UNAT
Ultra-small Nanohybrides for Advanced Theranostics

BIOEMTECH

Radiolabeling and imaging approaches for carbon-based nanohybrids

Antonis Skliris

Nanohybrides 18 – Bastia May-June 2022

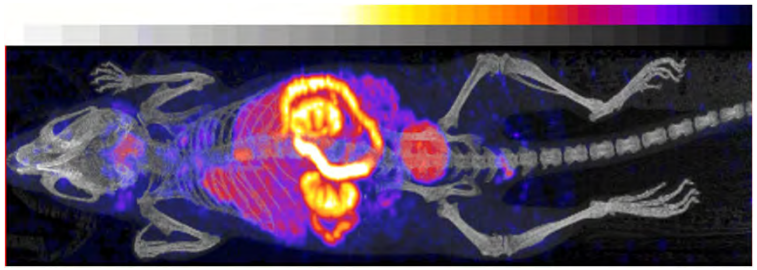
Who we are ?

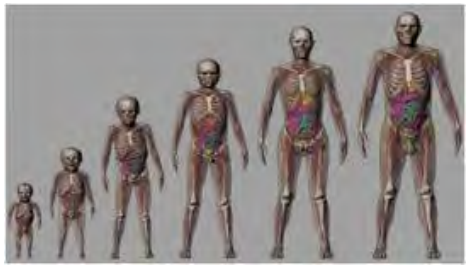
BIOEMTECH

BIOEMTECH is a SME who develops and offers innovative solutions in medical and pharmaceutical research in the **non-invasive *in vivo* imaging.**

We focus on **molecular imaging** and **biomedical engineering**:

- ✓ Design and construction of low-cost benchtop imaging devices
- ✓ Performance of preclinical imaging services in our imaging platform
- ✓ Computational simulations using Monte Carlo techniques





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BIOEMTECH Labs

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- R&D labs at the Technology Park "**Lefkippos**" in **NCSR Demokritos** since 2017.

BIOEMTECH | Labs

In-vitro Lab

In vitro testing (targeting, cytotoxicity, etc.)

Radiochemistry Lab

Radiolabeling of compounds & QC

Animal Hosting

Mouse models (oncology, etc.)

In-vivo Imaging Lab

2D/3D, static or dynamic PET/SPECT/CT imaging

Nanotechnology

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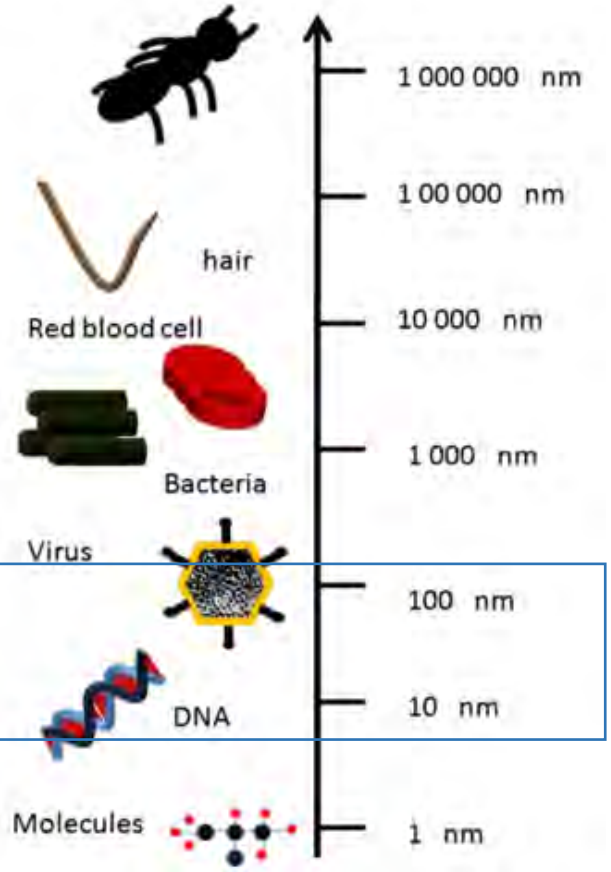
Nanoparticles

Structures where AT LEAST one dimension is between 1 and 100 nm (sometimes may be bigger)

Nanoscale

- Human: 1,800,000,000 nm
- Insect: 1,000,000 nm
- Hair: ~ 10,000 nm
- Blood cell: 8,000 nm
- Bacteria: eColi 2,000 nm
- Virus: 100 nm
- Protein: ~1-20 nm
- Molecule: ~1nm
- Hydrogen atom: 0.04 nm



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Multifunctional nanomaterials

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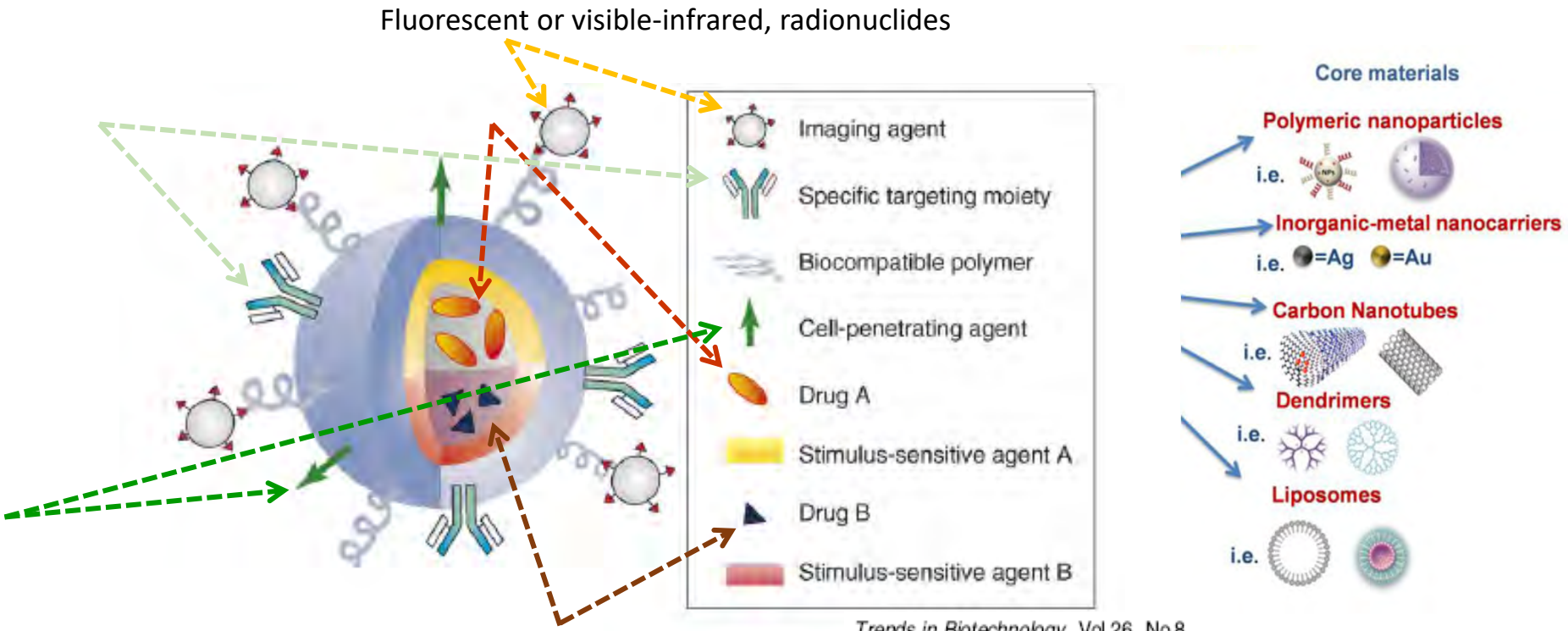

Trends in Biotechnology Vol.26 No.8

Advantages:

- Selected tumor/organ targeting;
- Low drug concentration in normal tissues
- Controlled drug release in target tumor/organ
- Minimization of side effects due to lower dose and targeted delivery

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Radiolabeling of compounds & QC

Labelling approaches

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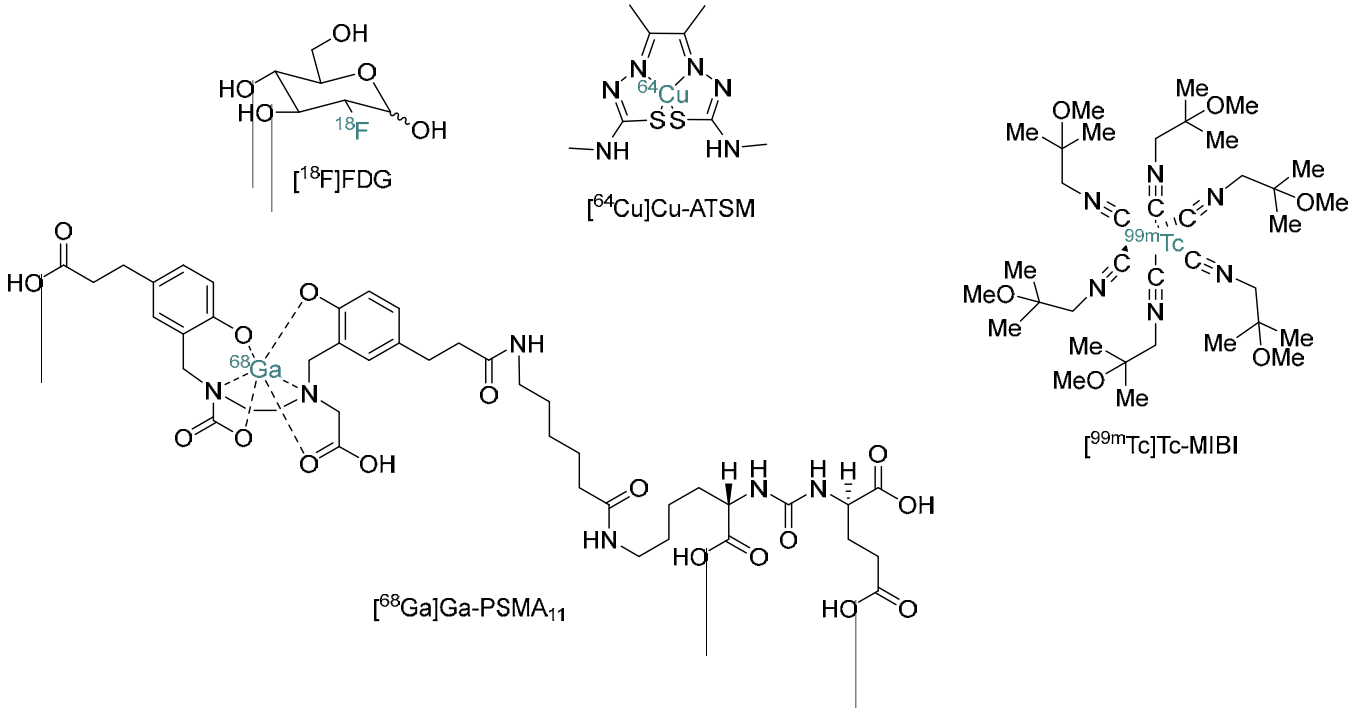
Metallic Isotopes: Use of precursors that chelate the radiometal, transmetallation reactions
Non-metallic isotopes: Use of organic chemistry reactions (SN, SN_2, SN_{Ar}, click reactions)



