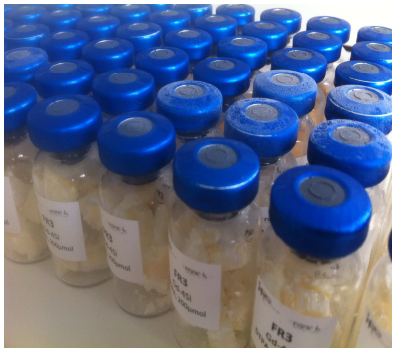
Functionalization by targeting ligands (Small peptides, antibodies, …)

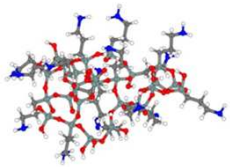


“Full options” multimodal AGuIX®

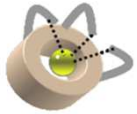


3-5 nm

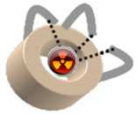




Polysiloxane network



DOTA(Gd)



DOTA(***radioactive species***)

NIR fluorophore

Functionalization

by targeting ligands

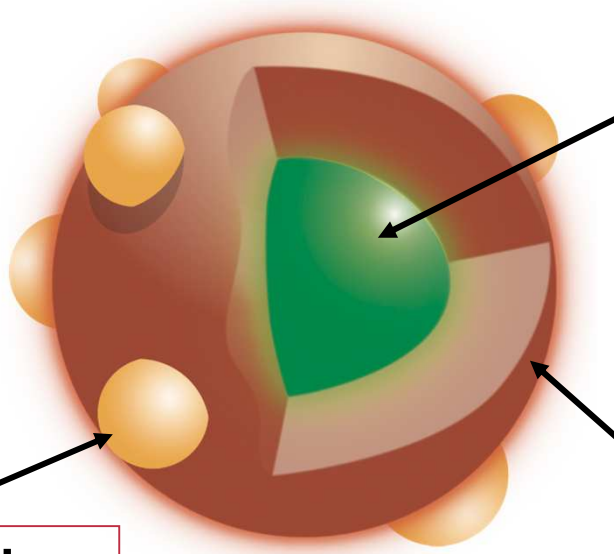
Few words about the

synthesis and fabrication

Top down process

applied on Nano-hybrid particles

$Gd_2O_3@SiO_x$

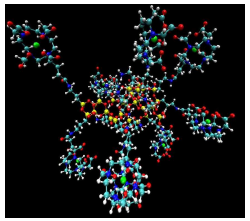


Core oxide
Lanthanide Sesquioxide
Gd_2O_3

Hybrid – shell
(*polysiloxane shell*)

Organic functionalization
with chelating species…

Core-shell synthesis : Roux *et al*, *J. Am. Chem. Soc.*, **2007,** *16,* 5076



AGuIX®

Synthesis protocol and material description

Synthesis of the lanthanide oxide core

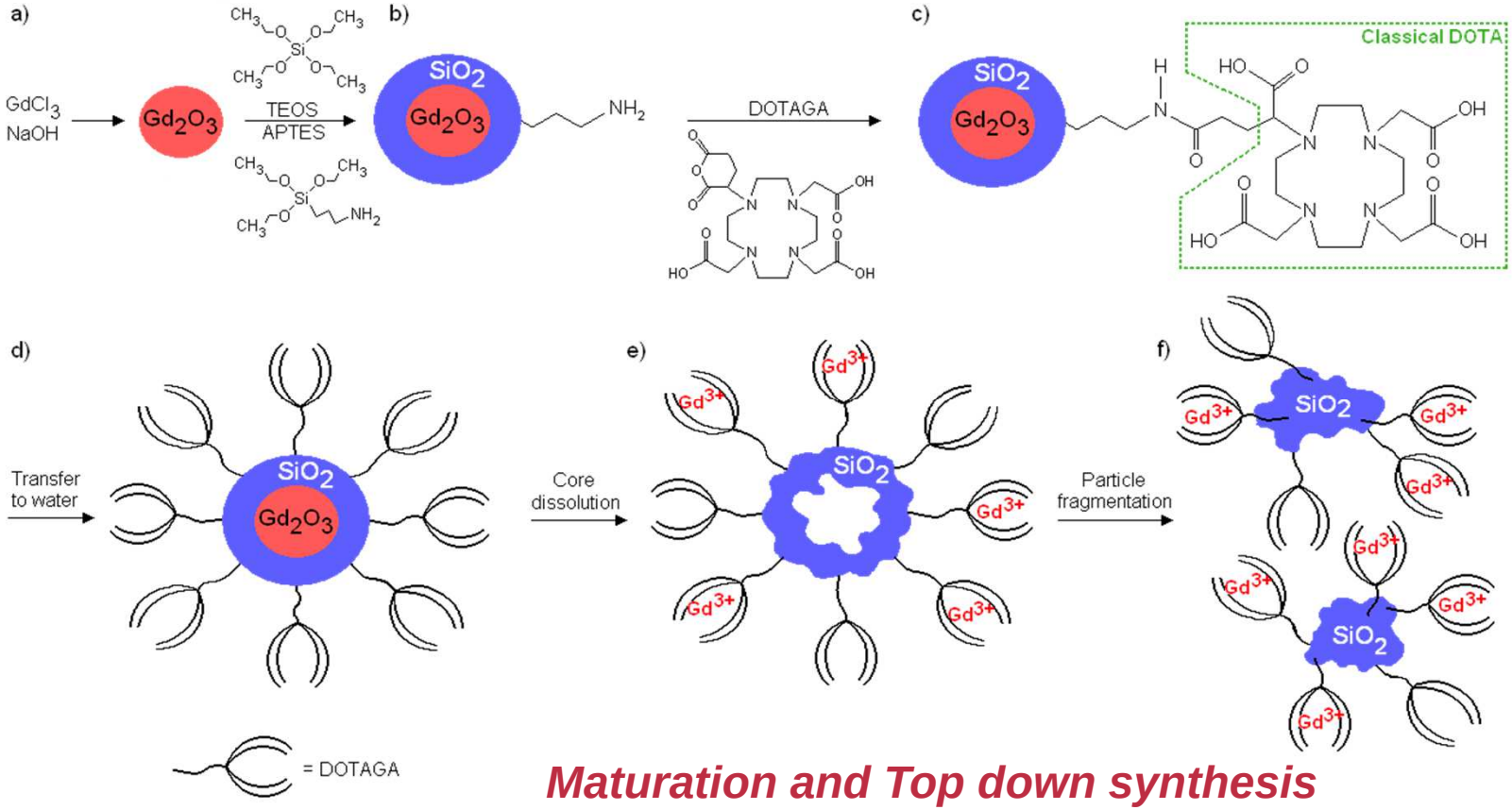
Polyol DEG: $GdCl_3$ + NaOH

Synthesis of the polysiloxane shell

Sol Gel: TEOS + APTES

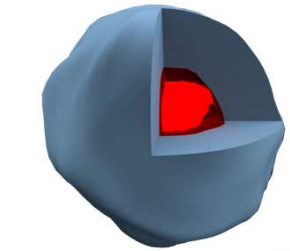
Grafting of hydrophilic Chelating Molecules

DTPABA or DOTAGA

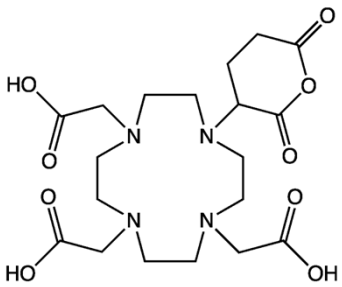


Maturation and Top down synthesis

Assembly with DOTAGA

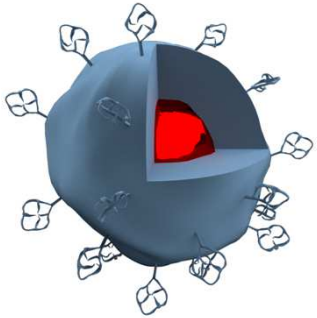


nano-h
Value-Added Nanotechnology



CheMatech
macrocycle design technologies

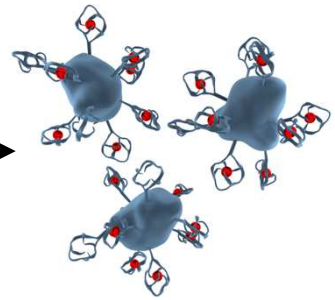
Core-shells
with DOTAGA



- Transfer to water
- Filtration
- Maturation

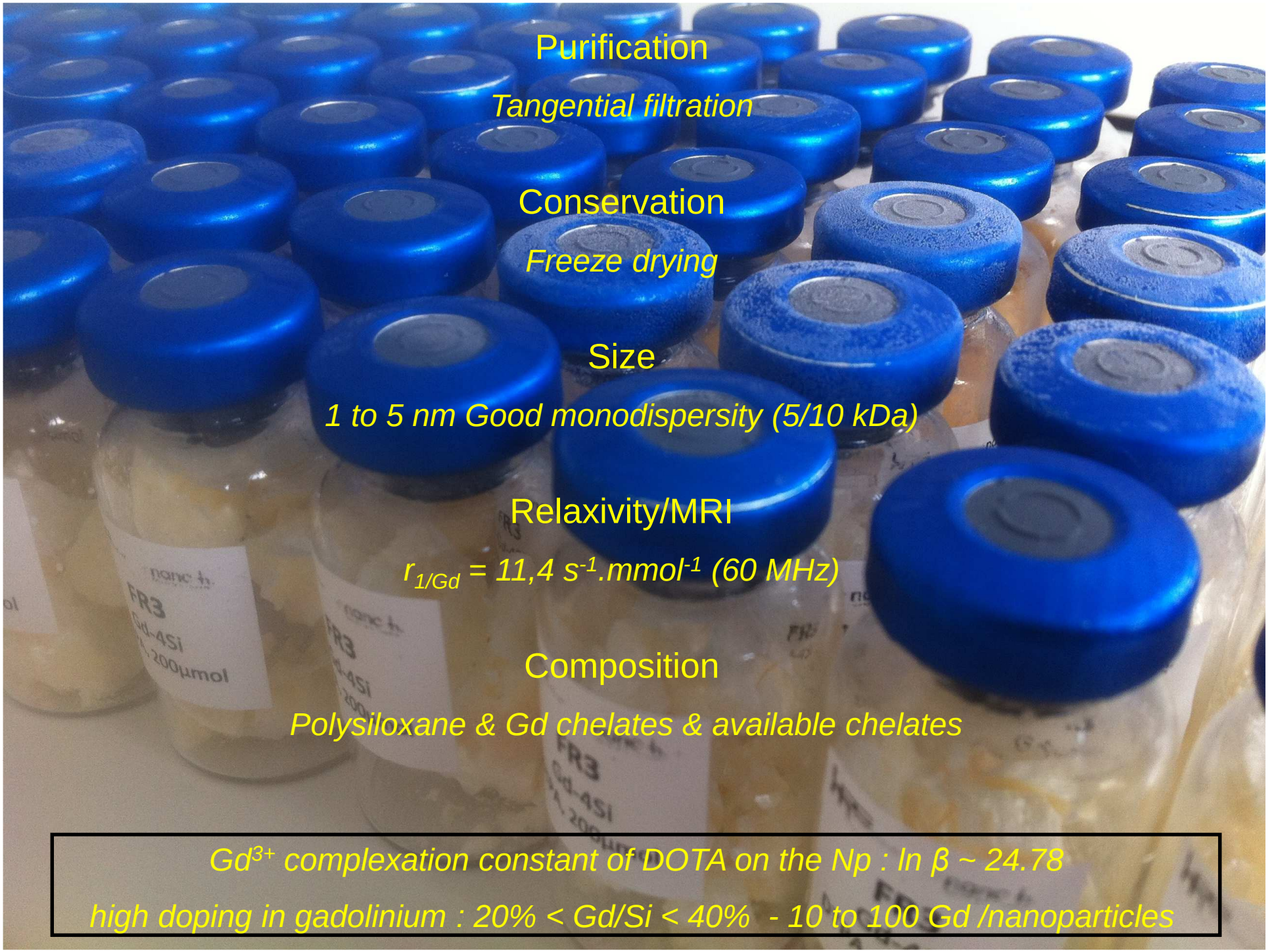
- Tangential flow filtration

AGuIX



Objectif…

→ Purification in GMP conditions



Purification

Tangential filtration

Conservation

Freeze drying

Size

1 to 5 nm Good monodispersity (5/10 kDa)

Relaxivity/MRI

$r_{1/Gd} = 11,4\ s^{-1}.mmol^{-1}$ *(60 MHz)*

Composition

Polysiloxane & Gd chelates & available chelates

Gd^{3+} complexation constant of DOTA on the Np : ln β ~ 24.78

high doping in gadolinium : 20% < Gd/Si < 40% - 10 to 100 Gd /nanoparticles

AGuIX®

Preclinical Multimodal probes

Imaging properties

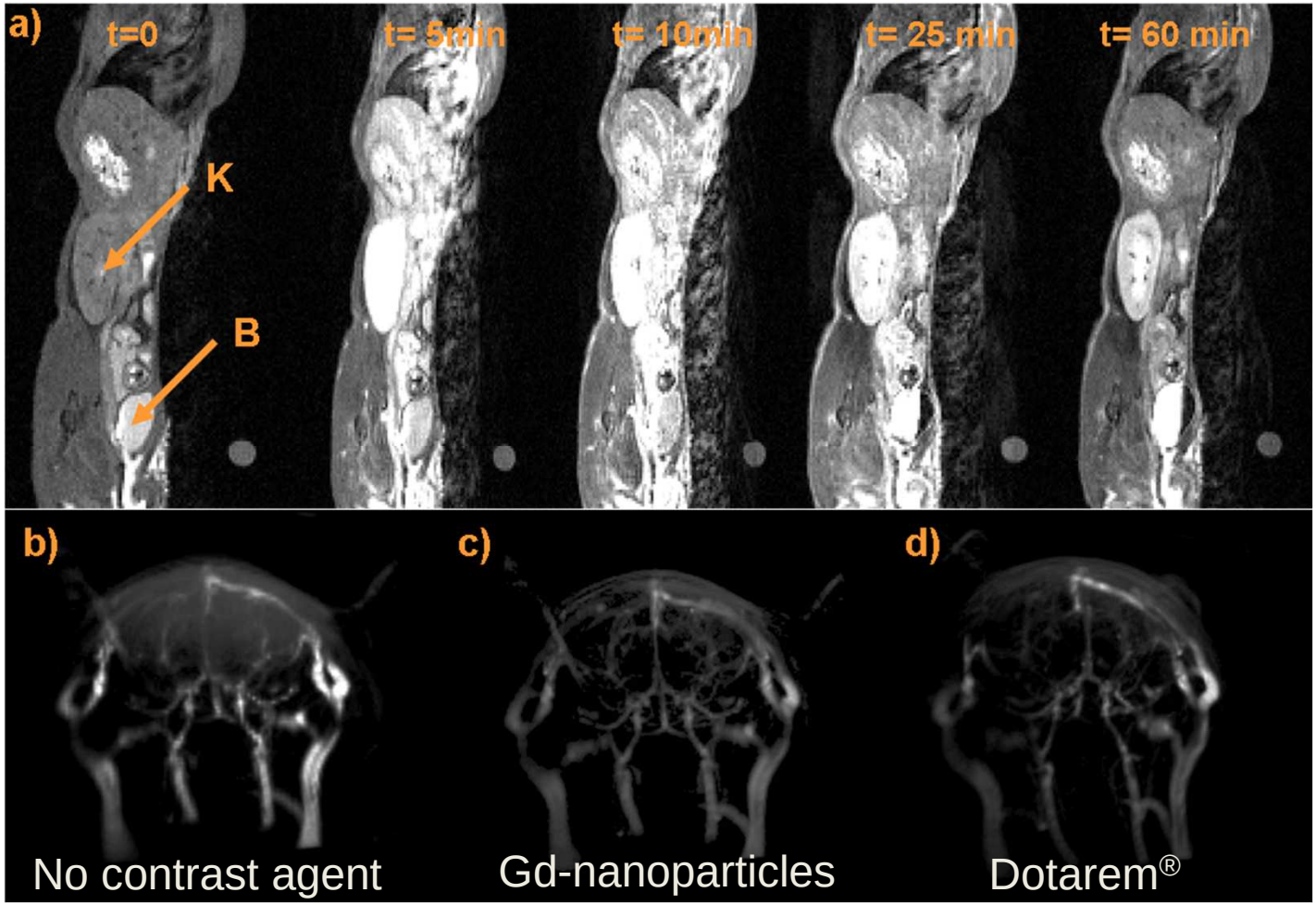
MRI

SPECT/PET

Fluorescence

X-ray tomography

MRI contrast agent



Male c57Bl/6J mouse, Injection: 80 µL at 40 mM in Gd

T1-Weighted images of a slice including a kidney (K) and the bladder (B) before and after injection