Radiolabelling approaches

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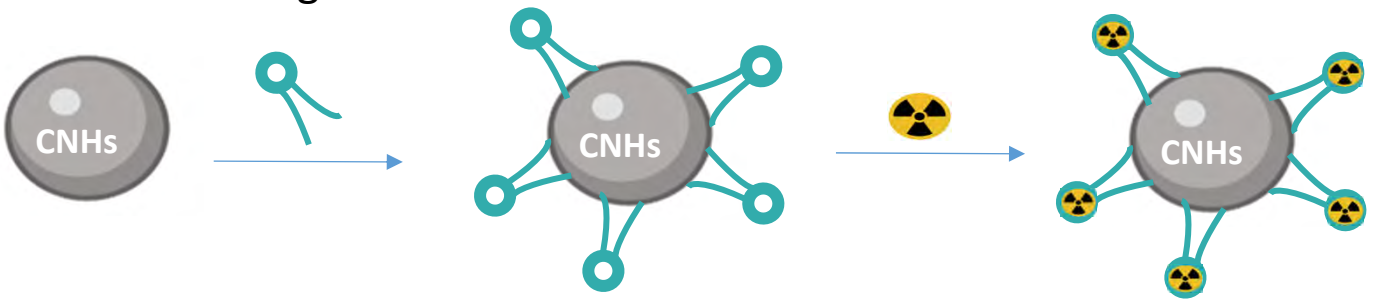
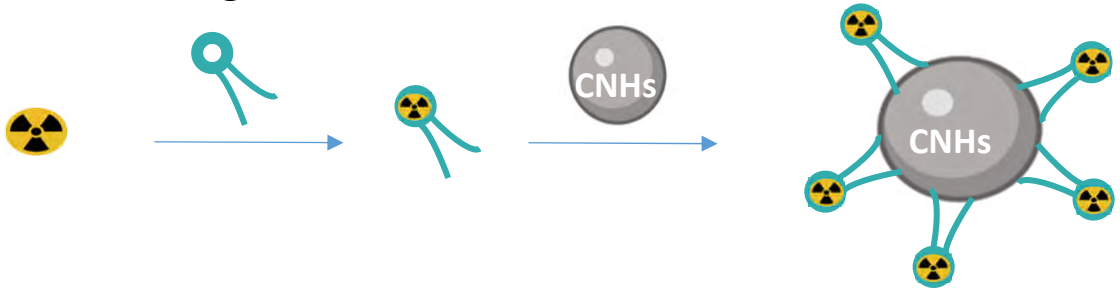
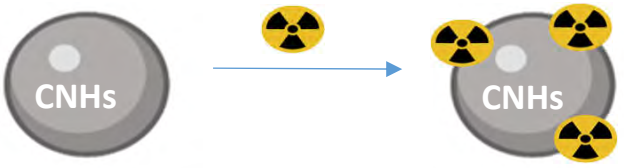
Post-radiolabeling



Pre-radiolabeling



Direct radiolabeling (no chelator)



Perform QC
Calculation of the
radiochemical conversion

Stockhofe et al. ,Pharmaceuticals 2014;7, 392-418

Types of radioisotopes

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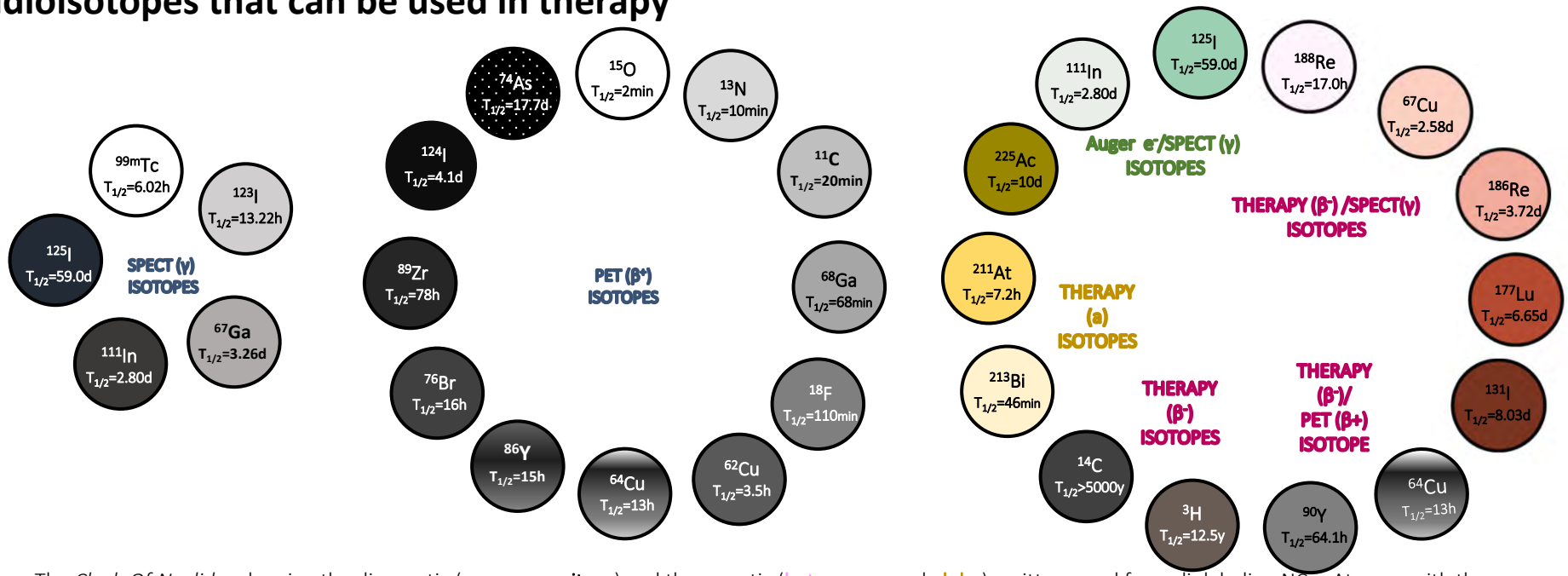
Long lived isotopes and Short lived isotopes

In medicine we usually use short-lived isotopes

Metallic radioisotopes and non metallic radioisotopes

ex. Metallic: technetium-99m gallium-68, copper-64 and zirconium-89; Non-metallic: carbon-11, fluorine-18

Radioisotopes that can be used in therapy



The *Clock-Of-Nuclides* showing the diagnostic (gamma, positron) and therapeutic (beta, auger and alpha) emitters used for radiolabeling NCs. At noon with the shortest physical half-life and ending with the longest physical half-life.

Analytical techniques

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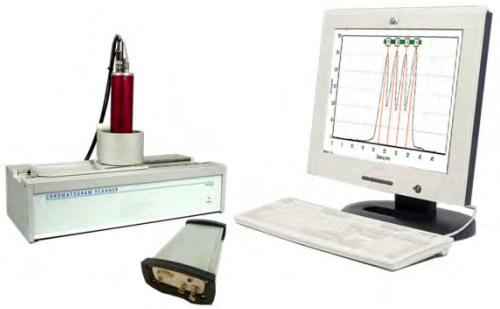
Radio-TLC



Radio-HPLC

γ-counter

Measures: the percentage of the incorporated radioisotope.
It is used: synthesis, stability tests

Measures: the activity per sample
It is used: metabolite analysis, tracer kinetic studies *in vitro*, and pre-clinical biodistribution studies

Scintillator detectors

QC of radiolabeled CNHs

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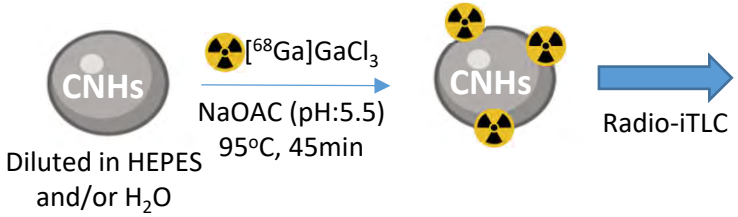
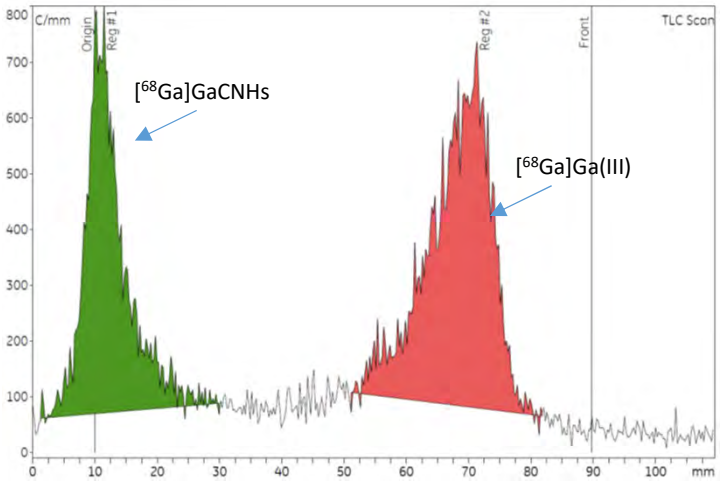
Why ? to ensure necessary chemical functionalization of the NHs for their successful in-vivo introduction

- Radiolabeling of different carbon nanohybrids (CNHs) received from the partners with [^{68}Ga]Ga(III) for PET imaging.

Nanomaterials tested	
CFO	carbon fluorooxide
CD3011	carbon nanodots
CDF19	carbon nanodots
S2	carbon nanodots

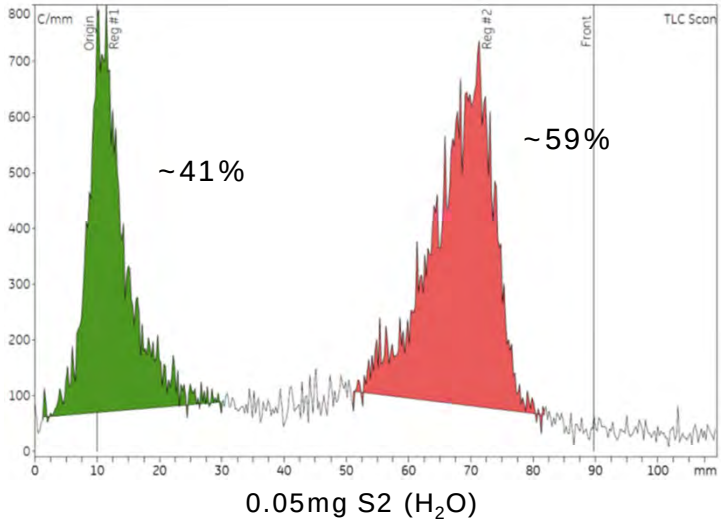


Whatman 3 MM, 0.1M EDTA

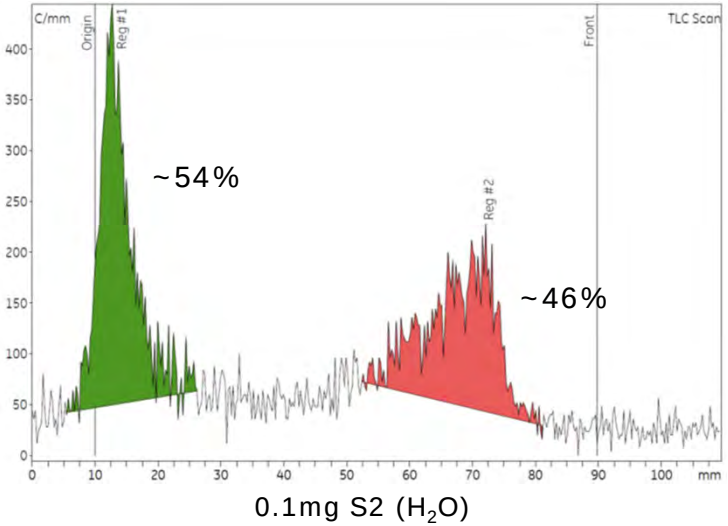


QC of radiolabeled CNHs

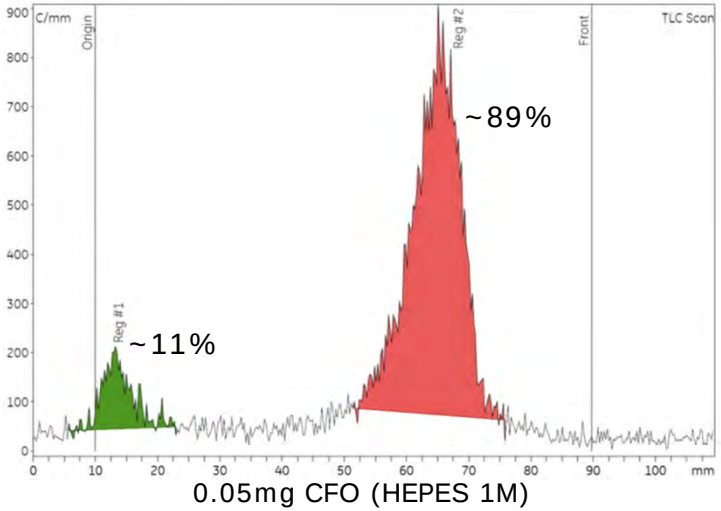
Radiochemical conversion (RCC)



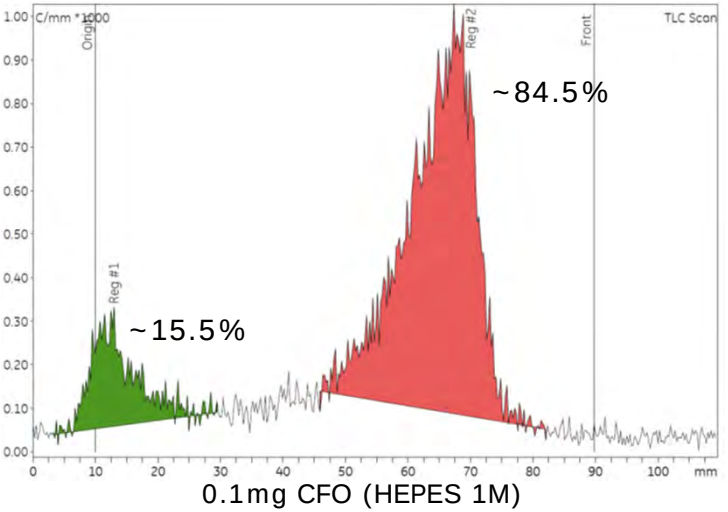
0.05mg S2 (H_2O)



0.1mg S2 (H_2O)



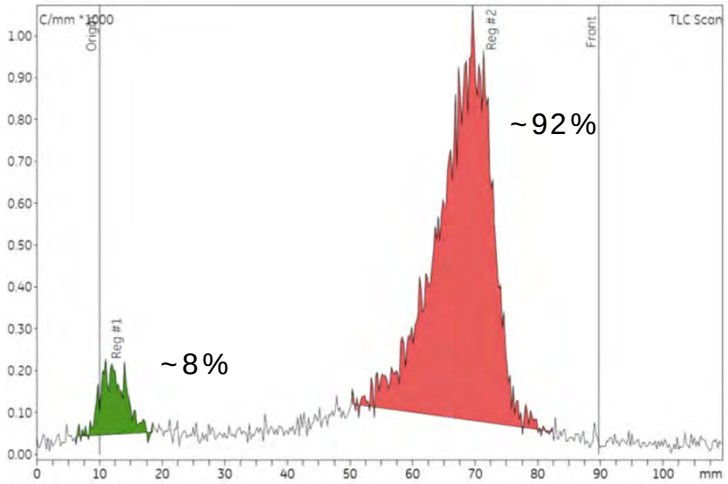
0.05mg CFO (HEPES 1M)



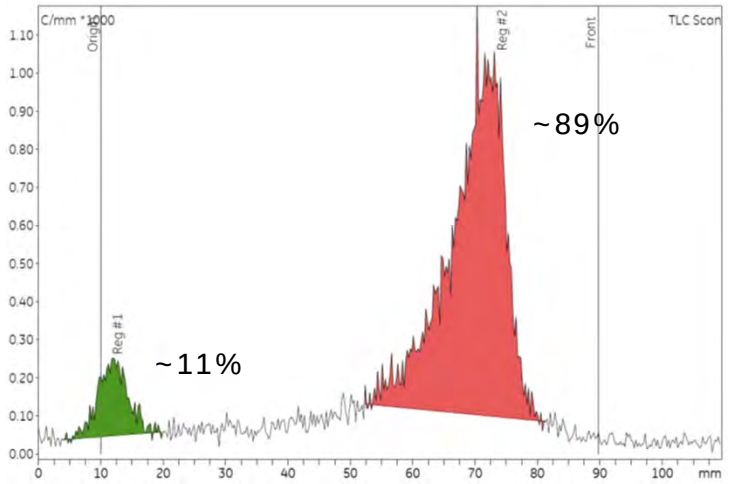
0.1mg CFO (HEPES 1M)

QC of radiolabeled CNHs

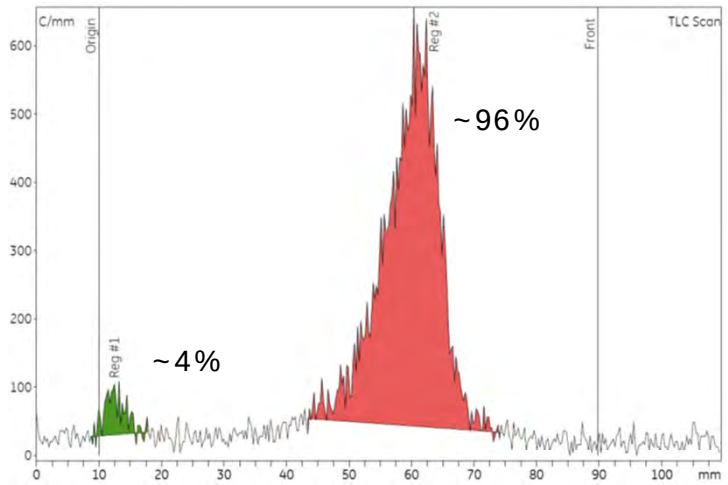
Radiochemical conversion (RCC)