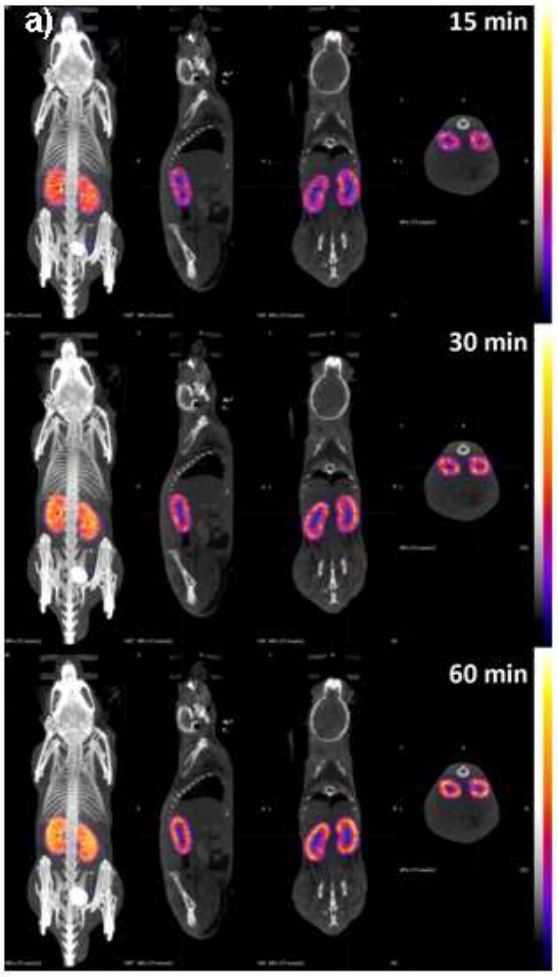
T1-Weighted images of the brain with no contrats agent (b), Gd-Nanoparticles (c) and Dotarem® (d)

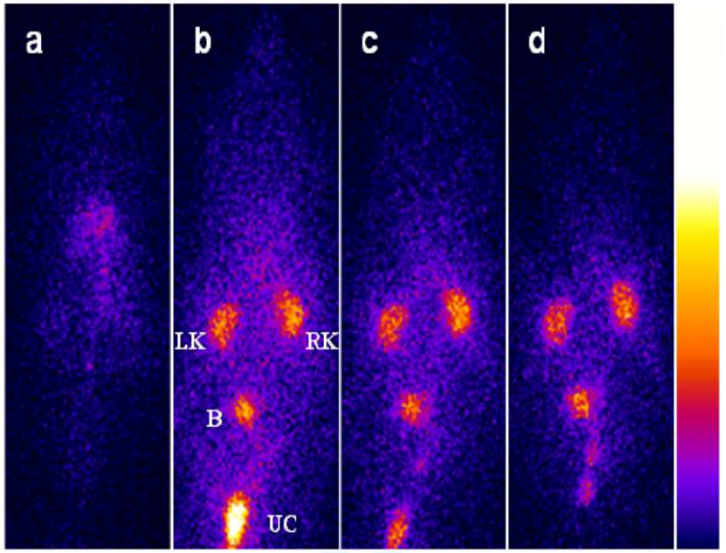
Efficient MRI contrast agent

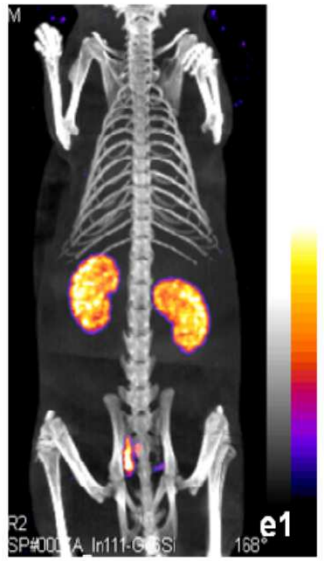
Collaboration V. Stupar, C. Rémy, E. Barbier (GIN)

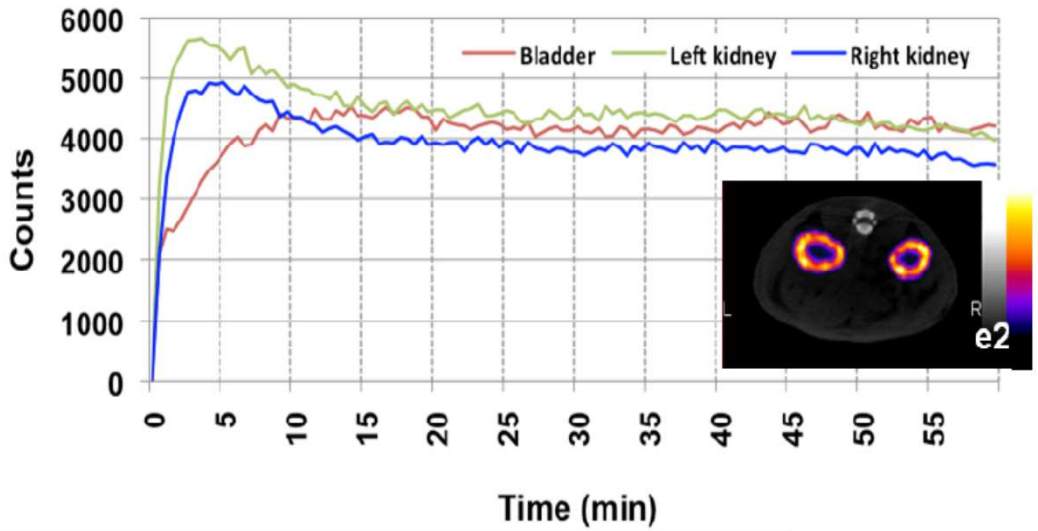
SPECT/CT imaging

Gd-Nanoparticles injected after chelation of ^{111}In









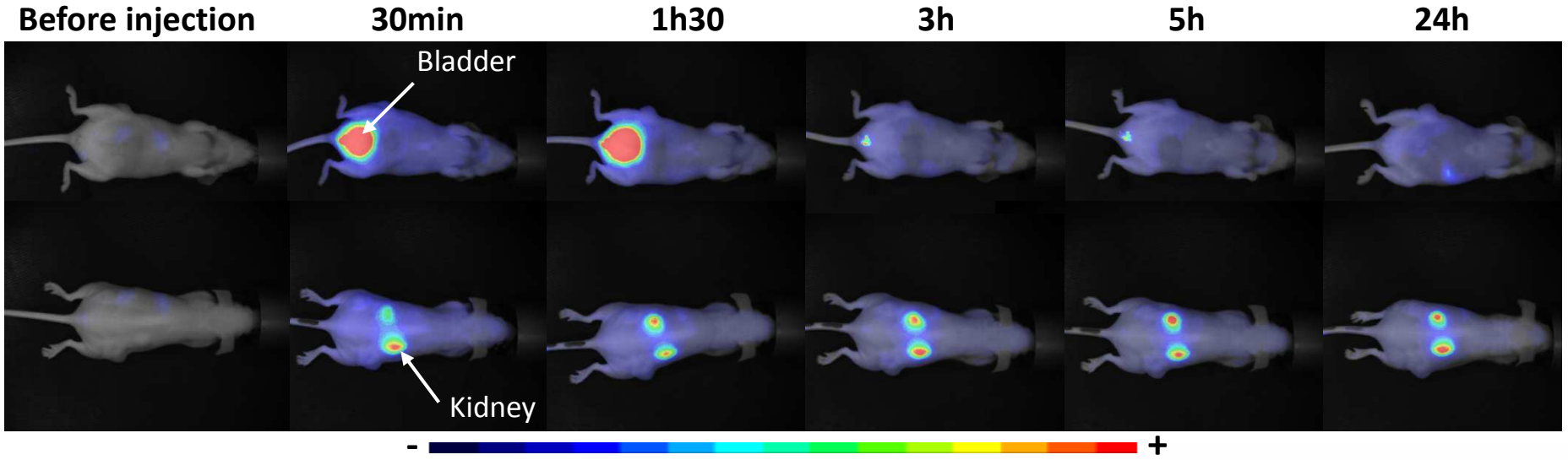
Interesting biodistribution, removed by renal excretion

Biodistribution Study of Nanometric Hybrid Gadolinium Oxide Particles as a Multimodal SPECT/MR/Optical Imaging and Theragnostic Agent,

Kryza et al., BIOCONJUGATE CHEMISTRY **Volume:** 22 **Issue:** 6 **Pages:** 1145-1152 **Published:** 2011

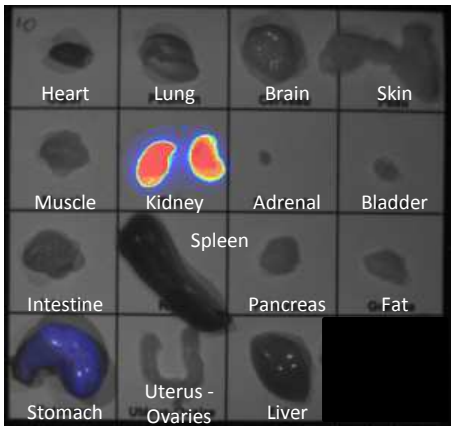
Fluorescence imaging

Cyanine 5.5 grafted to the amino functions of the polysiloxane shell

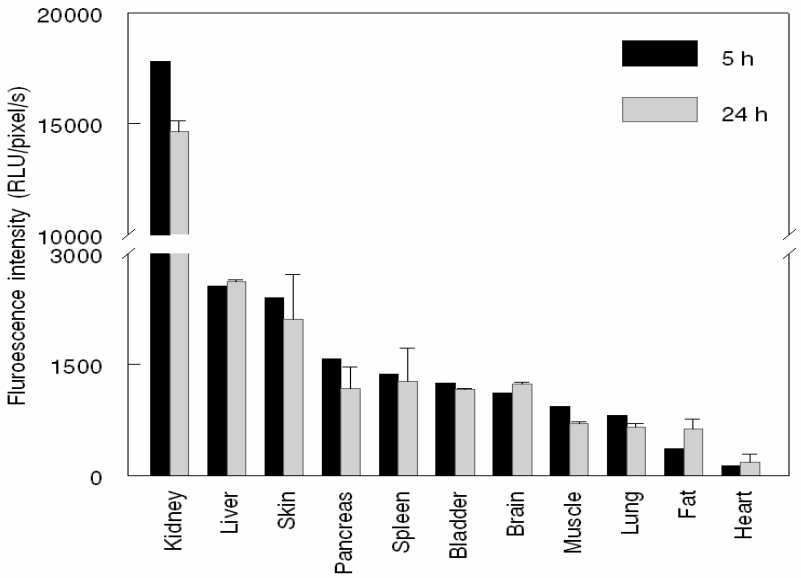


Organs

24h after injection

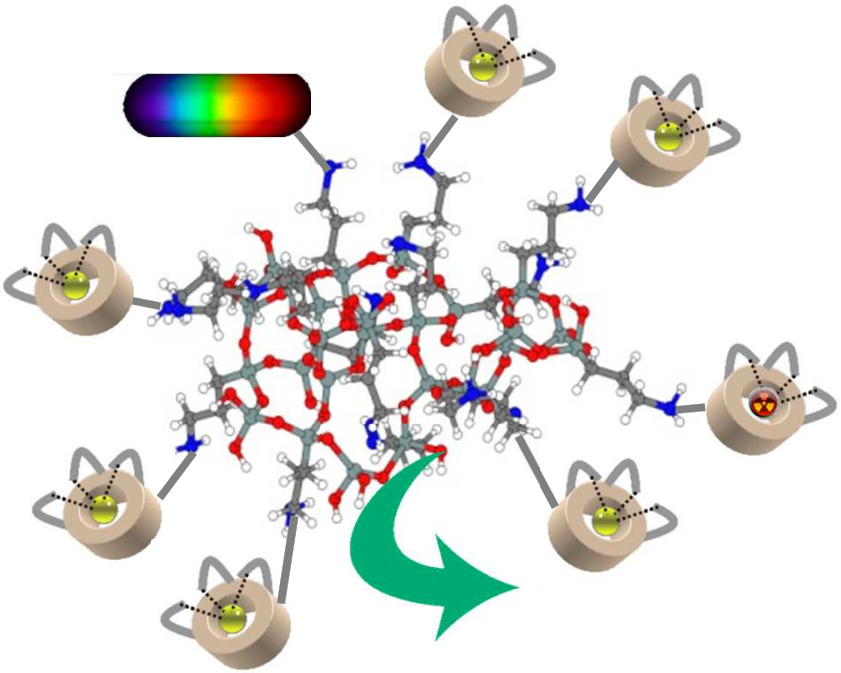


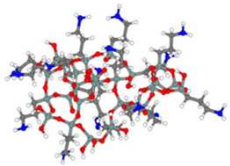
Similar pattern of biodistribution (Fluorescence/SPECT)



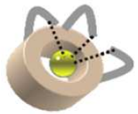
S. Dufort, J.L. Coll et al.

“Full options” multimodal AGuIX®

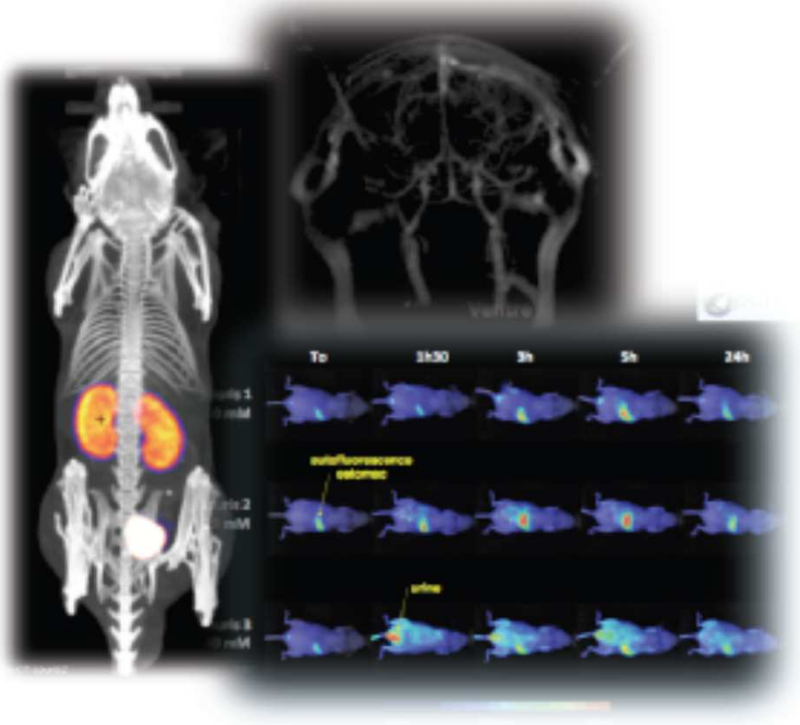




Polysiloxane network



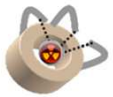
DOTA(Gd)



NIR fluorophore

Functionalization

by targeting ligands



*DOTA(**radioactive species**)*

AGuIX®

Tumor targeting

EPR – passive accumulation

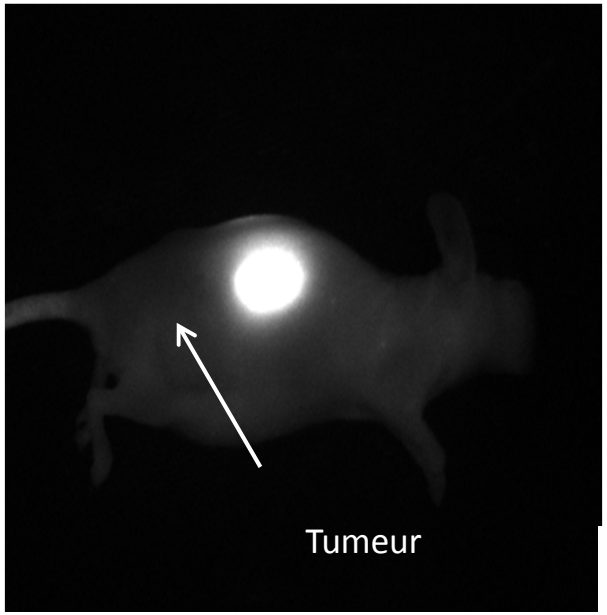
Active bio-targeting

Passive targeting: EPR

heterotopic tumor models (nude mice)