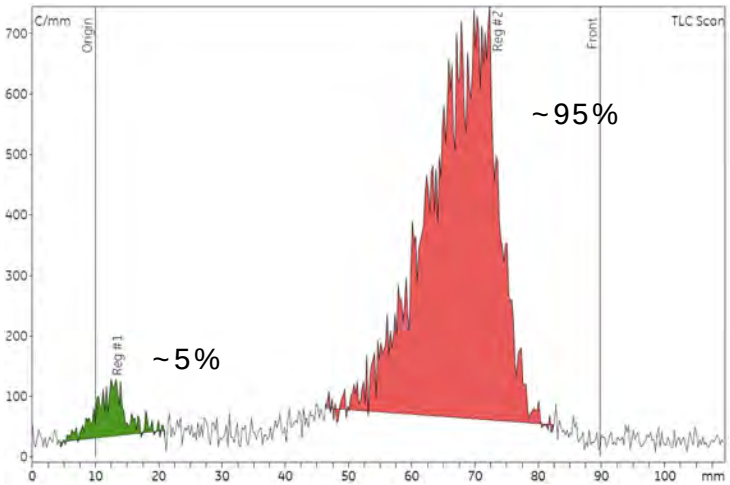
0.05mg CDF19 (H_2O)



0.1mg CDF19 (H_2O)



0.05mg CDF19 (HEPES 1M)



0.1mg CDF19 (HEPES 1M)

QC of radiolabeled CNHs
Radiochemical conversion

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Findings

- Radiochemical conversion up to ~54% (for S2)
- Low radiochemical conversion for the other CNHs (~3% - 15.5%)
- CNH dilution buffer & amount of CNHs affect radiolabeling

What is needed

- Optimization of the $[^{68}Ga]GaCl_3$ label protocol (pH, buffer ect.)
- Label nanomaterials with $[^{111}In]InCl_3$ for SPECT imaging

Next step

- Perform kinetics stability (temperatures, buffers)

QC of radiolabeled CNHs
Kinetic Stability Assays

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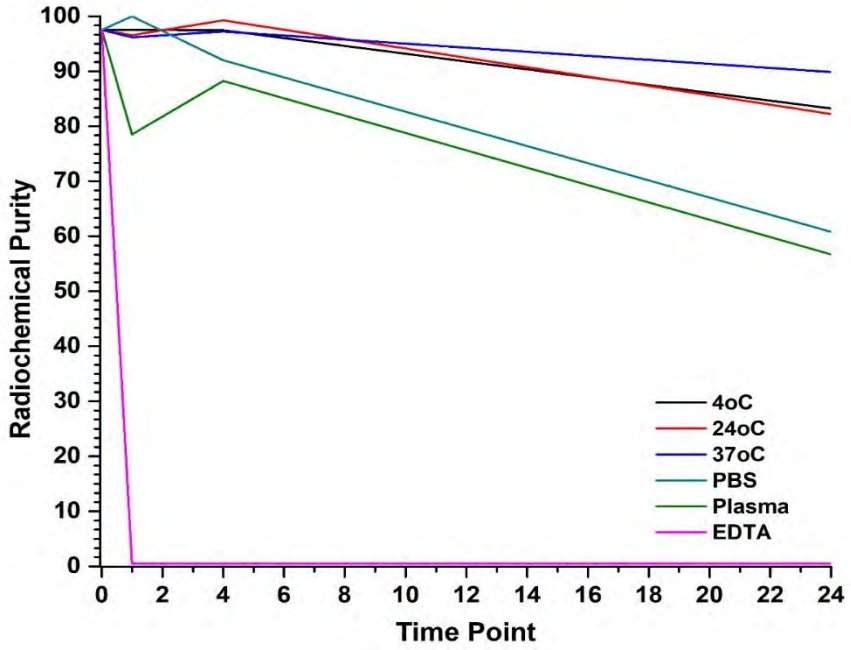
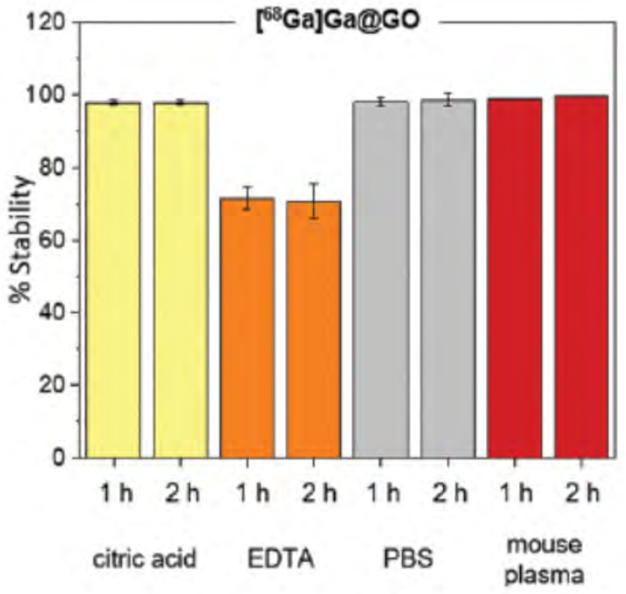
Aliquots of the reaction mixture were taken and incubated at different temperatures and different solvents
Radio-TLCs were taken at 1hr, 4 hrs and 24hrs post preparation.



Comparative diagram probing the potential *in vivo* stability of the **radiolabeled-NHs** at three time points post-preparation (p.p.) under various incubation conditions (PBS, EDTA, Plasma).



What's next

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If :

- RCC ≤ 95%
- Low stability over time

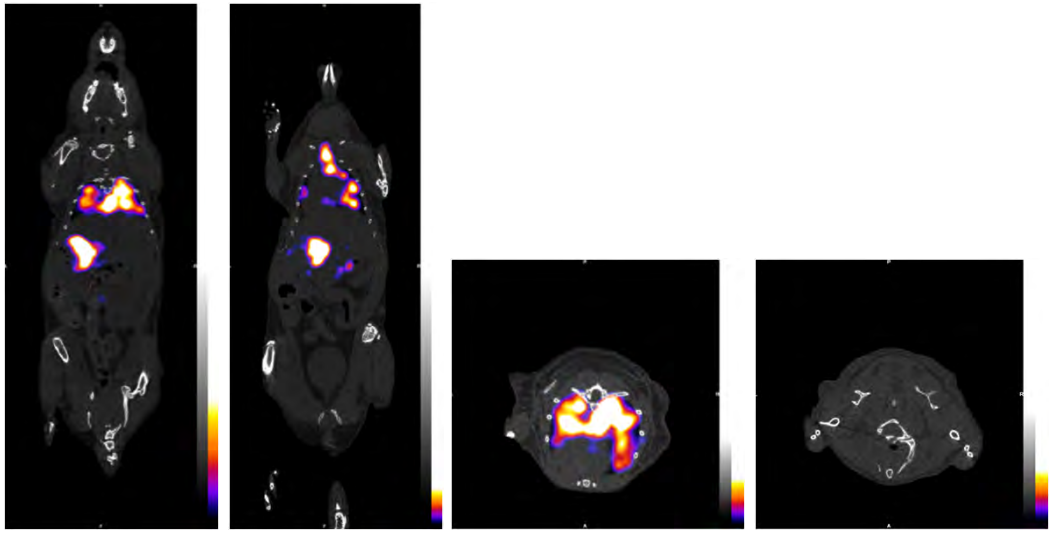
Then:

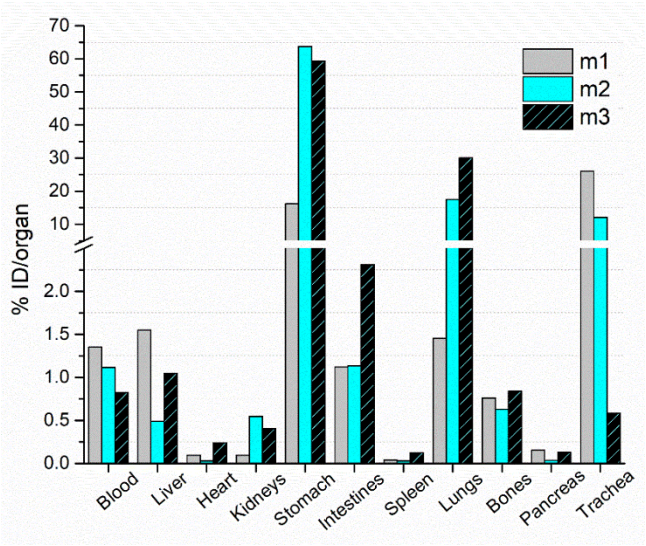
Return to nanomaterial preparation to improve characteristics and performance