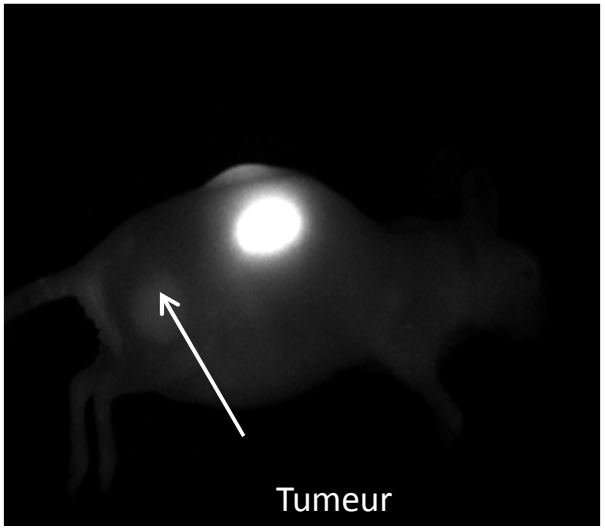
HEK(293)-β3

Humaine



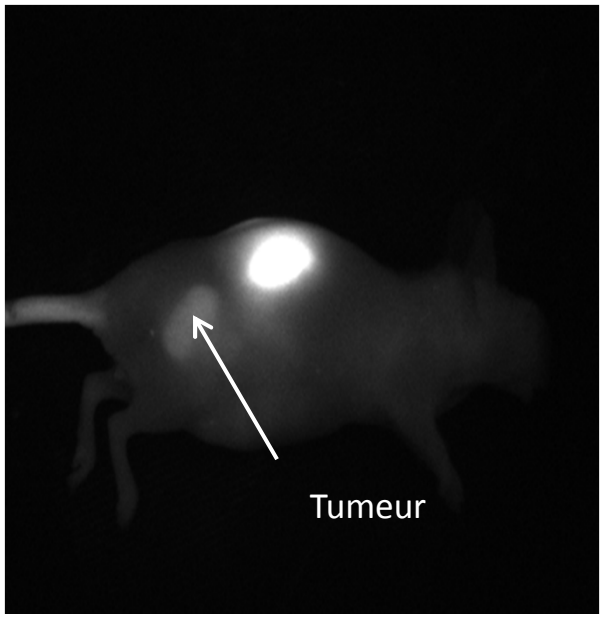
U87-MG

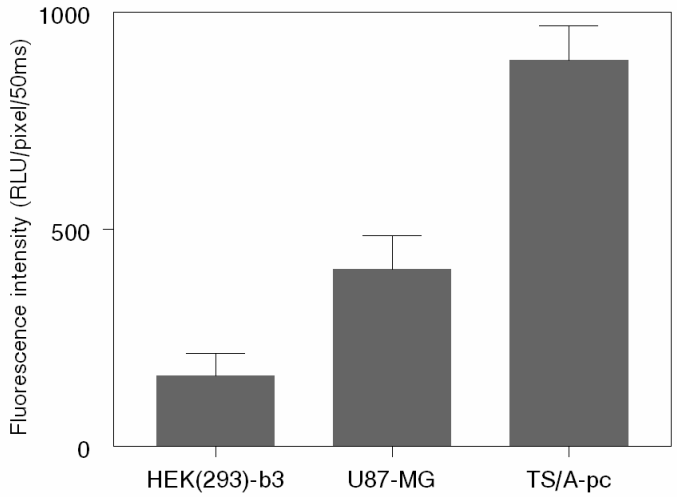
Humaine



TSA

Murine





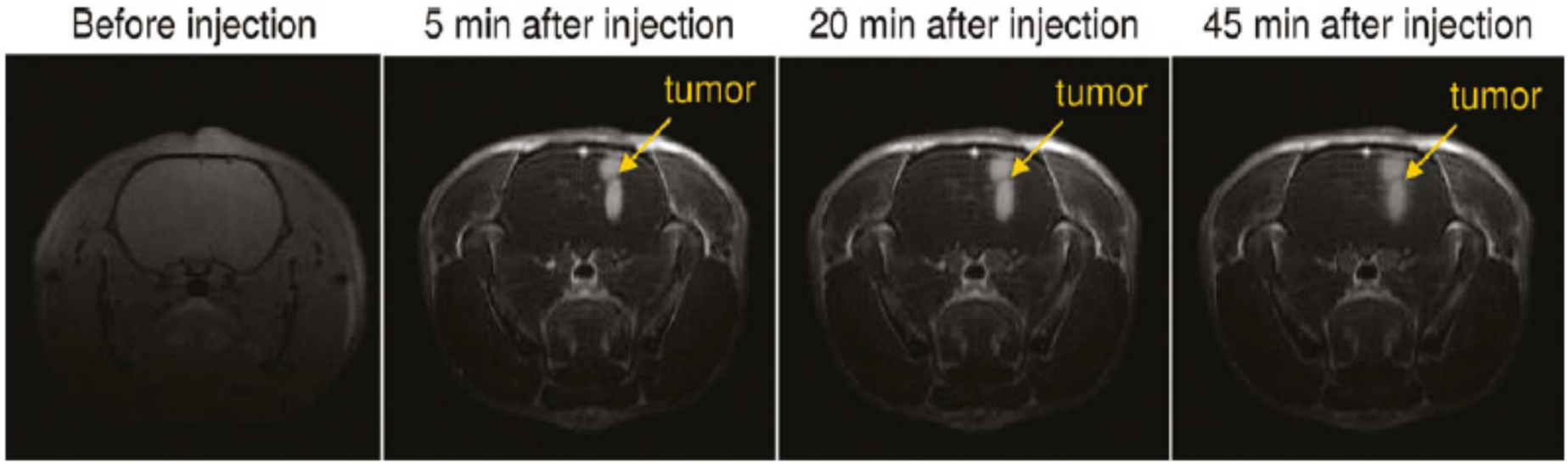
EPR ≈ 1% ID/g

S. Dufort, J.L. Coll et al.

Passive targeting: EPR

Orthotopic tumor model - glioma

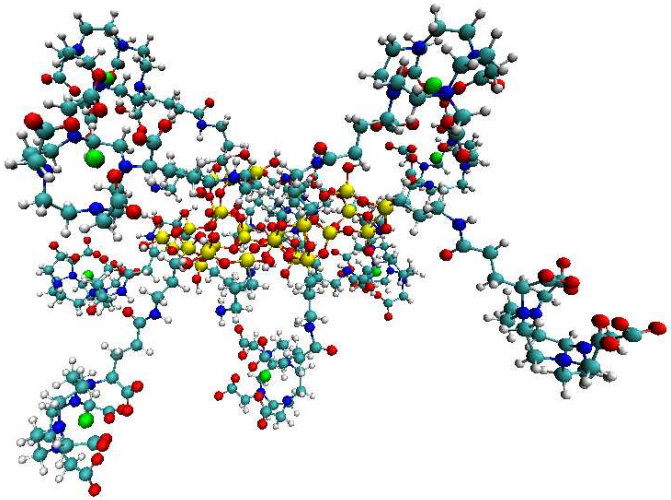
Brain's rat Fisher 344 + gliosarcoma 9L

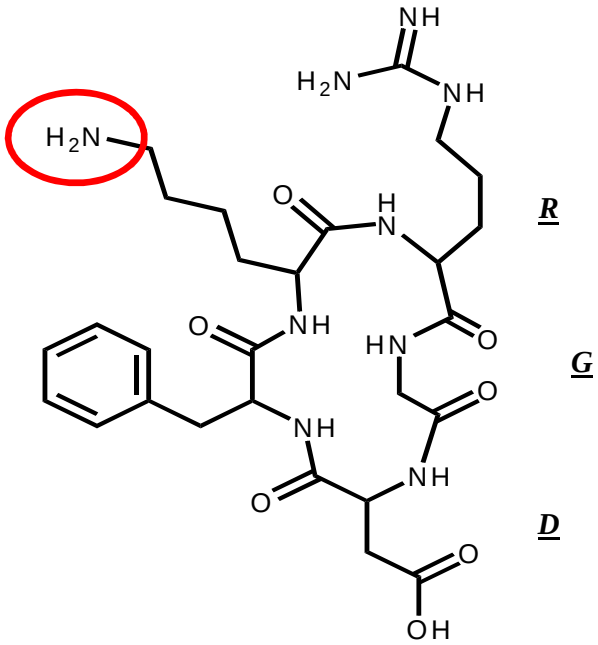


Rat nude + glioblastoma U87

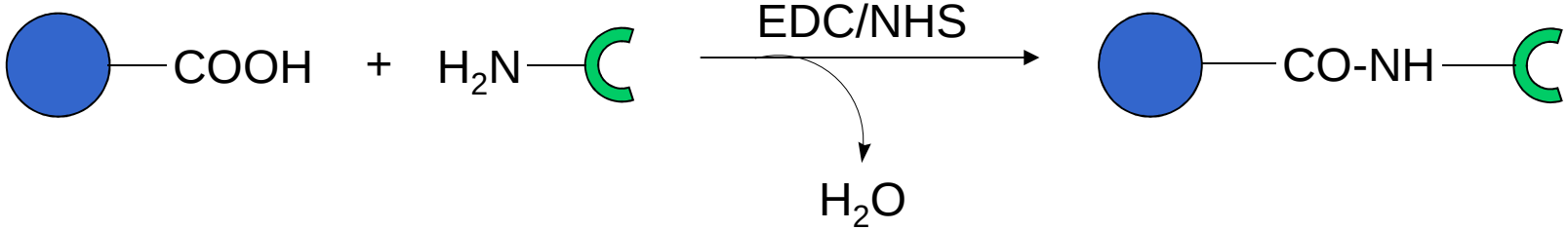
Active targeting

Example : via cyclic polypeptide cRGD

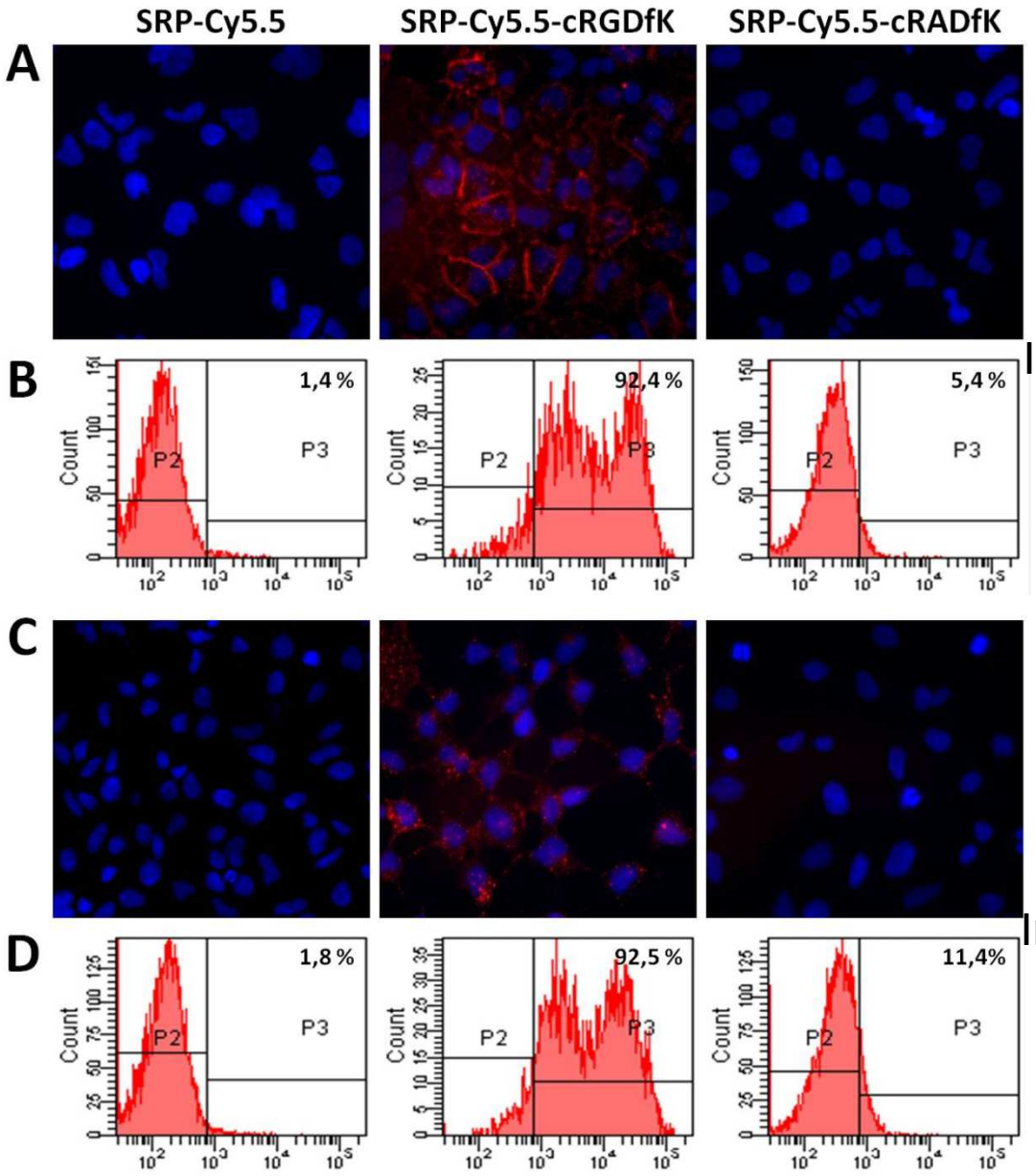




NP-cRGDfK



Active targeting via cyclic polypeptide RGD



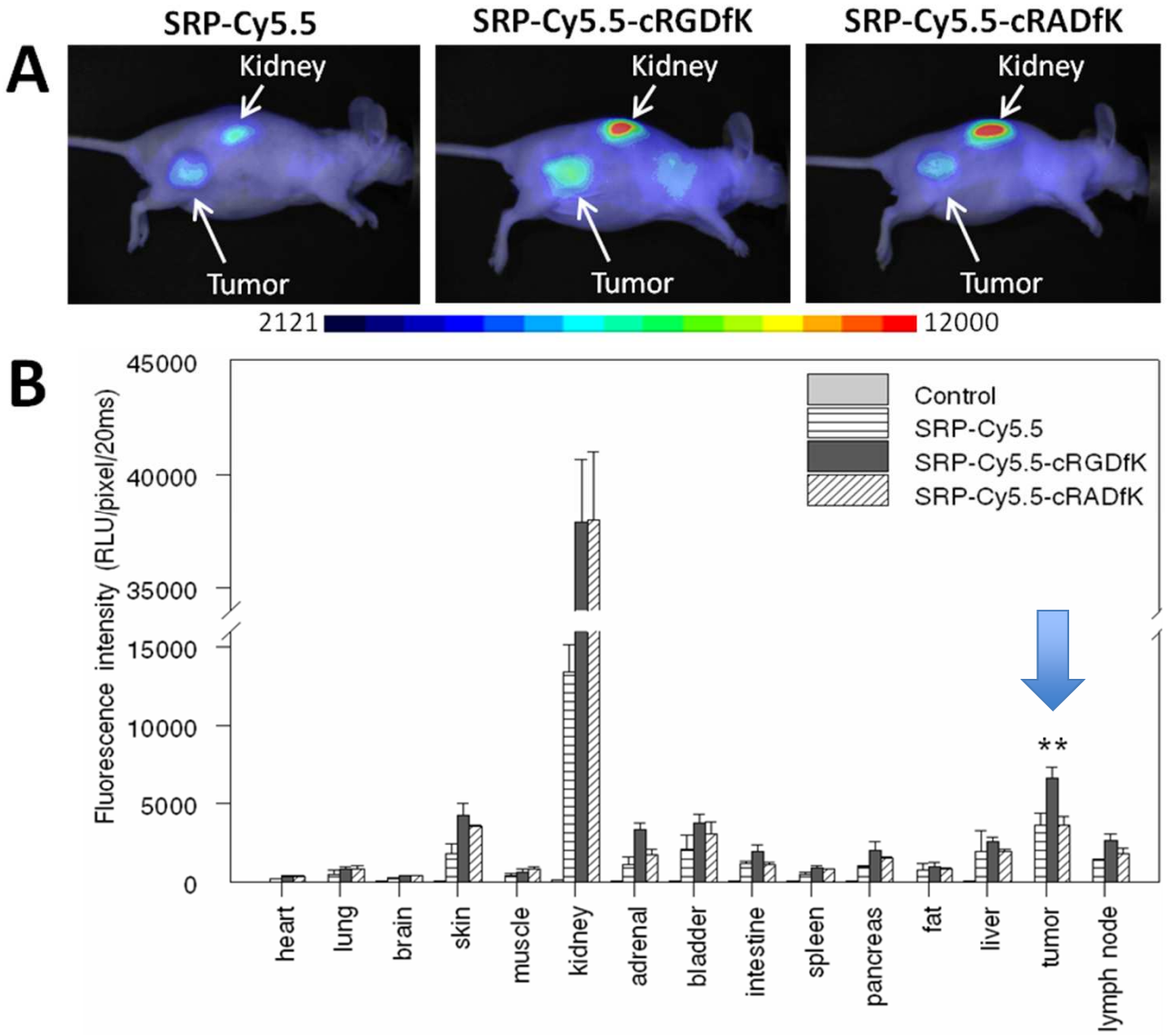
Hekβ3 cells

Incubation at 5℃

Incubation at 37℃

J. Morlieras, S. Dufort *et al, submitted*

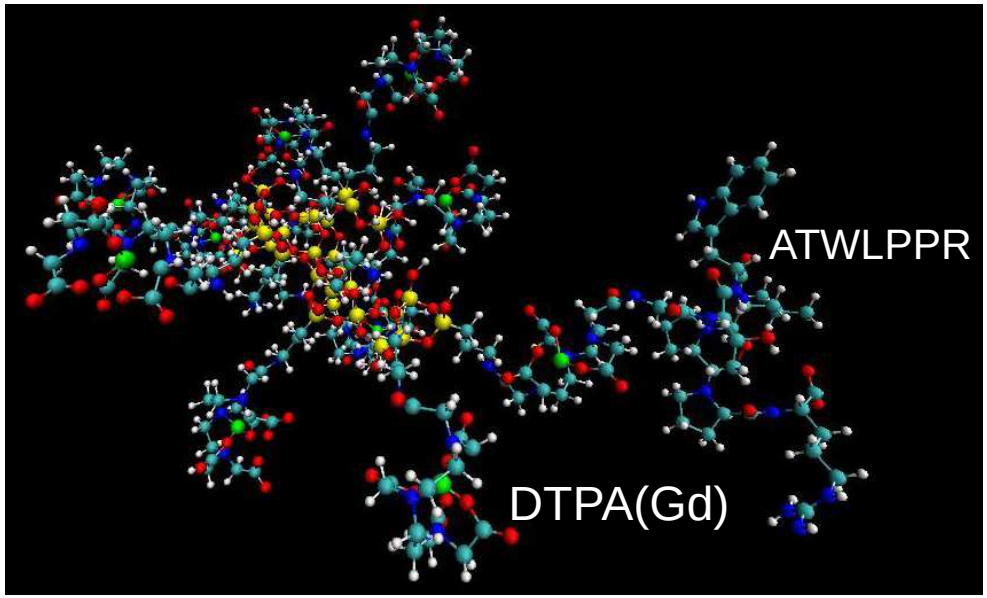
In vivo active targeting

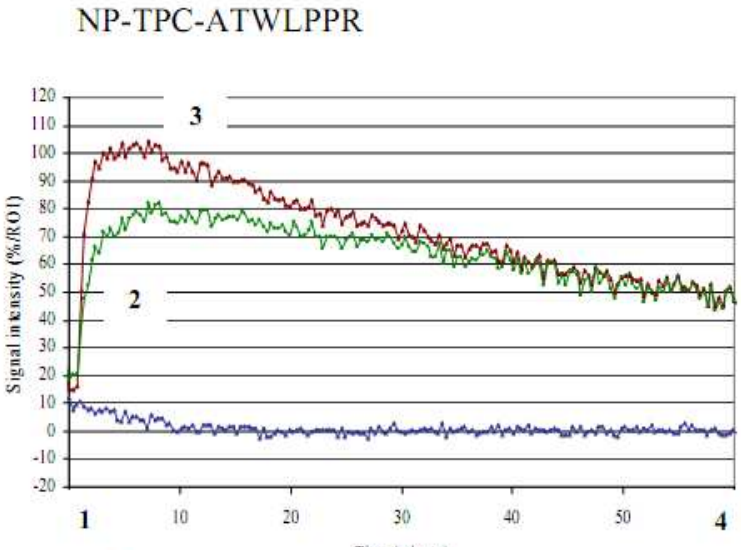


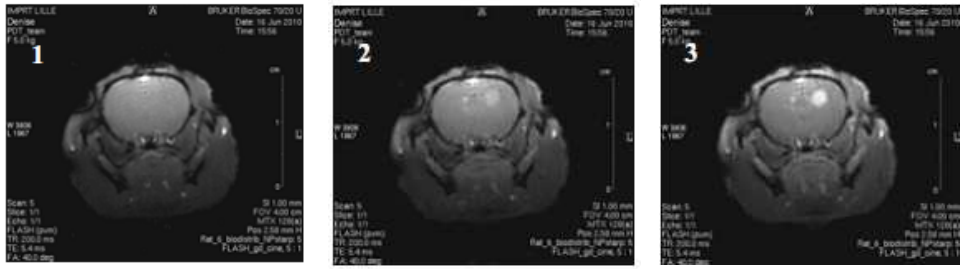
J. Morlieras, S. Dufort *et al, submitted*

Targeting of Np1 : ATWLPPR

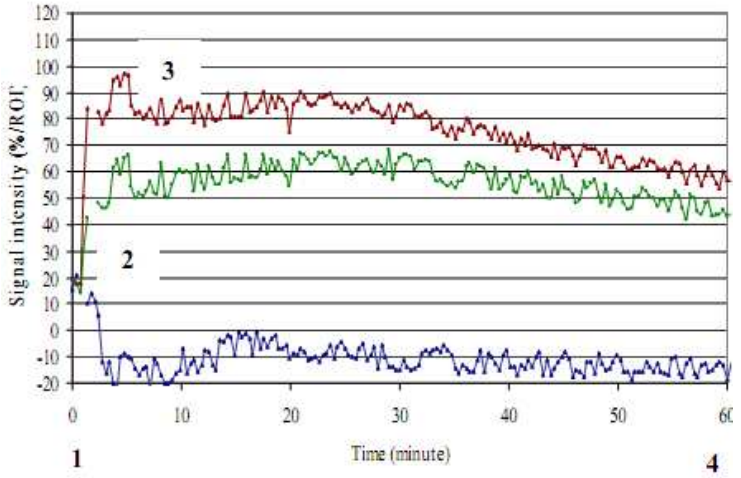