- RCC ≥ 95%
- Stable over time

In vivo administration

- Imaging studies
- Biodistribution studies





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Our Imaging Platform

Small animal imaging

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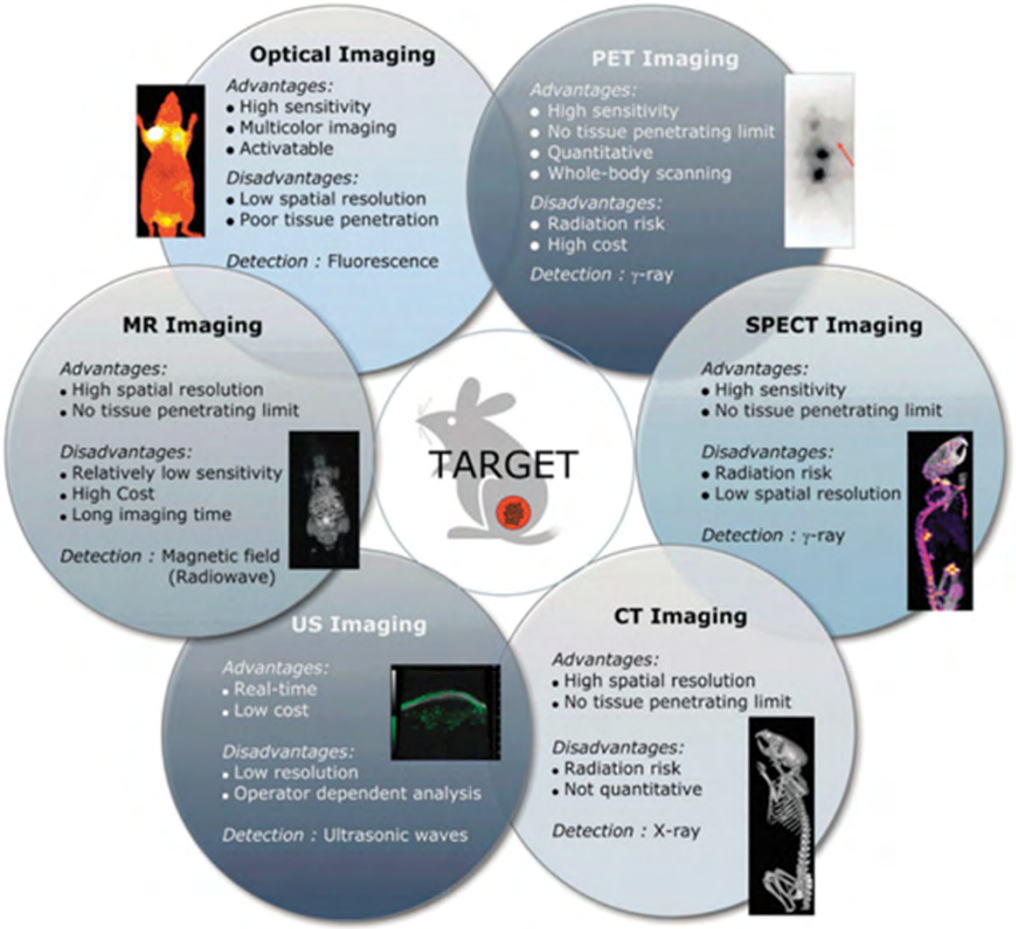
- In vivo ≠ In vitro
- Non-destructive. Repeated studies in the same animal
- Each animal serves as its own control
- We can efficiently image the entire animal simultaneously
- Imaging bridges the gap from cell to human studies
- Many potential targets
- A large variety of imaging techniques available



A Molecular Imaging Primer: Modalities, Imaging Agents, and Applications, Michelle L. James and Sanjiv S. Gambhir, Physiological Reviews, Vol. 92, No. 2, 01 Apr 2012.

Imaging modalities

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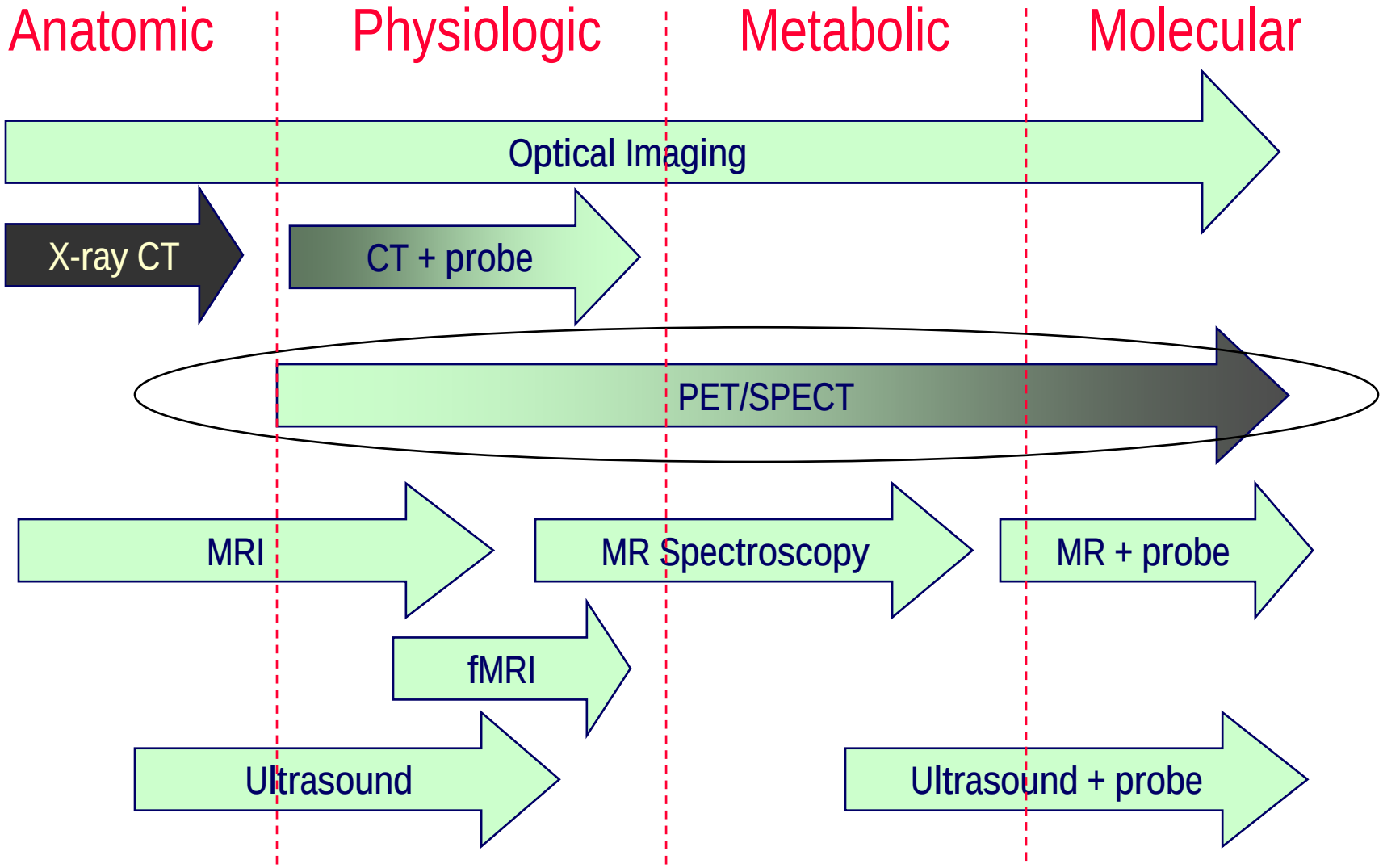

Imaging modalities

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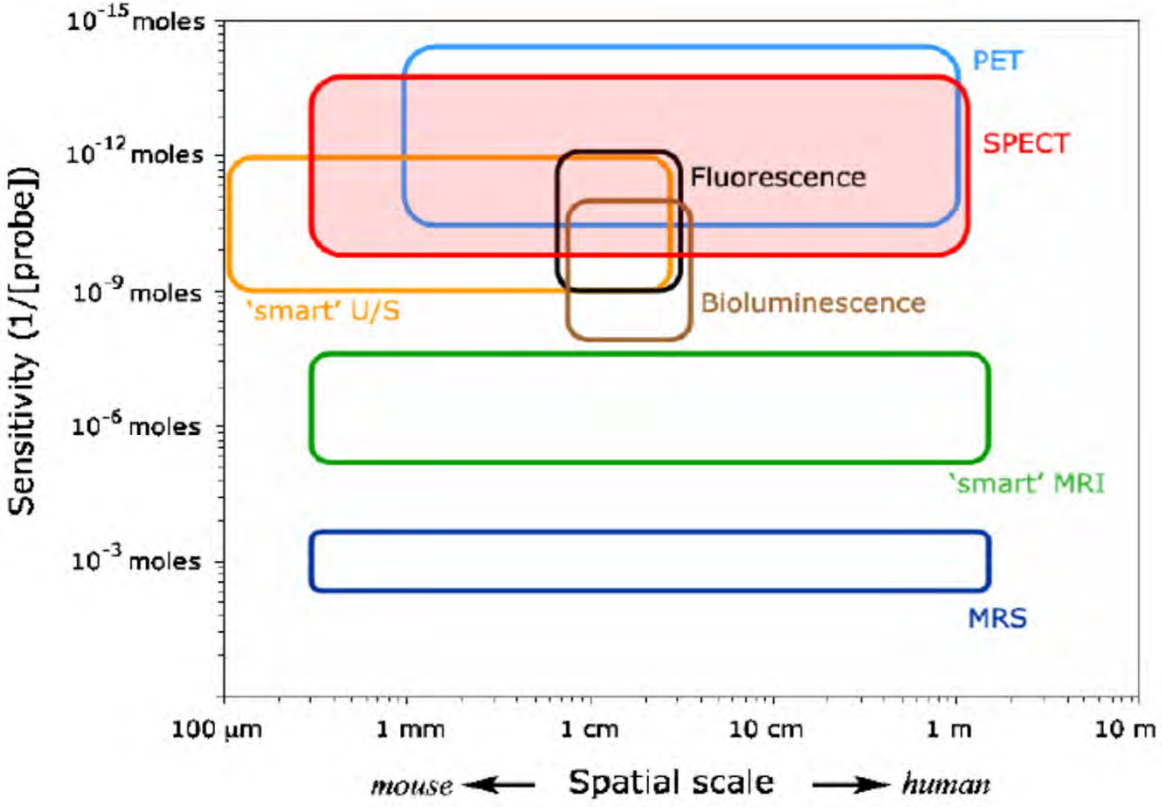

SPECT is among the most sensitive of the molecular *in vivo* imaging technologies and its spatial scale spans the resolution required for imaging small laboratory animals and the depth penetration required for imaging humans.