Nude rat – Glioblastoma U87







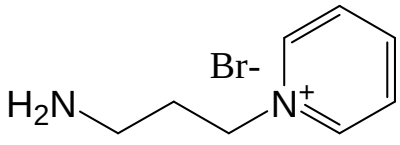
Avant IV **2 mn après IV** **10 mn après IV**

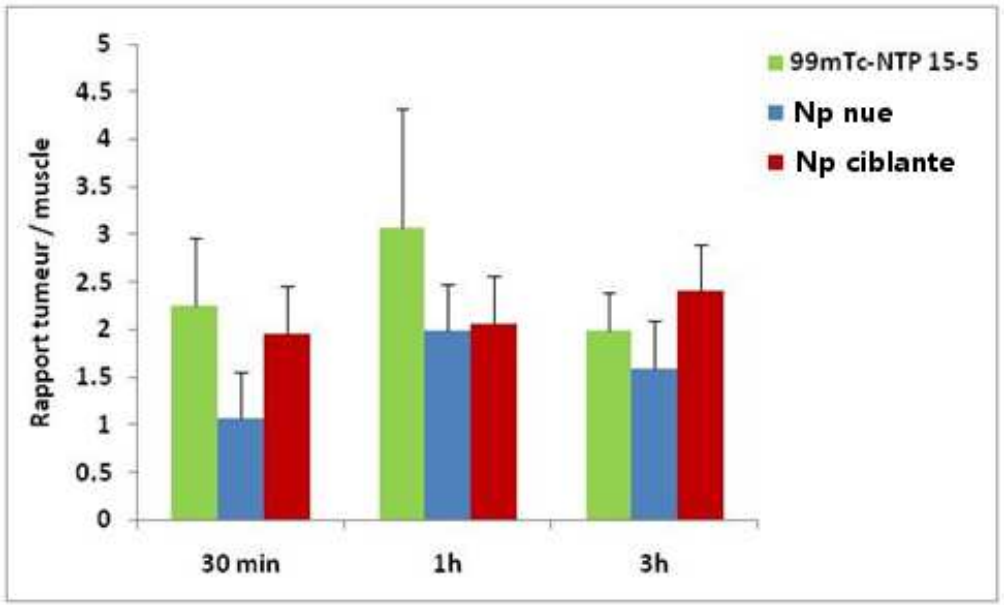


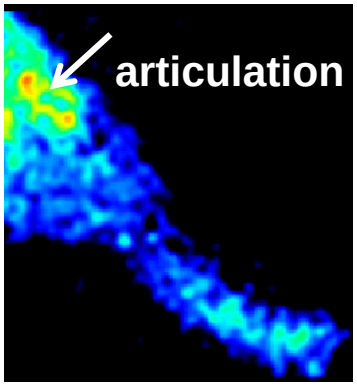
D. Bechet *et al.*, soumise

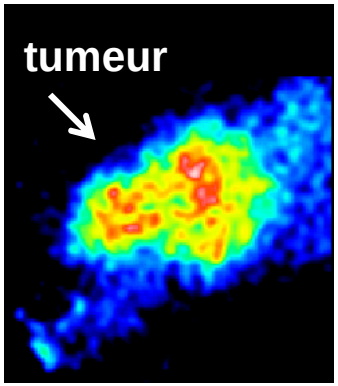
Active targeting

Example : via small charged molecules for chondrosarcomes



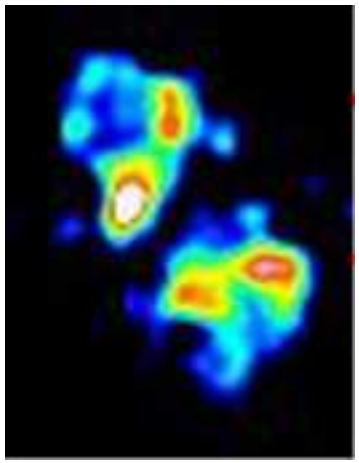


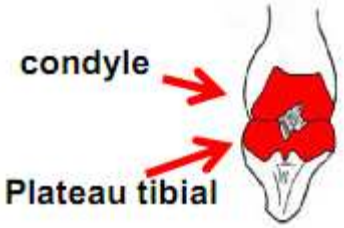




Test leg

Leg with chondro



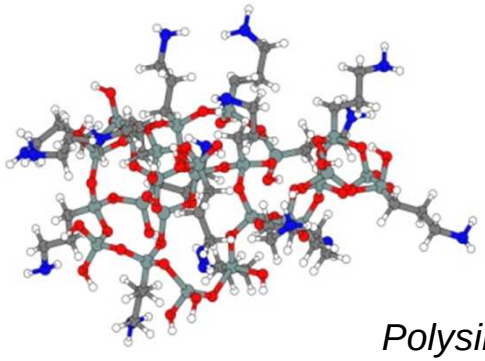


After dissection of test leg

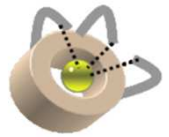
FR11 59856 2011 : *Chezal J. M. ; Miot-Noirault E. ; Morlieras J. ; Billotey C ; Janier M ; Tillement O.*

NANOPARTICULES FONCTIONNALISES POUR LE CIBLAGE DES PROTEOGLYCANES ET LEURS APPLICATION

Stabilité
Toxicité
Dégradation



Polysiloxane network

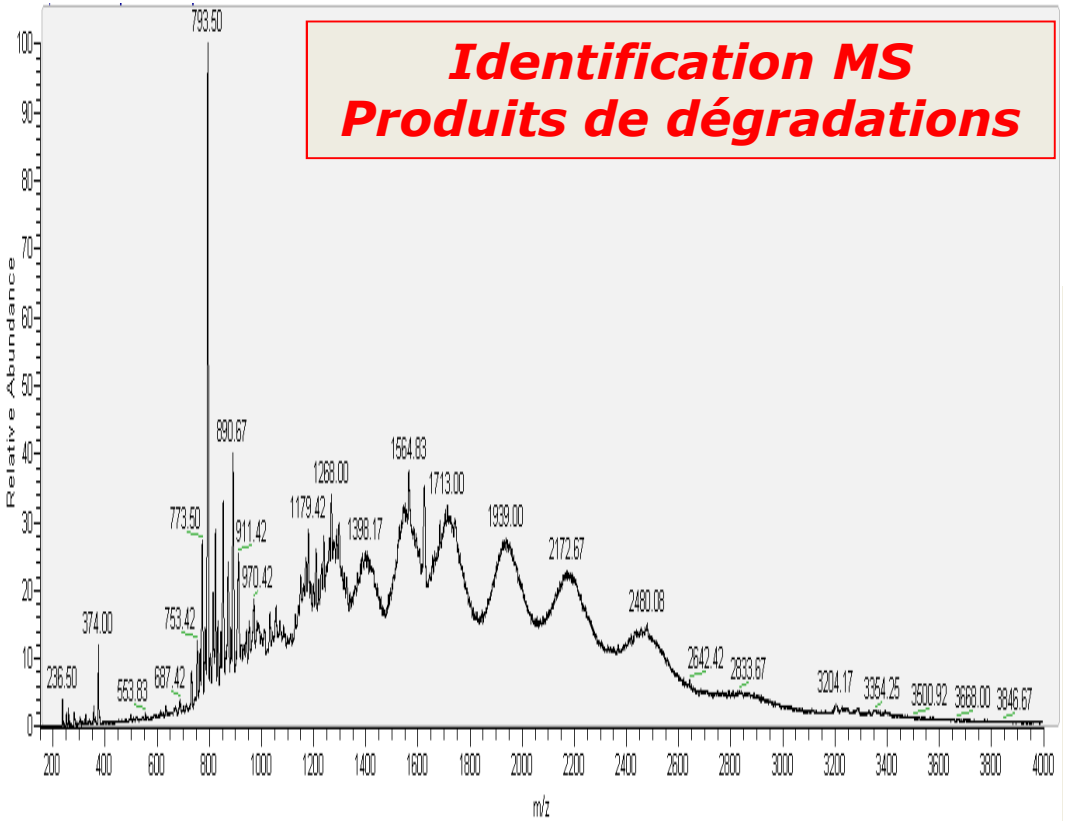


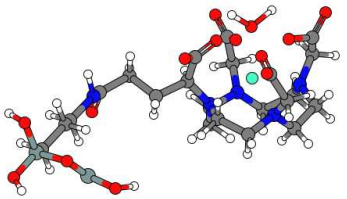
DOTA(Gd)

Etude de dégradation

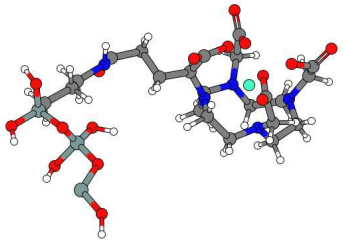
Expérience de dégradation
Dispersion des particules à 0,1 mM Gd - eau maintenue à 37℃ - 30 jours

Identification des produits de dégradation par Spectro de Masse

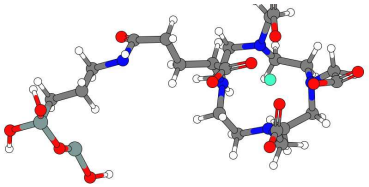




M= 813



M= 873

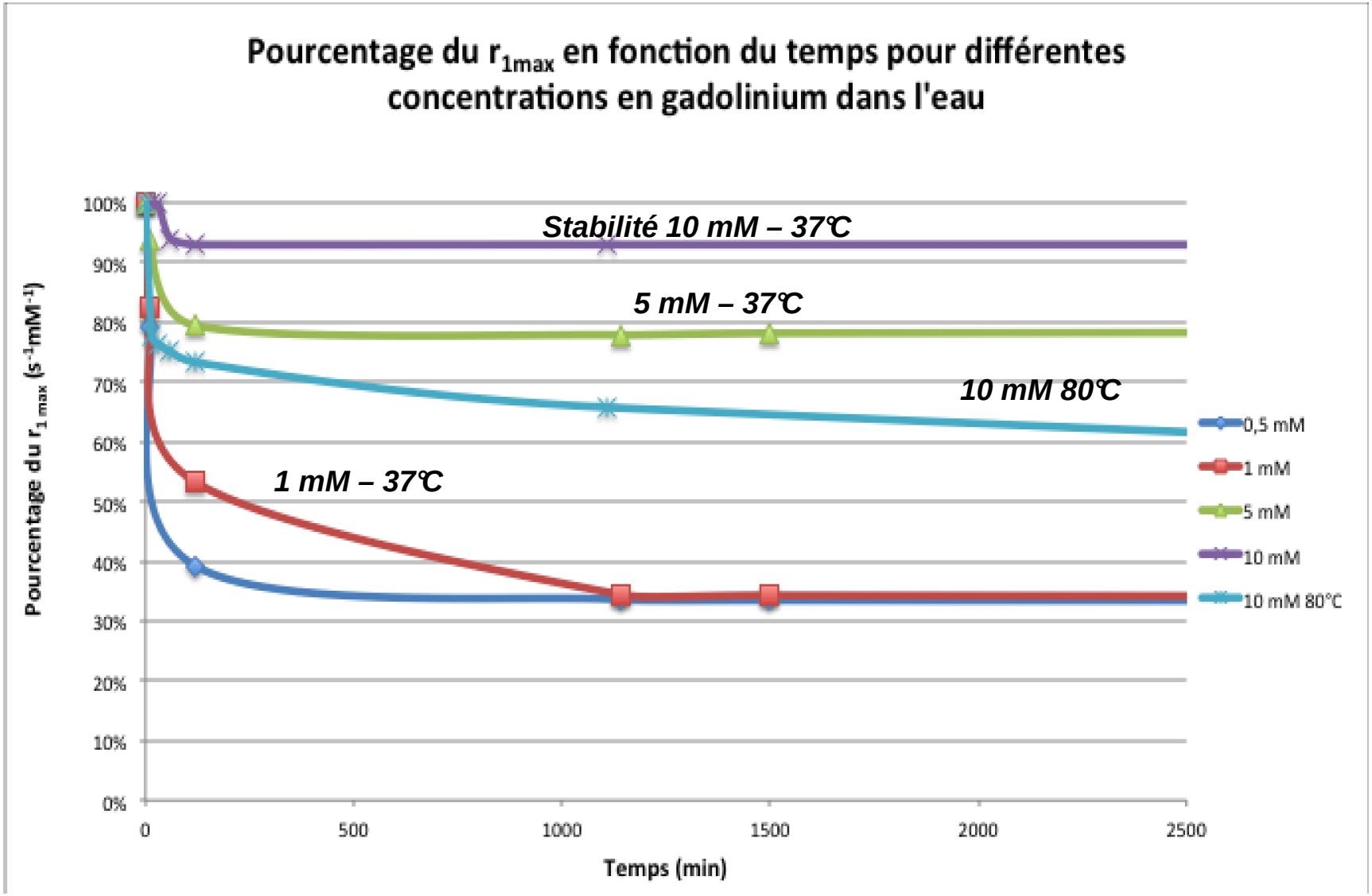


M= 795

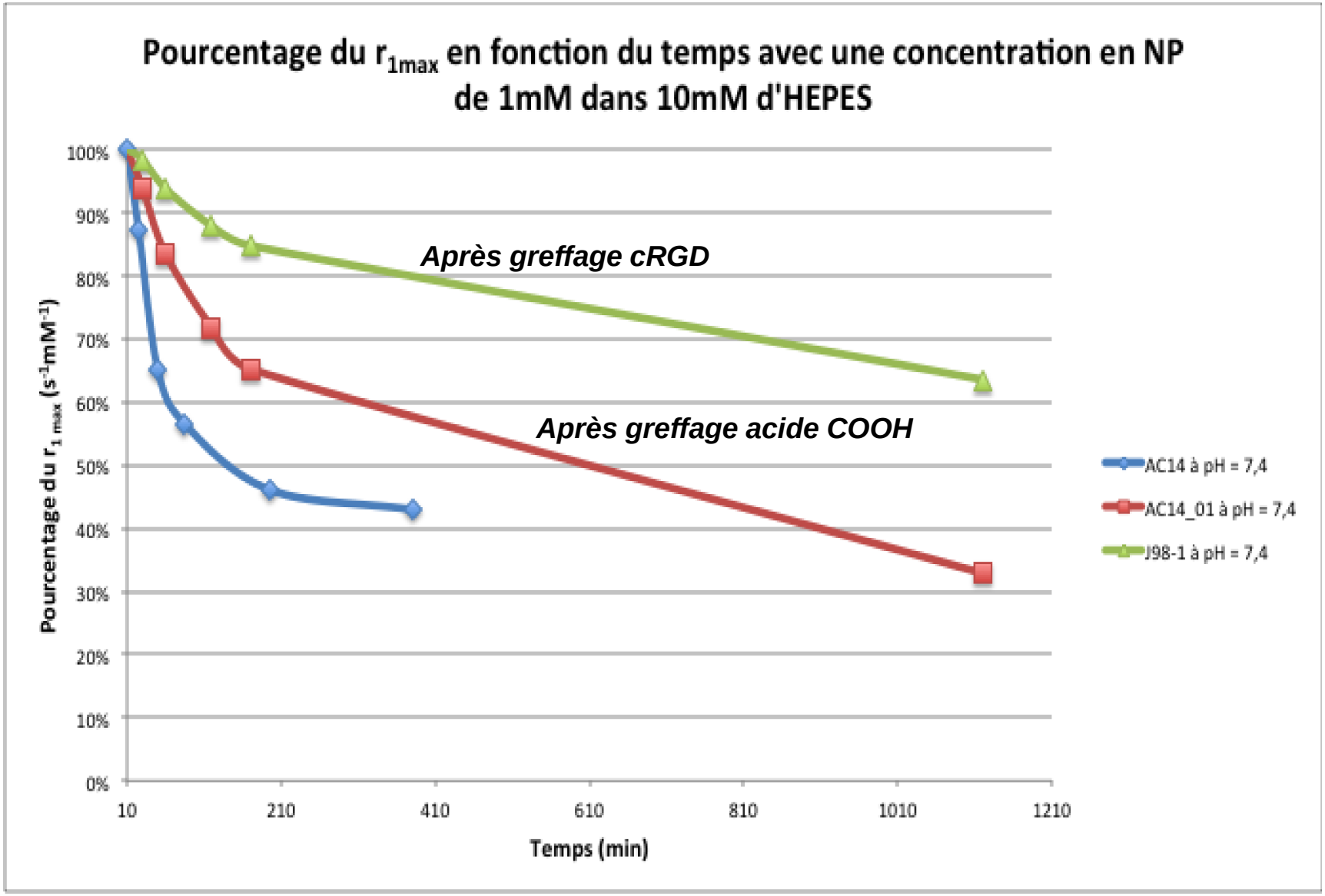
Etudes de stabilité :

particules à dégradation progressive

Suivis par relaxométrie… hydrolyse des polysiloxanes



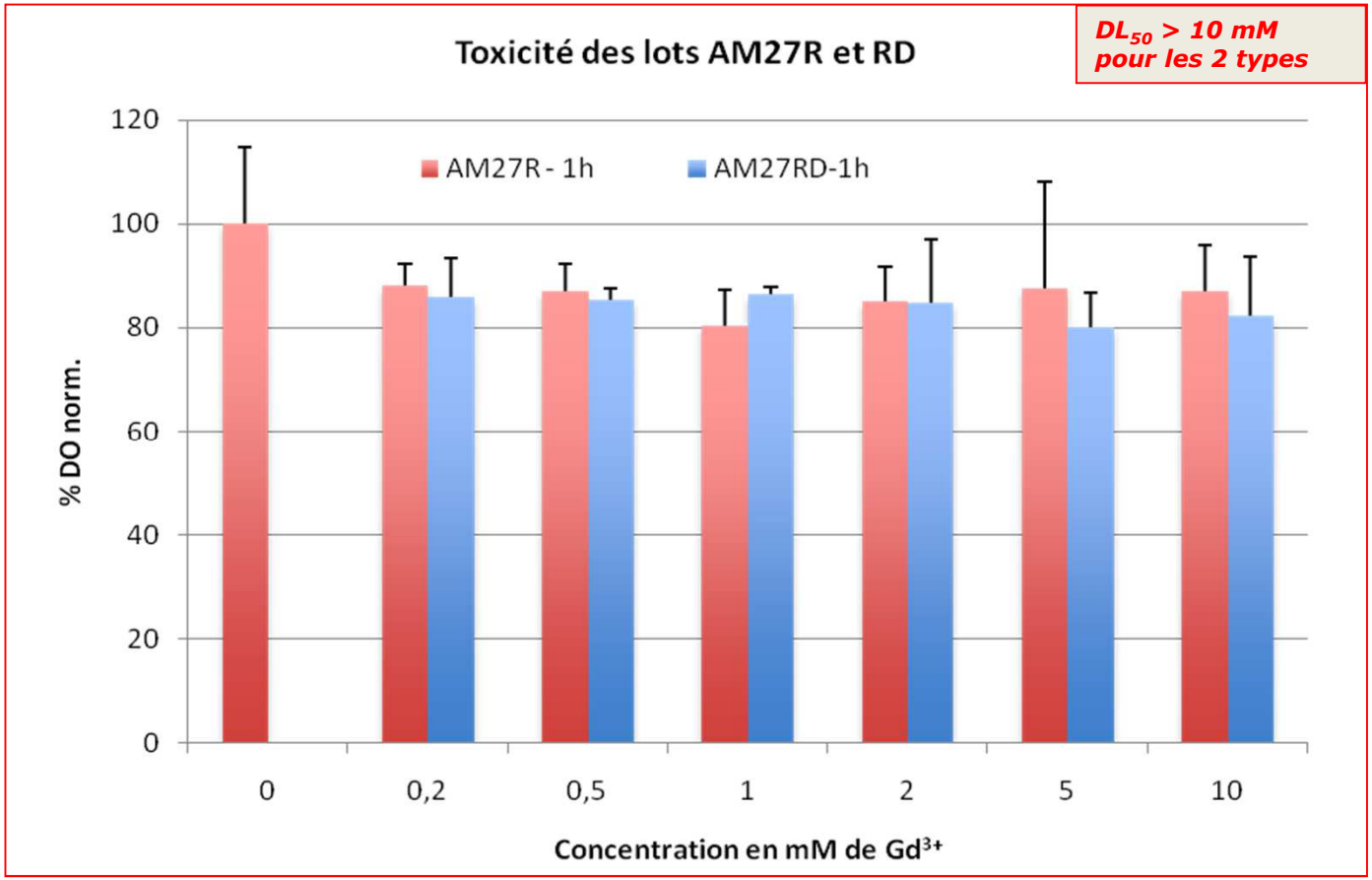
Augmentation de stabilité après fonctionnalisation



Pas de toxicité des produits de dégradation

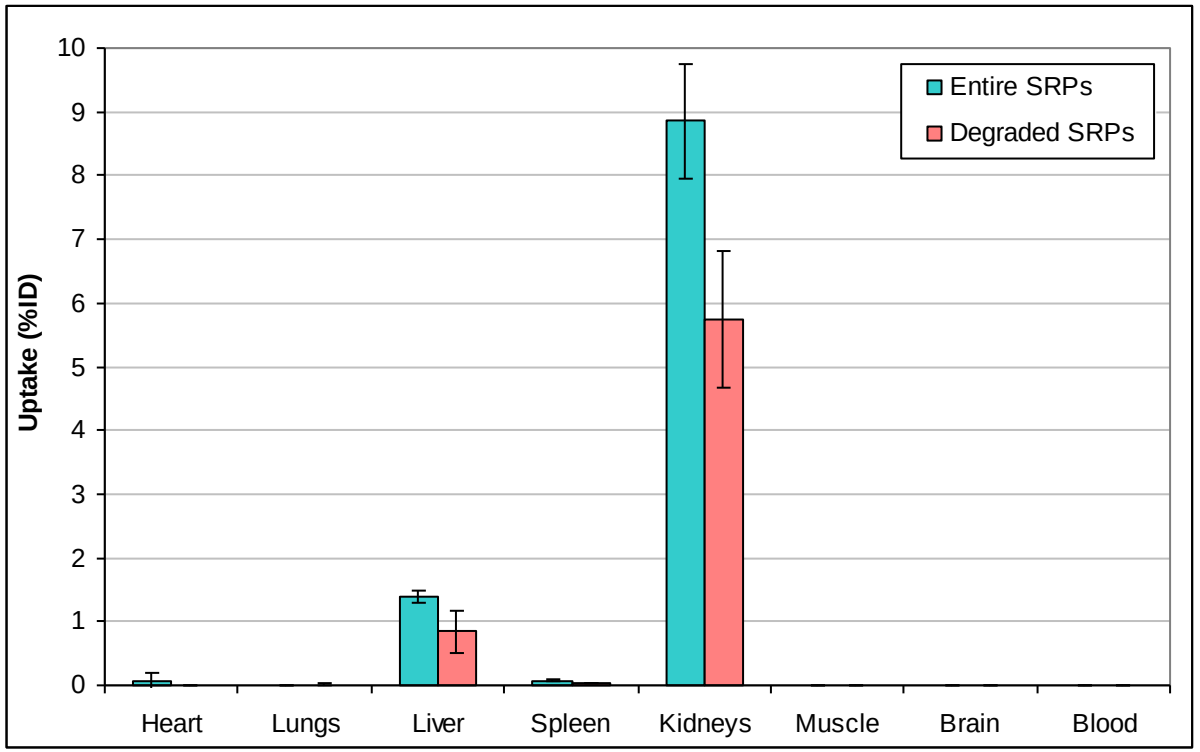
Pas de toxicité, ni de différence avec les particules primaires
Tests d'injection effectuées (pas de différences observées)
Tests in vitro (MTT)

Tests MTT sur Carcinome murin mammaire (TS/A-pc)
Particules initiales (AM27R) et particules après dégradation (AM27RD)



Produits de dégradations
In vivo

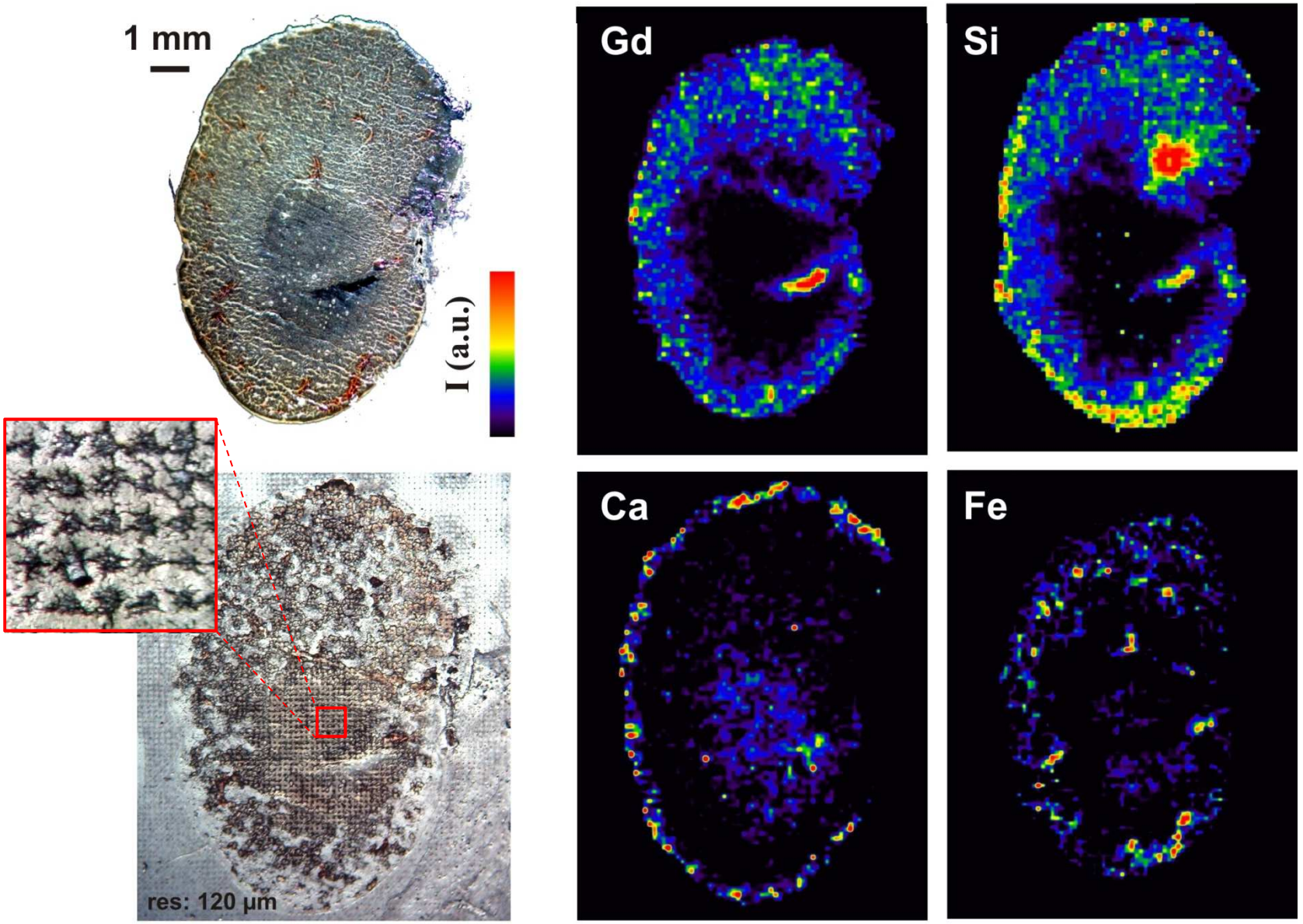
Elimination plus rapide



Gadolinium uptake in organs, 24 hours after injection.

Mapping nanoparticles in kidney using LIBS

Correlation between Gd and Si



Collaborations : V. Motto-Ros, L. Sancey, G. Panczer and J. Yu

Interest(s) of such ultra-small particles ?

Higher signal by object (*)

IRM, SPECT, PET… not enough

Multimodality (***)

PET-IRM

IRM-Fluo – Spect/PET-Fluo

Biodistribution (**)

Intravascular (low extravasation) and blood residential time

EPR, targeting (external)

(Cell tagging)

Theragnostic

Deux produits proposés

Special GDR-offer: buy one get one free !