Comparison of imaging technologies

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Technique	Resolution	Sensitivity	Depth	Time
MRI	10-100μm	μ-mMol	No limit	Min
CT	50μm	m-cMol	No limit	Sec
US	<50μm	mMol	mm	Sec
PET	1-2mm	p-nMol	No limit	Min
SPET	< 1mm	p-nMol	No limit	Min
FRI	1-2mm	p-nMol	< 1cm	Sec
FMT	1-2mm	p-nMol	< 10cm	Sec

Questions for in vivo imaging

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In-vivo imaging provides an answer to the following questions:

- ✓ Do nanoparticles reach the target?
- ✓ Are they concentrated in other organs/tissues?
- ✓ How long do they remain on the target?
- ✓ How long do they stay in blood circulation?
- ✓ Are they stable post injection?
- ✓ What happens at the first minutes post injection?
- ✓ What is the best injected concentration?
- ✓ How shall we prepare the animals?
- ✓ When is the highest concentration in target?
- ✓ When is the best time point for tomographic imaging?
- ✓ When is the best time for biodistribution points?
- ✓ Which is the best injection route?

NPs and targeting

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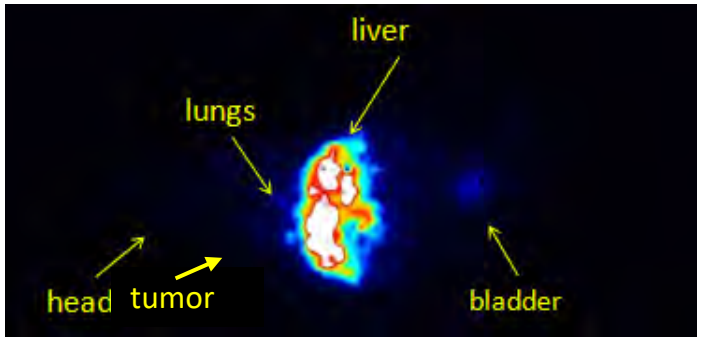
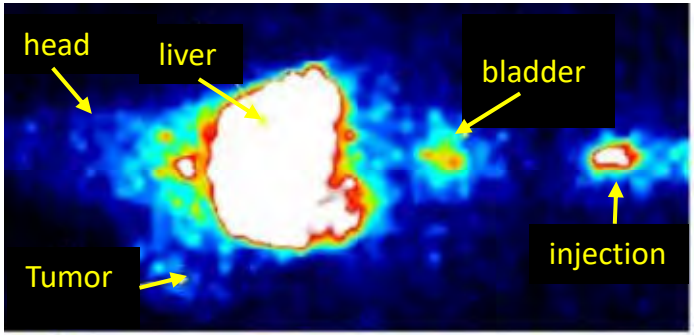
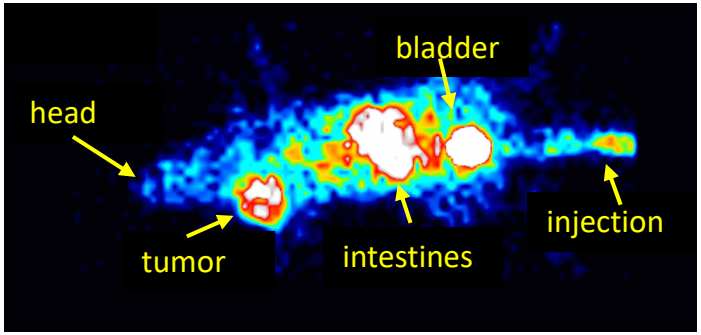
Assesment of **tumor targeting** does **not** need **tomographic imaging** for preliminary assessment



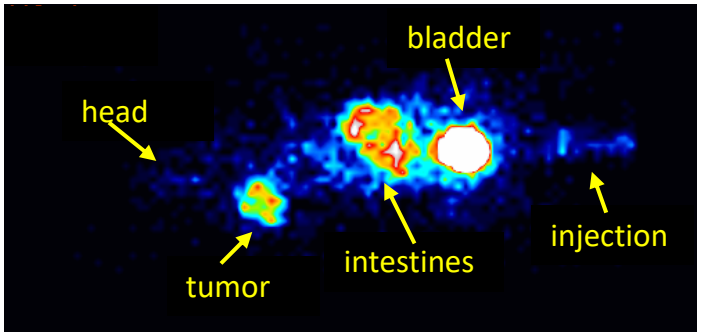
No concentration on tumor



Low concentration on tumor



Good concentration on tumor



High concentration on tumor

Different administration routes

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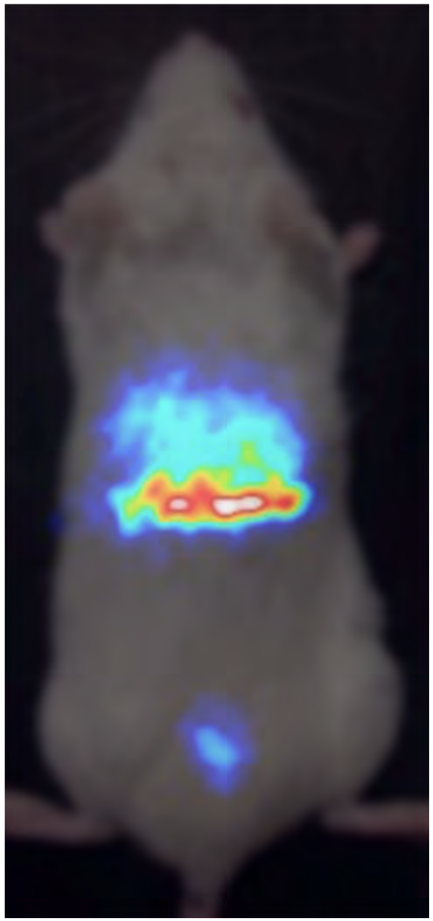
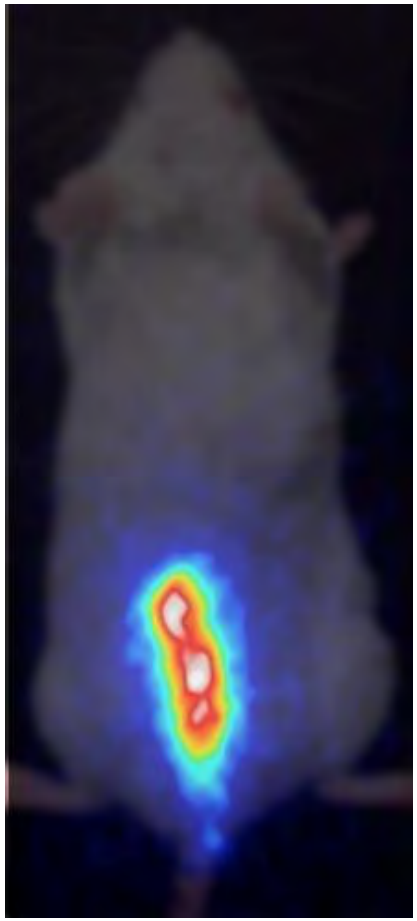
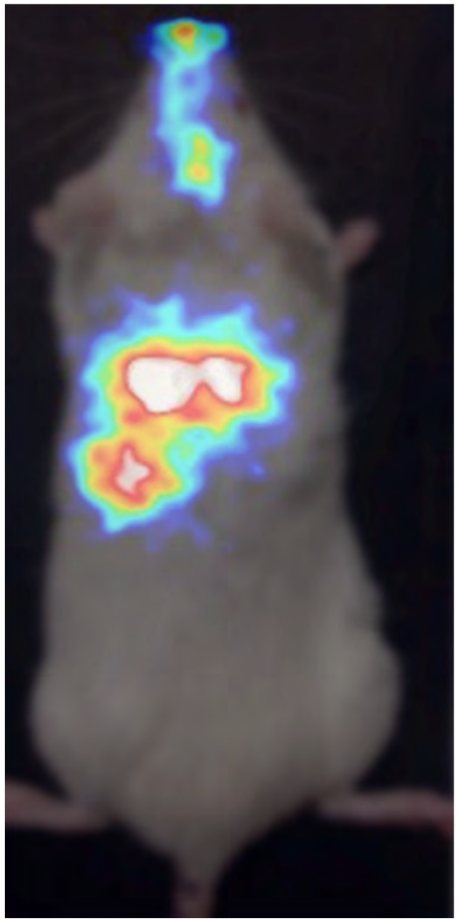
Retro-orbital



Intra-rectal



Intra-tracheal



Optimize protocol parameters

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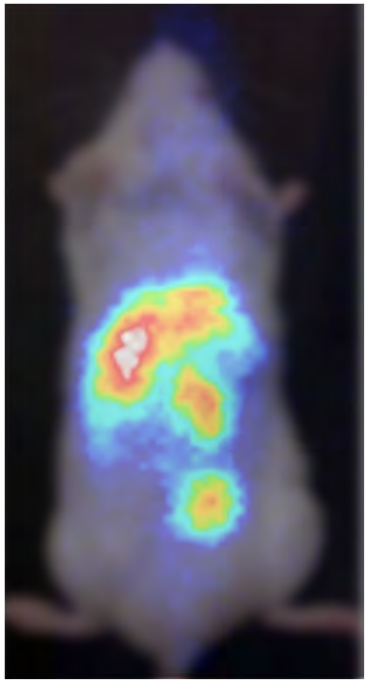
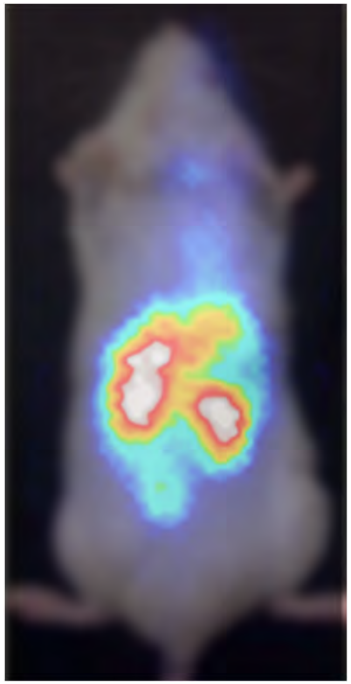
Different concentrations