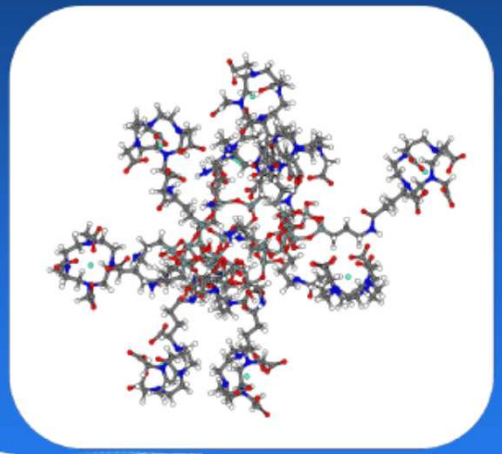
CheMatech
macrocycle design technologies

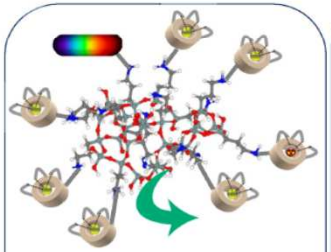
nano-H
Value-Added Nanotechnology

AGuIX®

Preclinical Multimodal Probe

Theragnostic Nanoparticles
(MRI-SPECT/PET-fluorescence-Therapy)





Ultrasmall size
4±1 nm - renal excretion
MW 8.5±2 kDa

Polysiloxane composition
Easy further functionalization

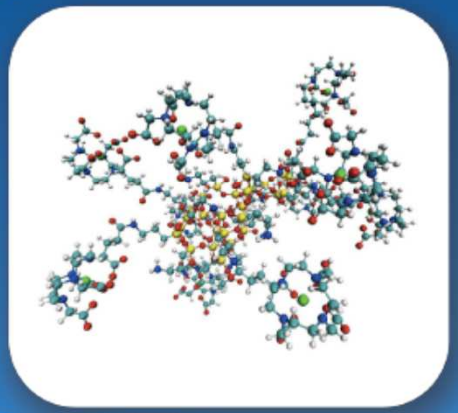
DOTA (Gd) (MRI - Radiotherapy)
FDA approved
About 10 DOTAs/nanoparticle

Radiometals (M*) chelation
PET, SPECT, Therapy

Gado-H®

Preclinical MRI Gd probes for cellular labeling

Paramagnetic multimodal hybrid sub-5 nm particles
High efficient T1 MRI Cell Tracking



Cell labeling
Stem Cells, Lymphocytes, Dendritic cells, Fibroblastes, Macrophages, Tumors cells, etc...

Freeze dried
Easy handling and ready to use

Storage
Stable for months

Reproducible synthesis
Since 2004 in Nanosynthesis business

Simple labeling process
No cytotoxicity, no impact on Stem Cell differentiation

Ultrasmall size
4±1 nm

Polysiloxane and Gadolinium chelates platform
High Gd loading Gd/Si >0.2

Low biological interferences

High colloidal stability in biological buffer

Multilabeling access
- fluorescence,
- nuclear imaging

High relaxivity
>10 $mmol^{-1}.s^{-1}$ per Gd^{3+} (1.4T)

Therapeutical activation

Radio-sensitization

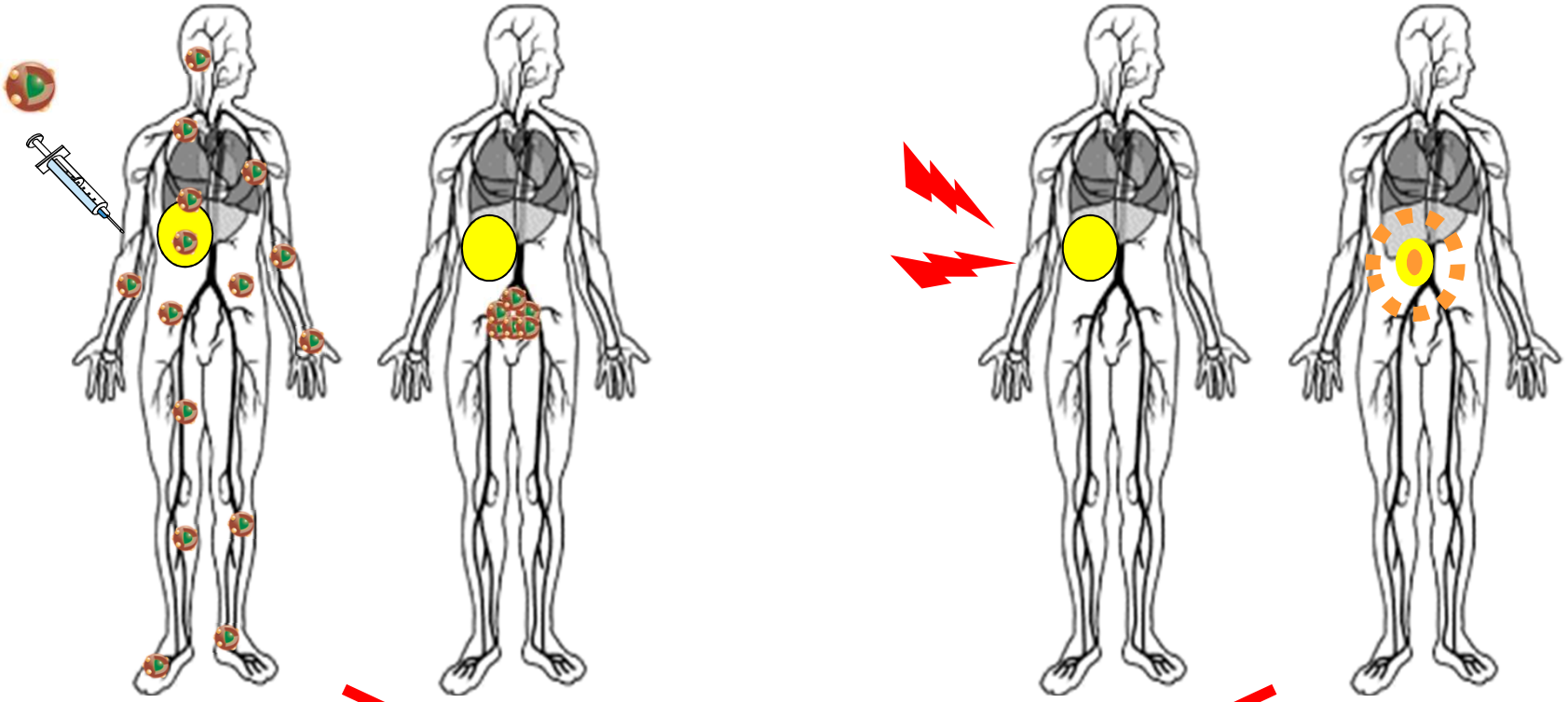
Towards an increase of the efficiency of radiotherapies

Radiosensitizers

Nanoparticles injection & radiotherapy

Nanoparticles
Injection (IV or IT)
MRI monitoring possible

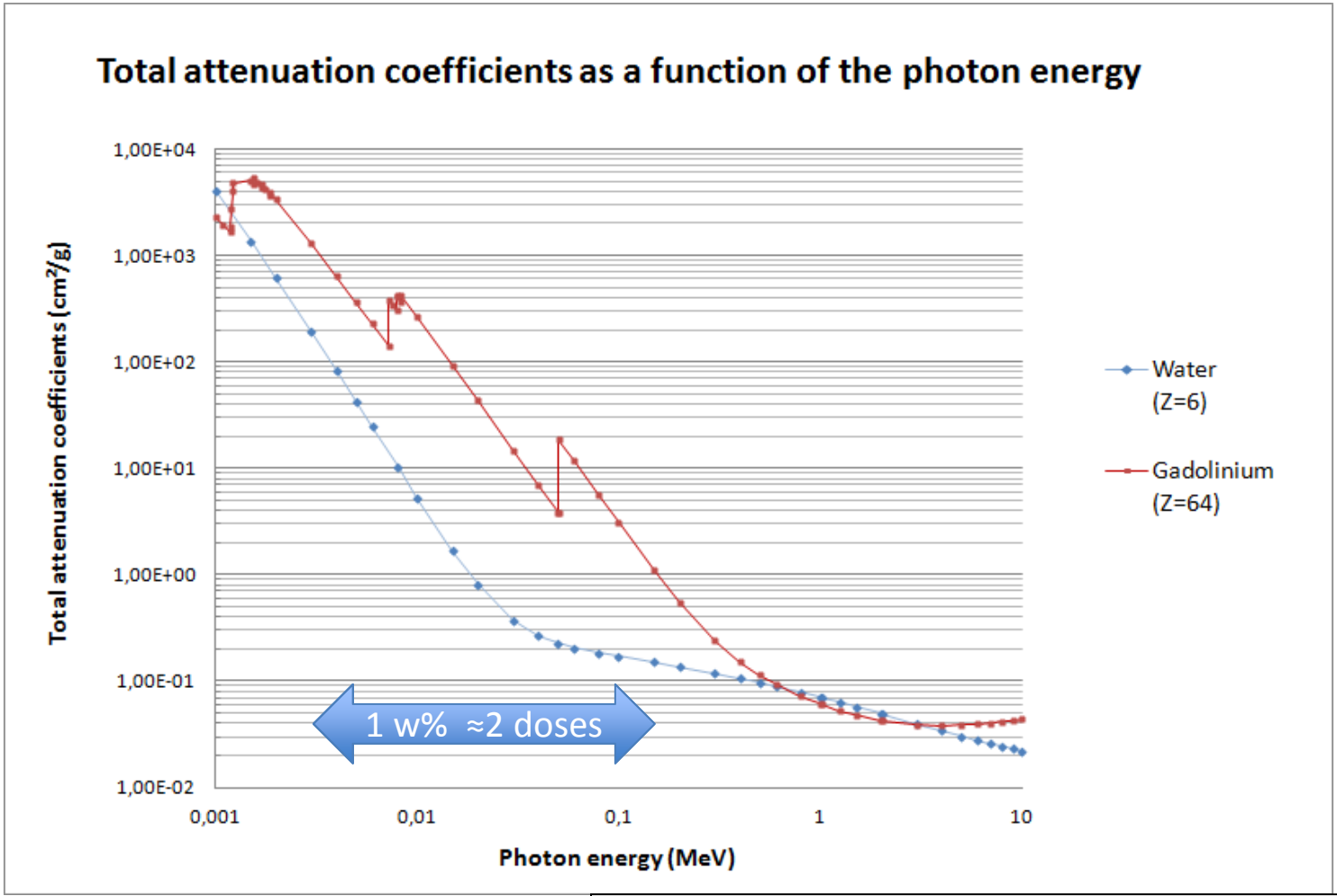
Irradiations
May be insufficient
Risk of high dose for adjacent tissues



ASSOCIATION

Dose enhancement can be expected with the presence of Gd (Z=64) due to their greater X-ray absorption (attenuation coefficient)

1% by mass combined with keV X-rays have been suggested to increase the dose deposited by a factor of two (1 w% i.e. 10 g/l or 1000 ppm)



In the 5-150keV energy range, the interaction probability of the photons with high Z atoms strongly increases by comparison with light atoms (water, tissues…).

In vitro tests

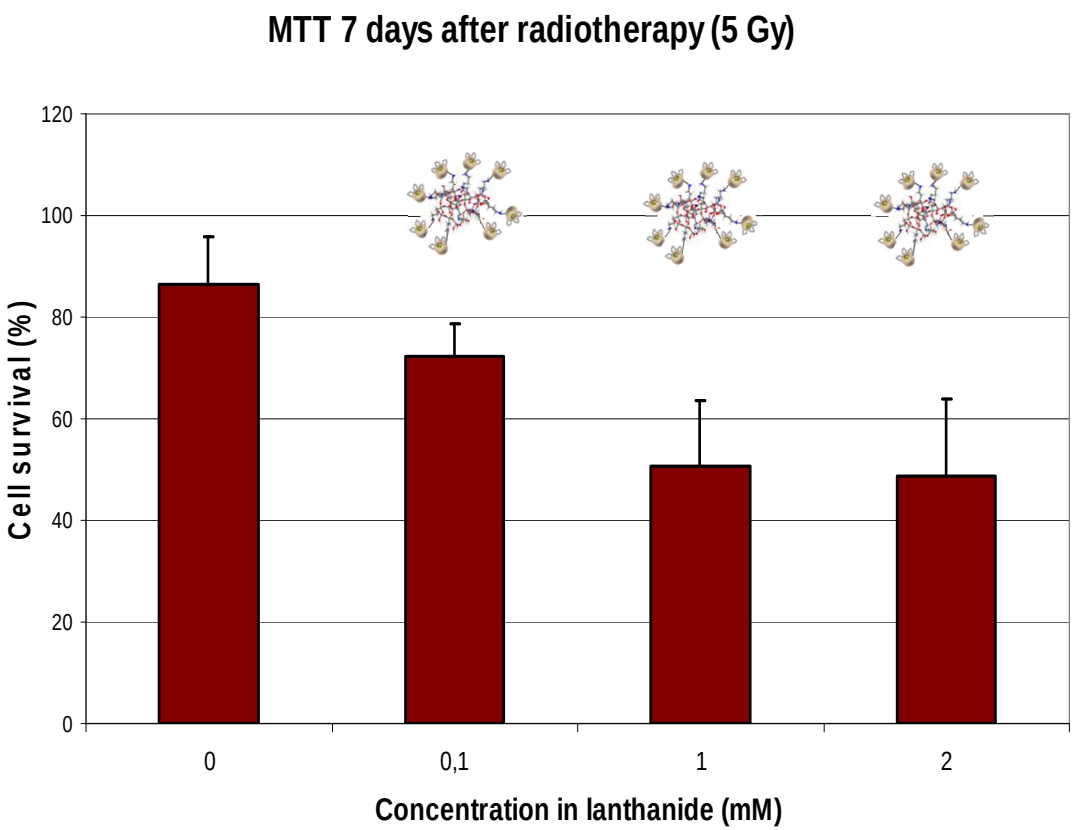
Radio-sensitization

Small animal irradiator (SQ20B)
Various clinical high energy irradiators (U87)

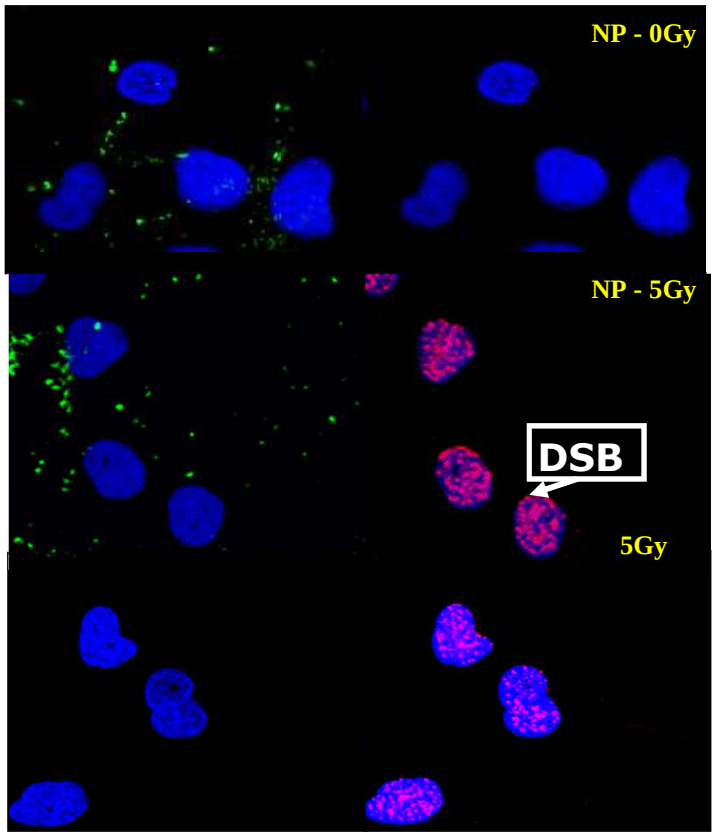
ESRF irradiation and Co (F98 Rat Glioma)
Hadrons (C^{6+} - H^{+})
Neutrons

Radiosensitization with U87
radioderistant glioblastoma cells

2-4 MeV X irradiation



660 keV γ-irradiation



DAPI, FITC-NP γ-H2AX, DAPI

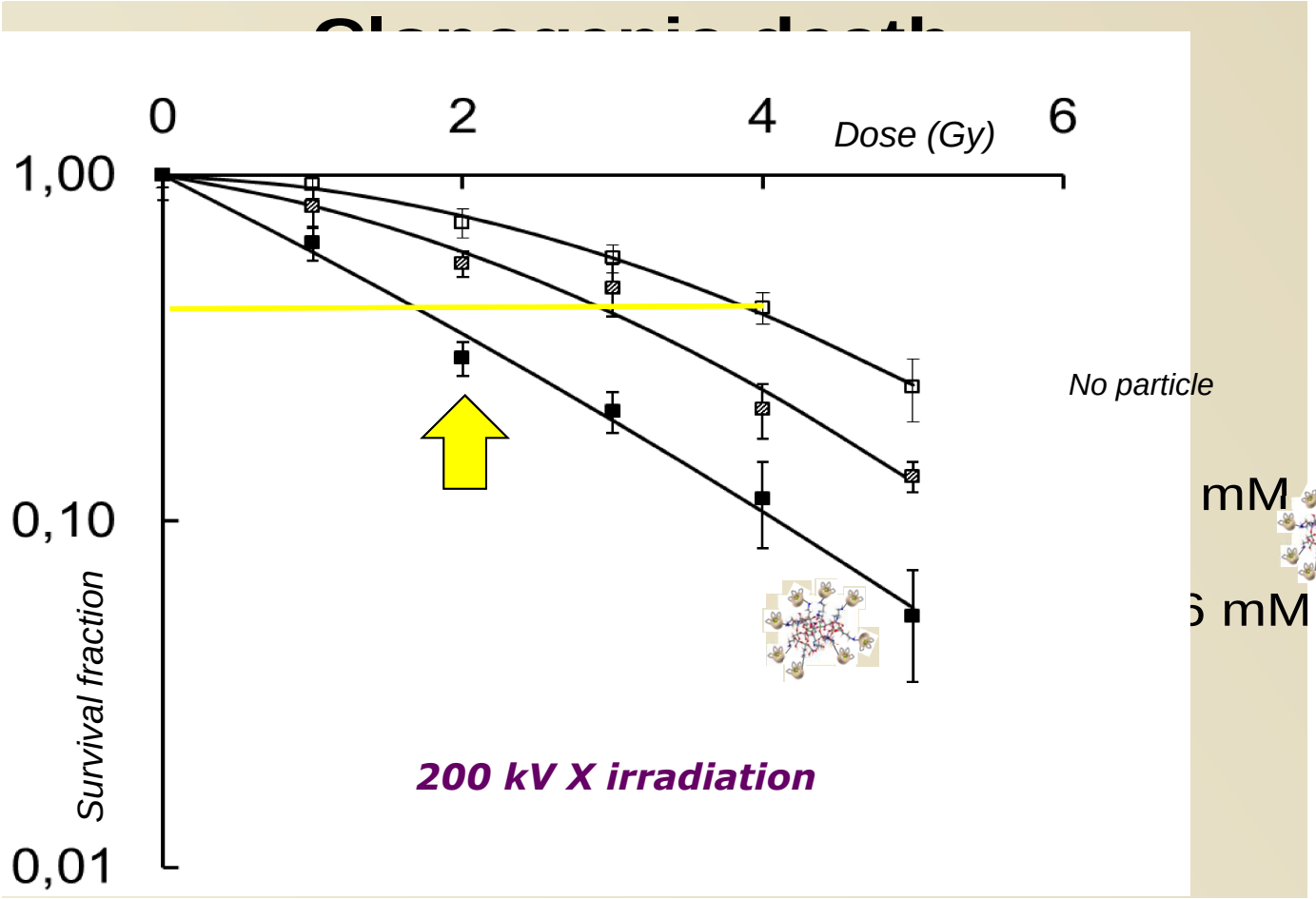
Red points: γH2AX

Double Strand DNA Breaks +80%

P. Mowat *et al.*, J. Nanosci. Nanotechnol., **2011,** 11, 7833

Radiosensitization with SQ20B

Radioresistant tumours, Head and Neck carcinoma



Radiosensitization with particles

1 h incubation with ~1 mg/ml (0.1 w%) SER $_{2Gy}$ ~ 2.7

More effects with 0.1 w% Gd Nano and 2 Gy than 4 Gy without Nano

I. Miladi *et al.*, thesis 2012

In vivo tests

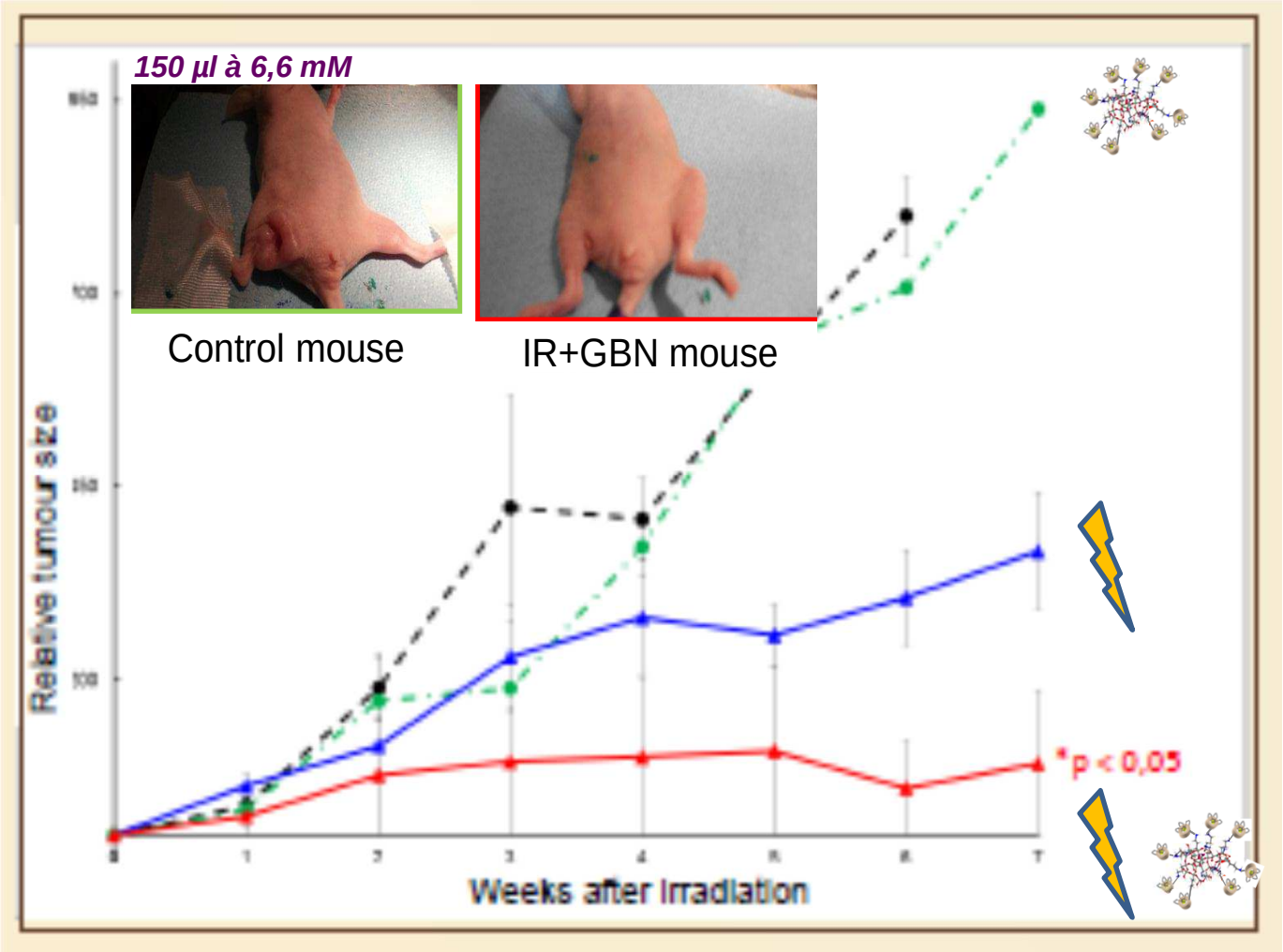
Radio-sensitization

SQ20B
(animal irradiators)

Glioblastoma
(ESRF MRT & animal irradiators)

In vivo irradiation SQ20B heterotopic

Irradiation 200 kV 10 Gy after AGuIX IT injection



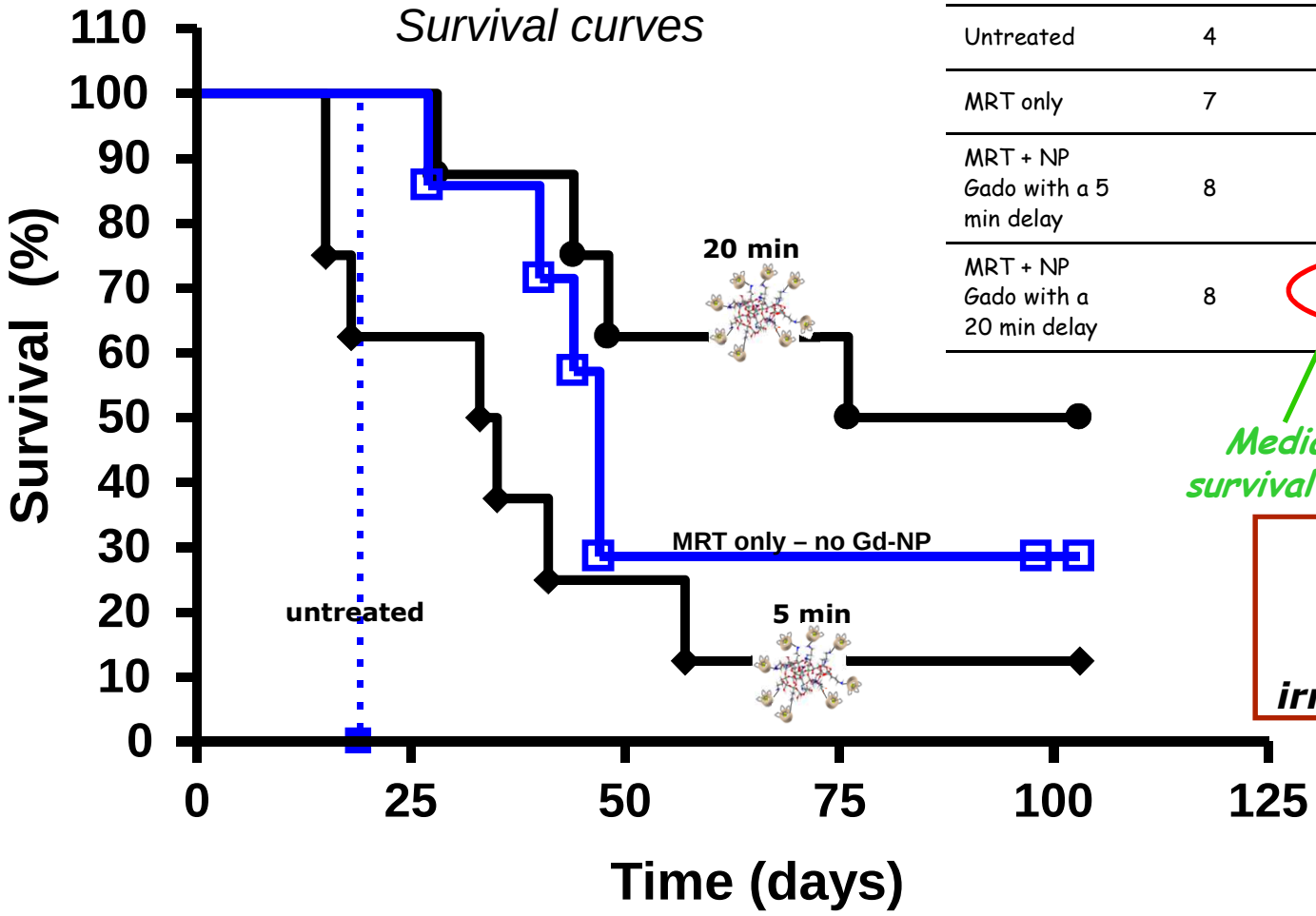
Major radiosensitizing effect of gadolinium based Nanoparticles on radioresistant tumours, Head and Neck Carcinoma

I. Miladi *et al.*, thesis 2012

In vivo irradiation Gliosarcoma 9L orthotopic

Irradiation MRT after AGuIX IV injection

Series	Number of rats	MeST days	ILS %	Survivors 100 days post implantation
Untreated	4	19	n/a	0/4
MRT only	7	47	147%	2/7
MRT + NP Gado with a 5 min delay	8	34	78%	1/8
MRT + NP Gado with a 20 min delay	8	90	373%	4/8



Median survival time

Life time

Efficiency

Factor 2 compared to irradiation alone

In vivo evidences of high Radiosensitizing effect…

G. Le Duc *et al., ACS Nano,* **2011**, *5*, 9566-9574

Mechanisms
Radio-sensitization

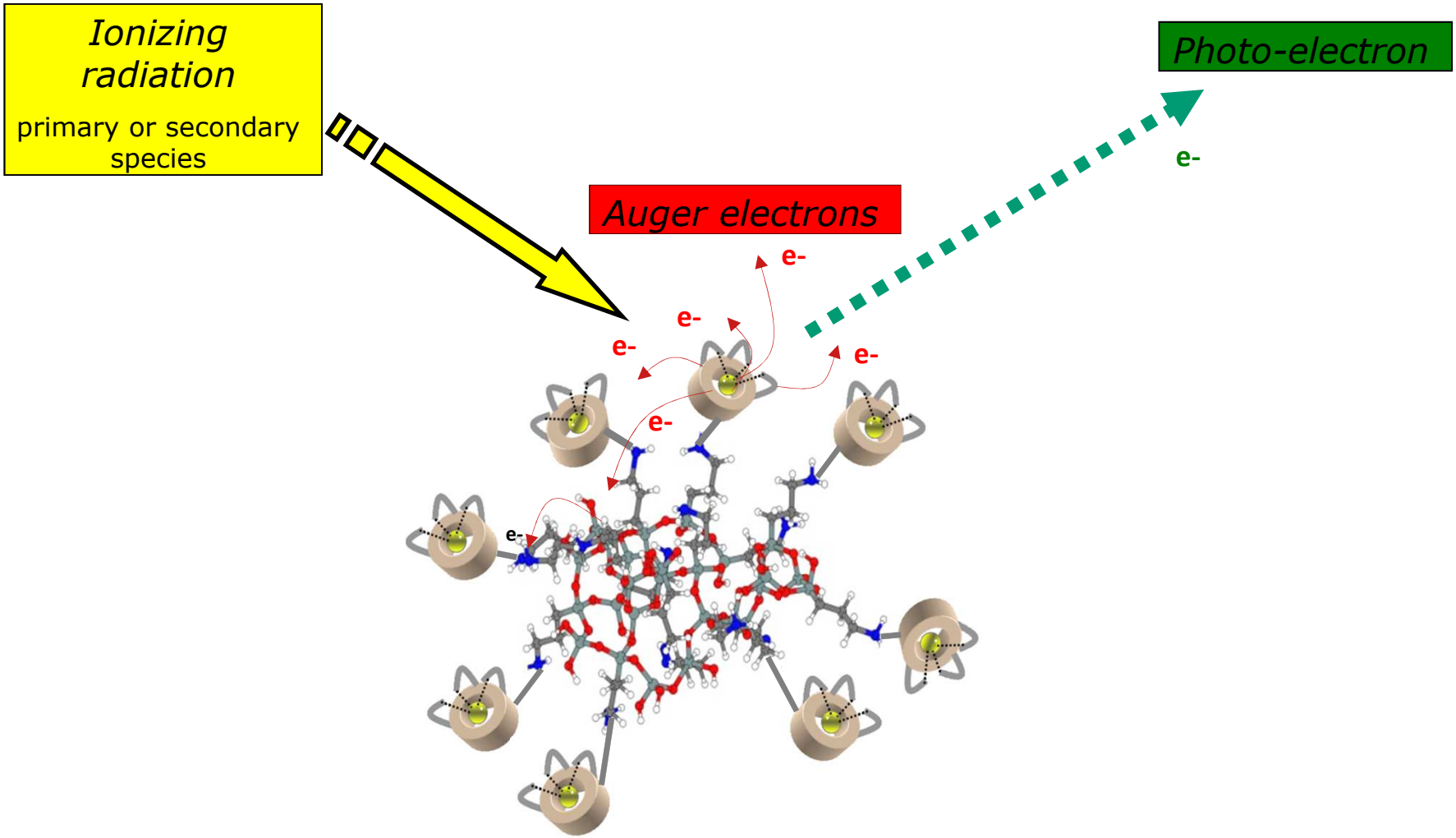
Surprising very high efficiency

Efficient with
low concentrations,
large panel of Ionizing species,
large panel of tumour cells

A possible mechanism story

Interaction with Ionizing radiation and a gadolinium

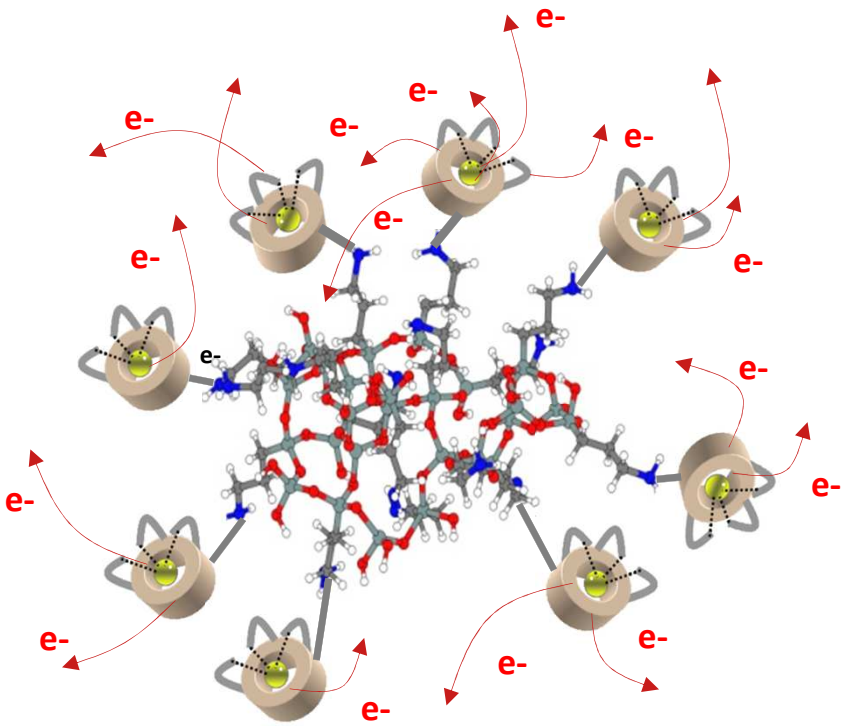
Initiation of a photon electron and some Auger electrons