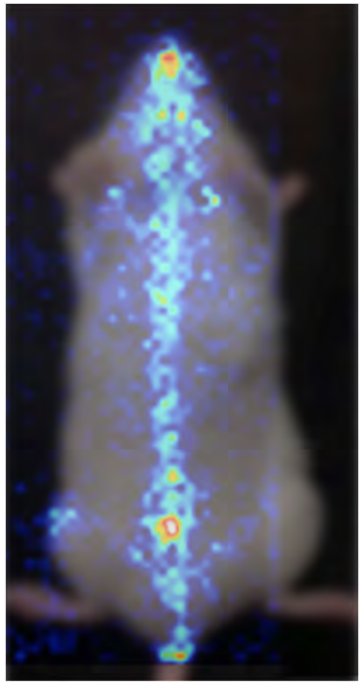


[^{99m}Tc]Tc-MIBI
100uCi
almost no heart signal

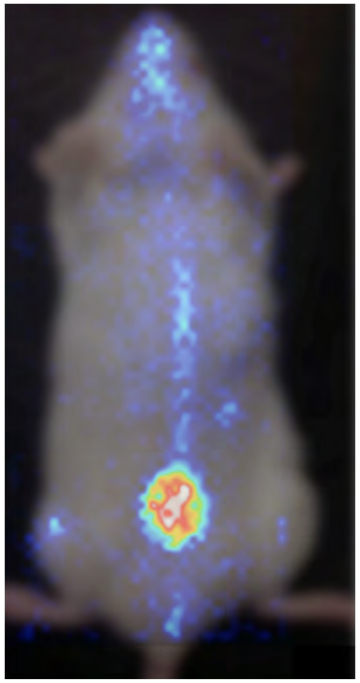


[^{99m}Tc]Tc-MIBI 2mCi
noticeable heart signal

Different preparation conditions



[^{99m}Tc]Tc-MDP after water fasting
Bones clearly visible



[^{99m}Tc]Tc-MDP under normal feeding conditions – need to "burn" image to see the bones

Identify unsuccessful tests

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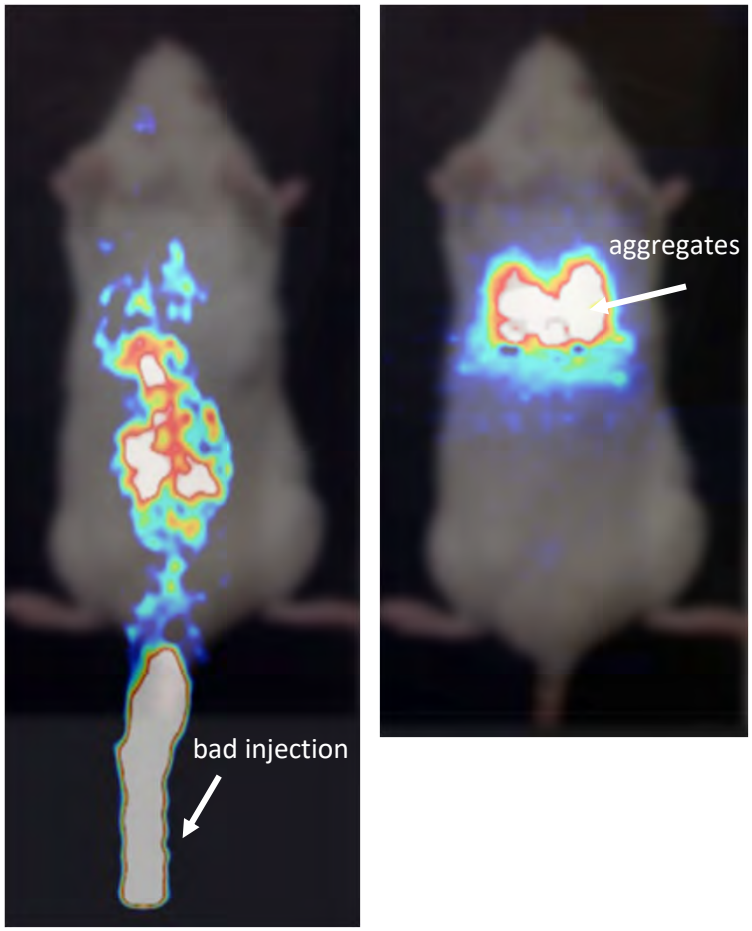
- Image <u>all</u> mice that will participate in a biodistribution study before dissection
- Bad injections can be identified and excluded to improve statistics
- Aggregations or other unexpected concentrations are visible
- **Animals with obvious "errors" are excluded from biodistributions**



Reduction of animals

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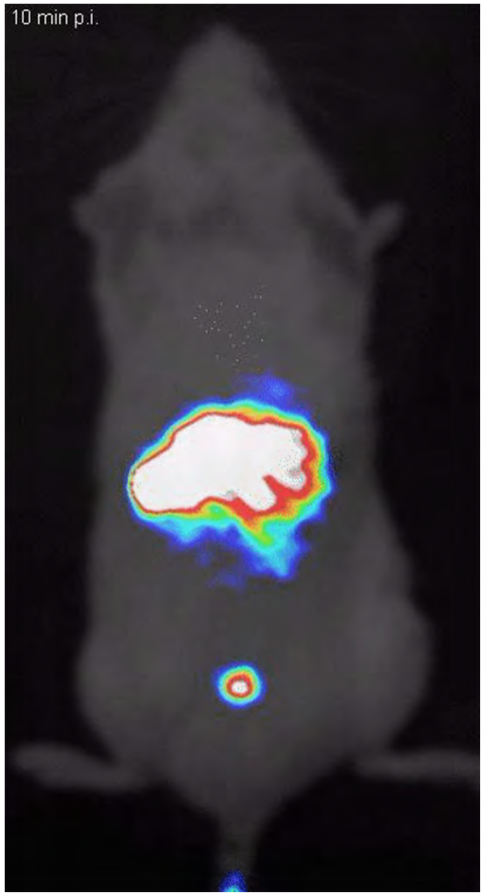
Scan the same mouse easily and fast over multiple time points, with frames down to few seconds. **Full bio distribution data with one animal**



Nanoparticles Biodistribution

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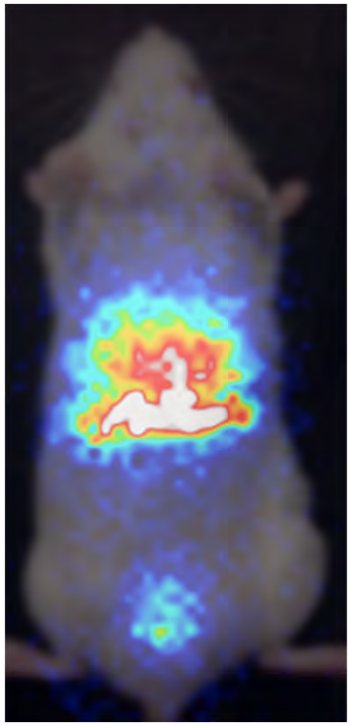
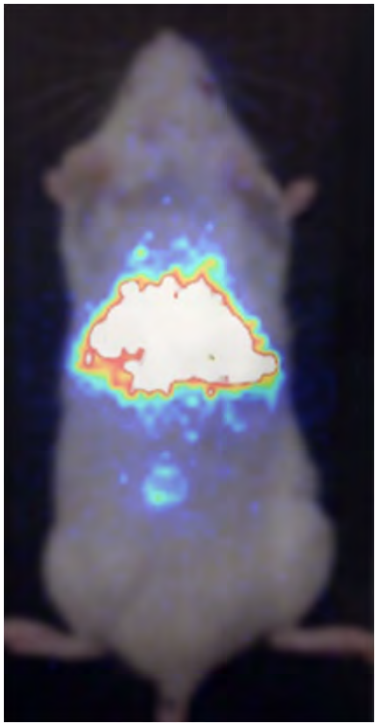
Magnetic Nanoparticles



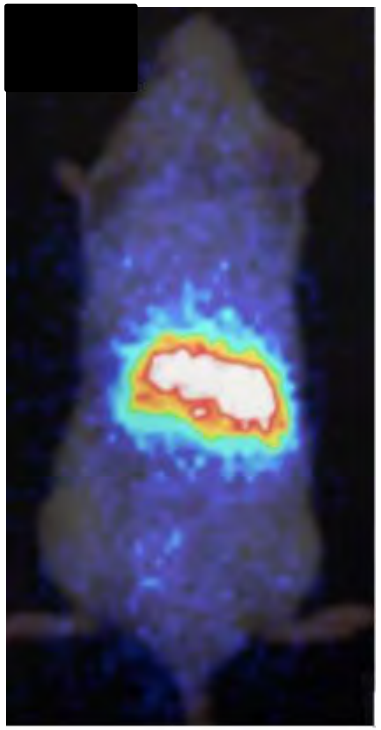
100uCi of [^{99m}Tc]Tc-MDP
in 100ul - 400μg FeCaP
20 min acquisition

Selenium Nanoparticles



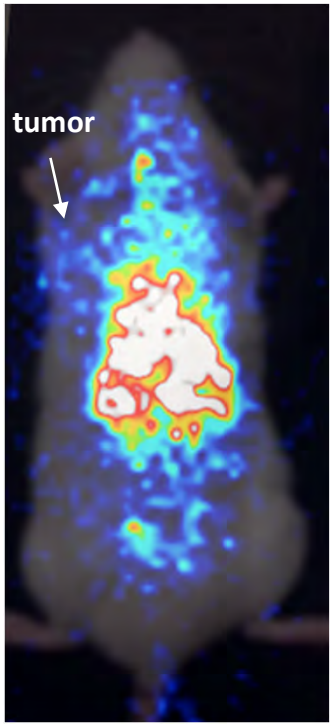
[^{99m}Tc]Tc-PLGA/SeNp
100 ul with 100 uCi
20 min acquisition