Propagation to neighbour High Z species

Nano particle effect

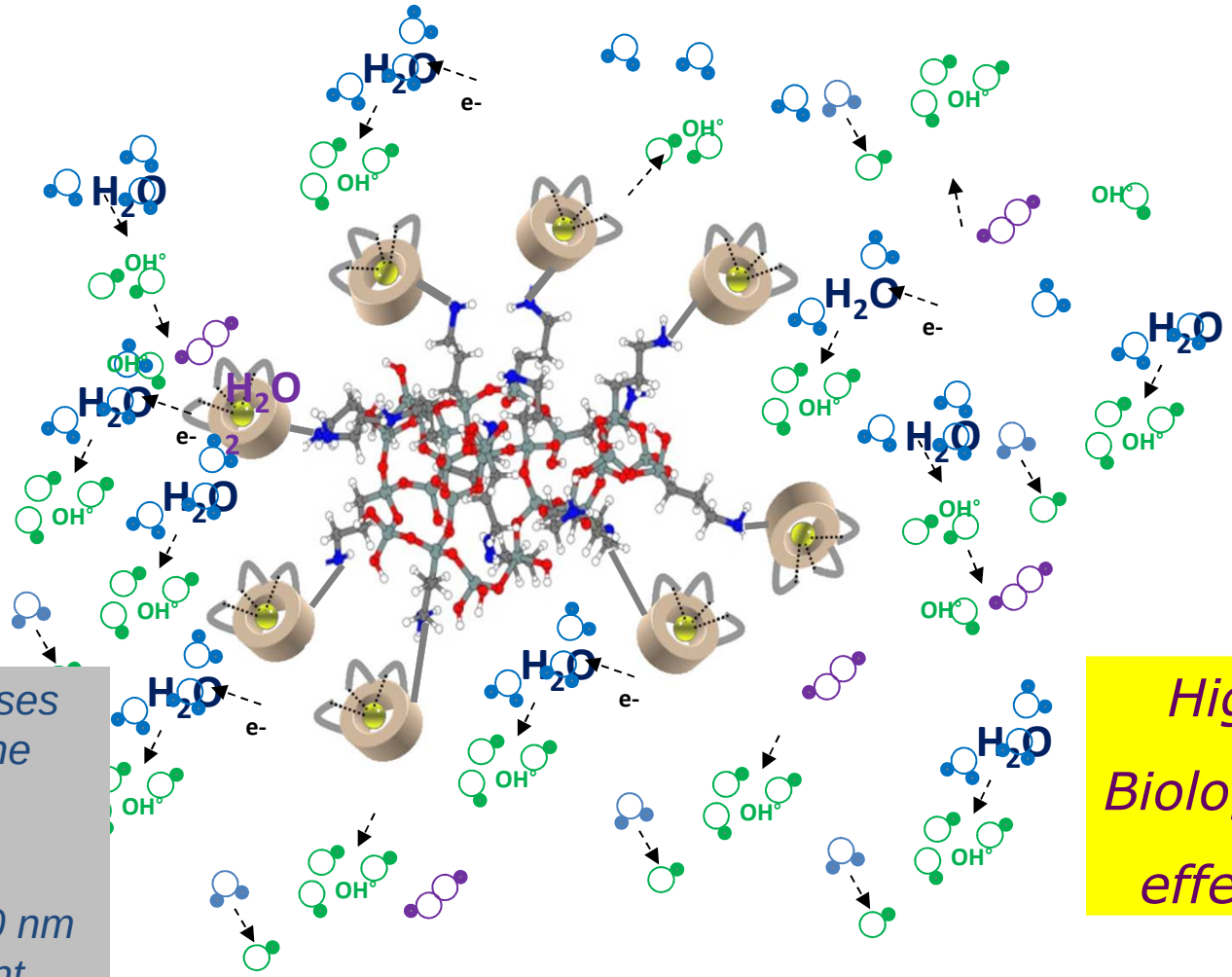
Auger shower propagation



Delivery of high doses in the local zone around nanoparticles

Formation of high concentration of active species

(radicals, peroxides,…)



Extremely large doses are observed in the vicinity of the Nanoparticles.

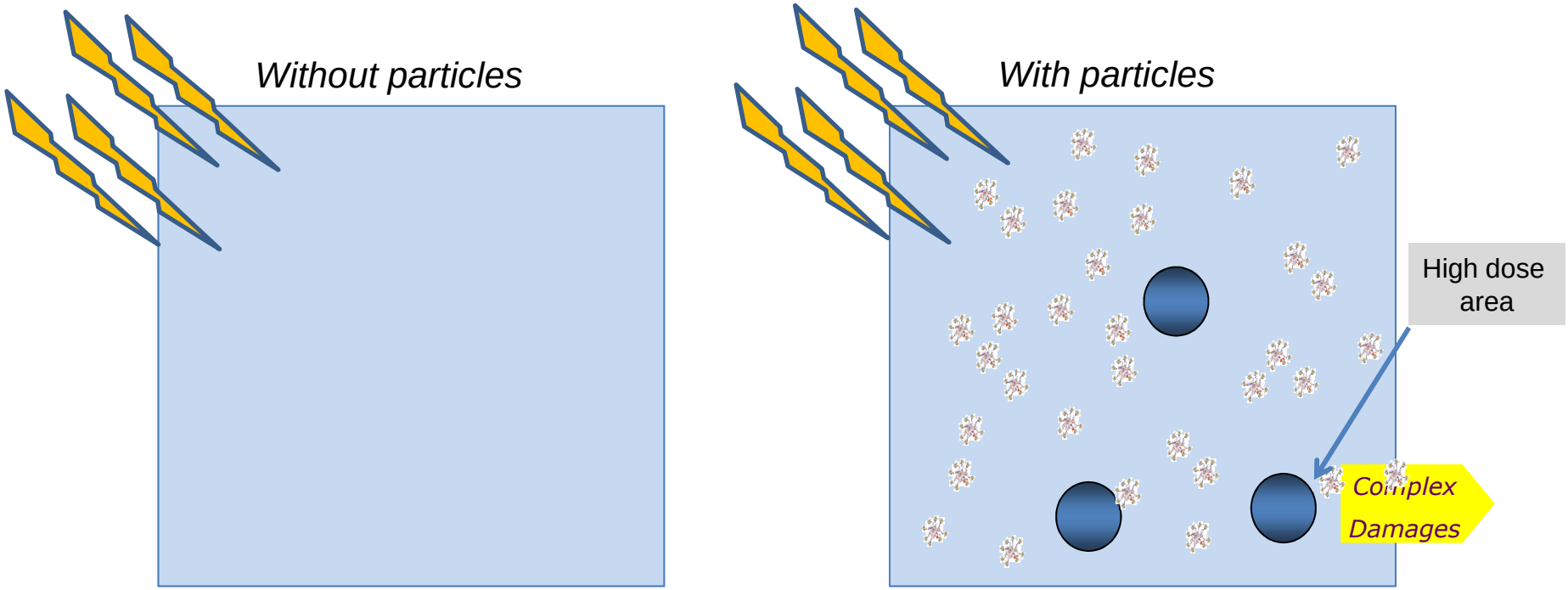
Hundreds of Gy <50 nm after 1 ionizing event…

High Biological effects

Average dose delivery is the same

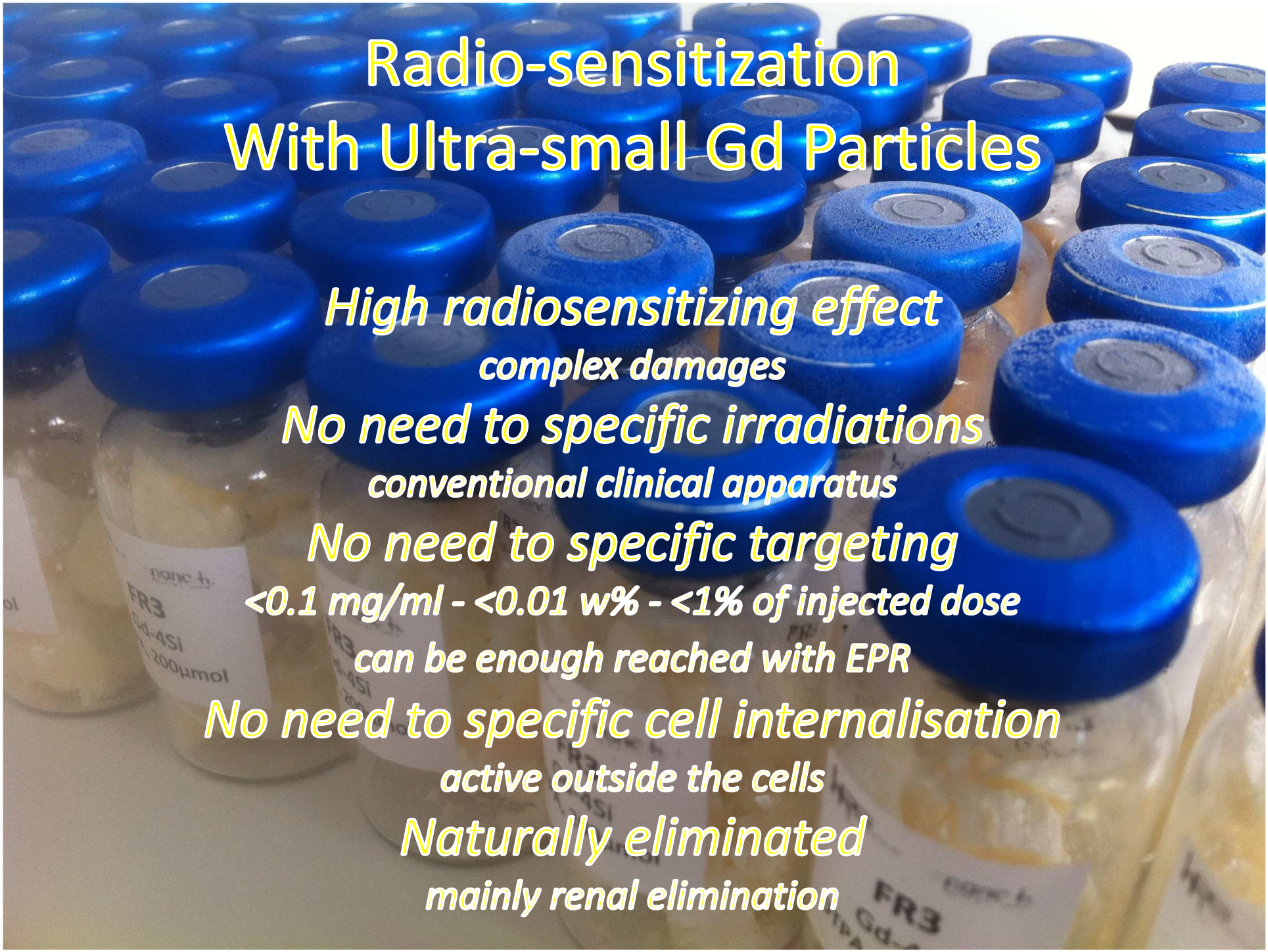
But the spatial repartition is very different

Delivery of high dose in the local zone around nanoparticles



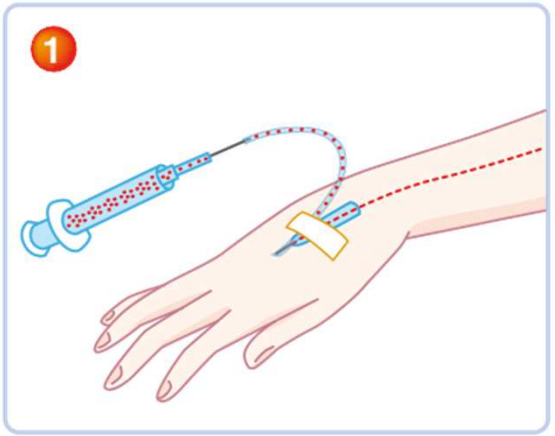
The biological effect could then be "similar" to the effect of dose inhomogeneity in heavy ion therapy: applicability of the LEM (Local Effect Model) ?

S. McMahon *et al.,* Scientific Reports 2011, *with Gold nanoparticles*

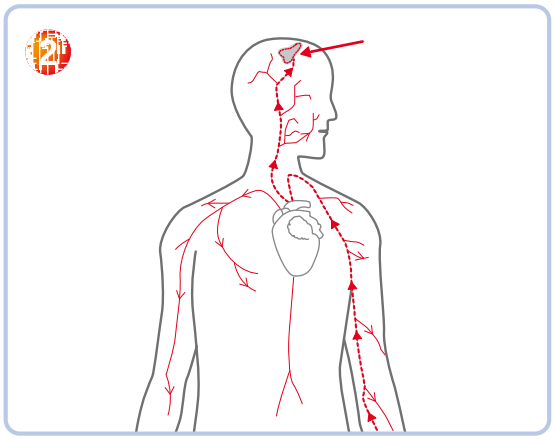

Radio-sensitization
With Ultra-small Gd Particles
High radiosensitizing effect
complex damages
No need to specific irradiations
conventional clinical apparatus
No need to specific targeting
<0.1 mg/ml - <0.01 w% - <1% of injected dose
can be enough reached with EPR
No need to specific cell internalisation
active outside the cells
Naturally eliminated
mainly renal elimination

Theragnostic compounds

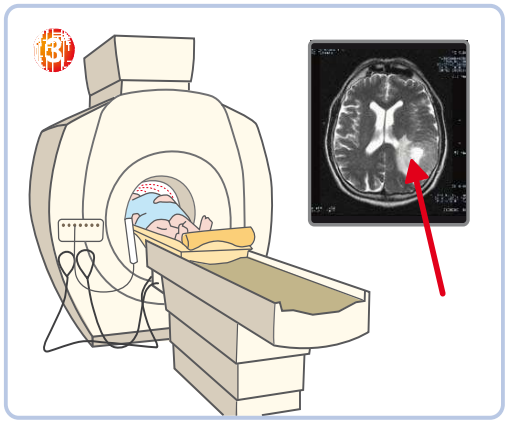
Radiosensitizer & MRI



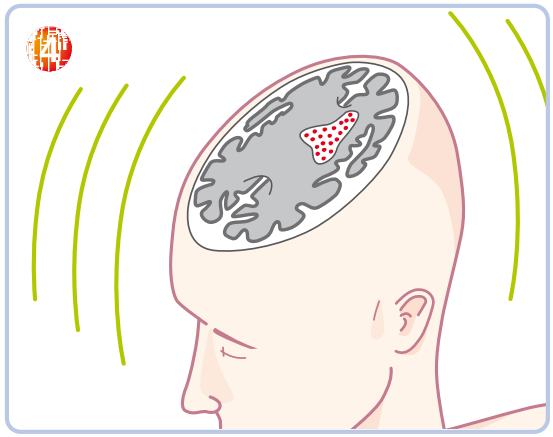
- Injection des particules en intraveineuse.



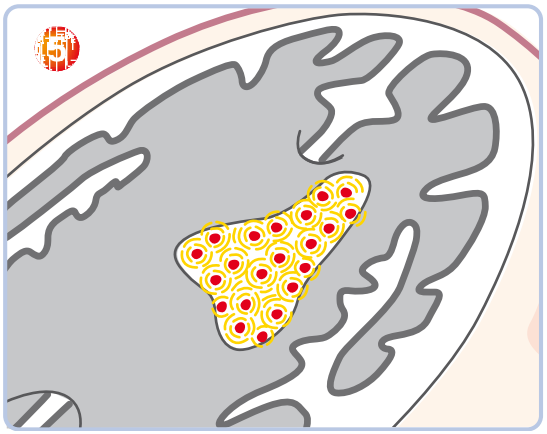
- Une fois injectées, les particules circulent dans le sang très rapidement et une partie s'accumule progressivement dans la tumeur.



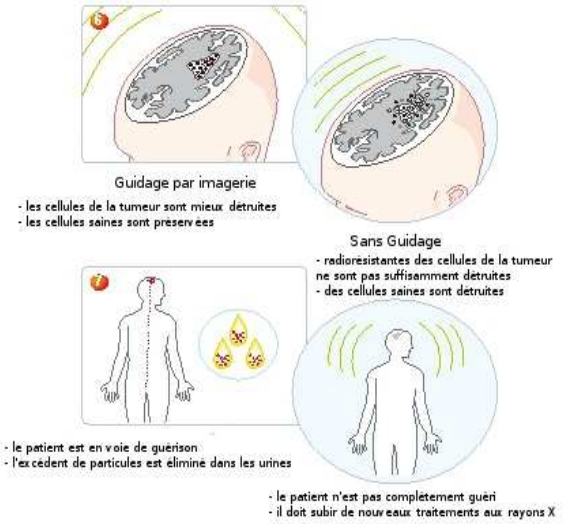
- Le patient est suivi par IRM. La tumeur apparait en blanc. Les particules ne circulent plus et une partie reste logée dans la tumeur.



- Le patient est soumis aux rayons X lors d'un traitement de radiothérapie localisé sur la zone et adapté à ce cancer.



Effet radiosensibilisant local de la particule qui Augmente l'efficacité du traitement



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Jean-Luc Coll
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Sandrine Dufort

Géraldine Le Duc

M. Dutreix

Vasile Stupar
Chantal Rémy
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Philippe Dugourd

Isabelle Deschamps
Françoise Chuburu
Gilles Lemercier

Denise Bechet
Muriel Barberi-Heyob

... et al.