Gold Nanoparticles



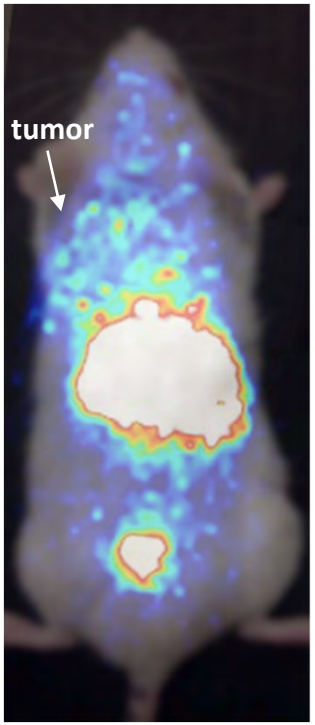
[^{68}Ga] Gold nanoparticles
100 ul with 10 uCi
10 min acquisition

Liposome Nanoparticles



U-87MG mouse, 100 μCi
of [99mTc]Tc-NT Lipo-Cys
20 min acquisition

Silver Nanoparticles



U87MG mouse, Ag-^{99m}Tc (100 μCi)
20 min acquisition

Imaging examples of nanoparticles

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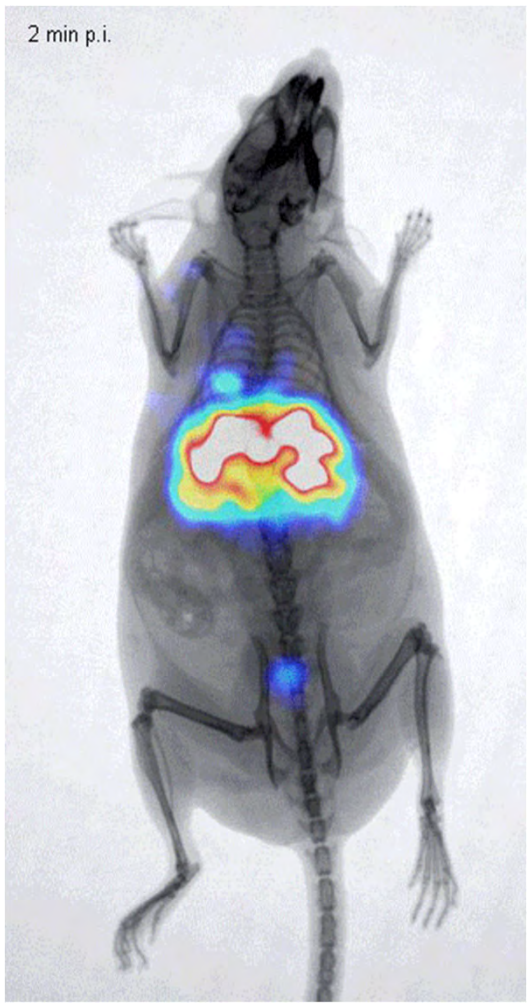
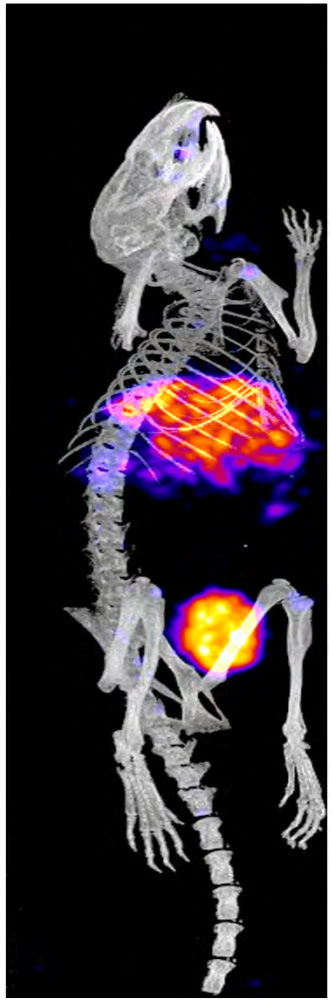
Nanoparticles

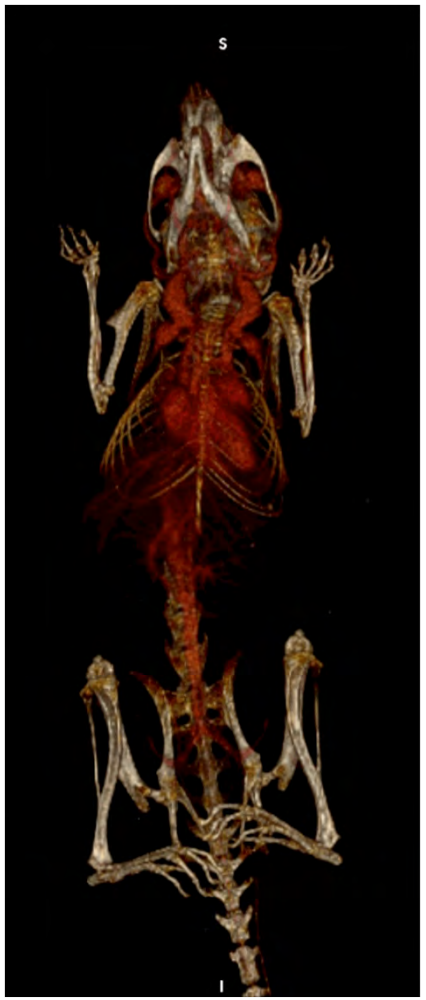
i. Dynamic imaging of silver NPs

ii. SPECT imaging of magnetic NPs

iii. CT imaging of gold NPs







Indicative preclinical studies

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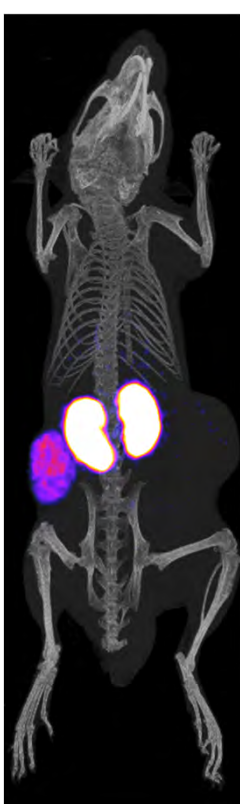
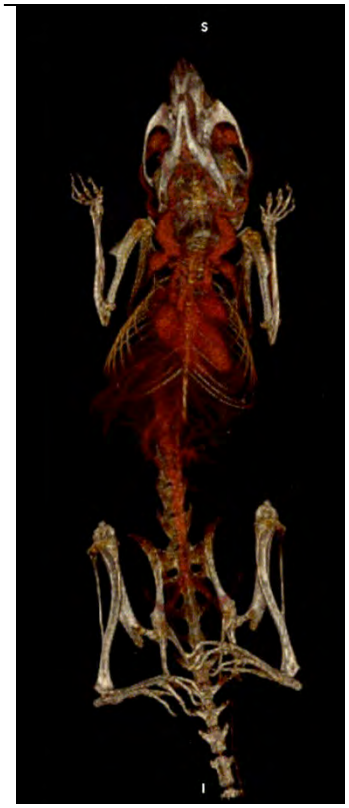
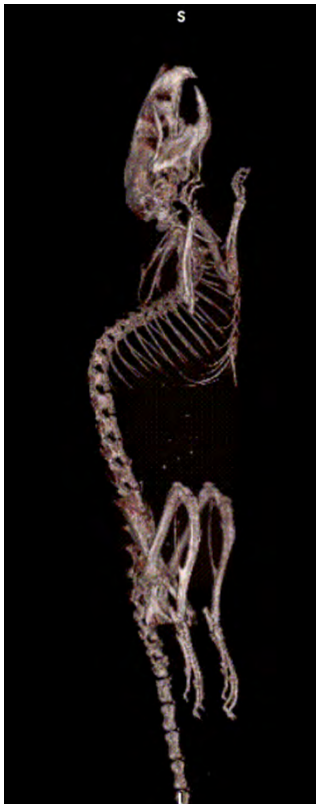


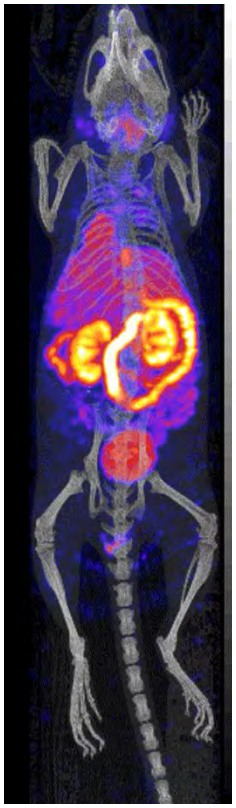
Tumor imaging



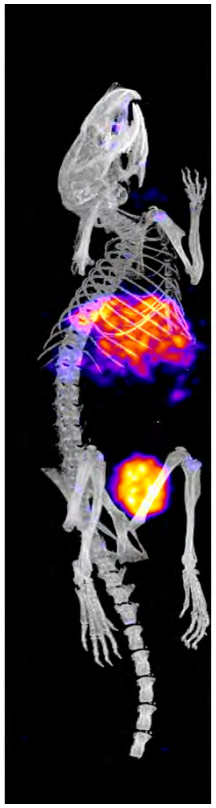
Contrast agents



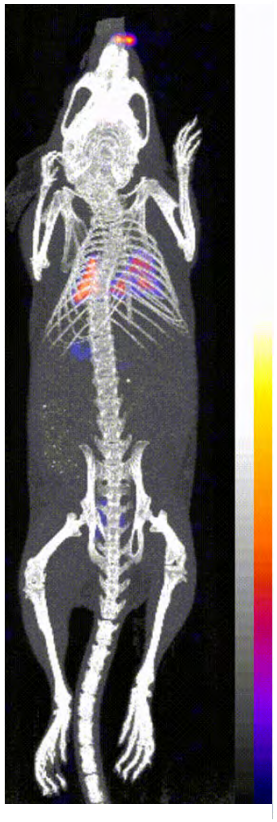
Bone imaging



Cardiac imaging



Nanoparticle imaging



Lung imaging

Imaging examples (I): Oncology

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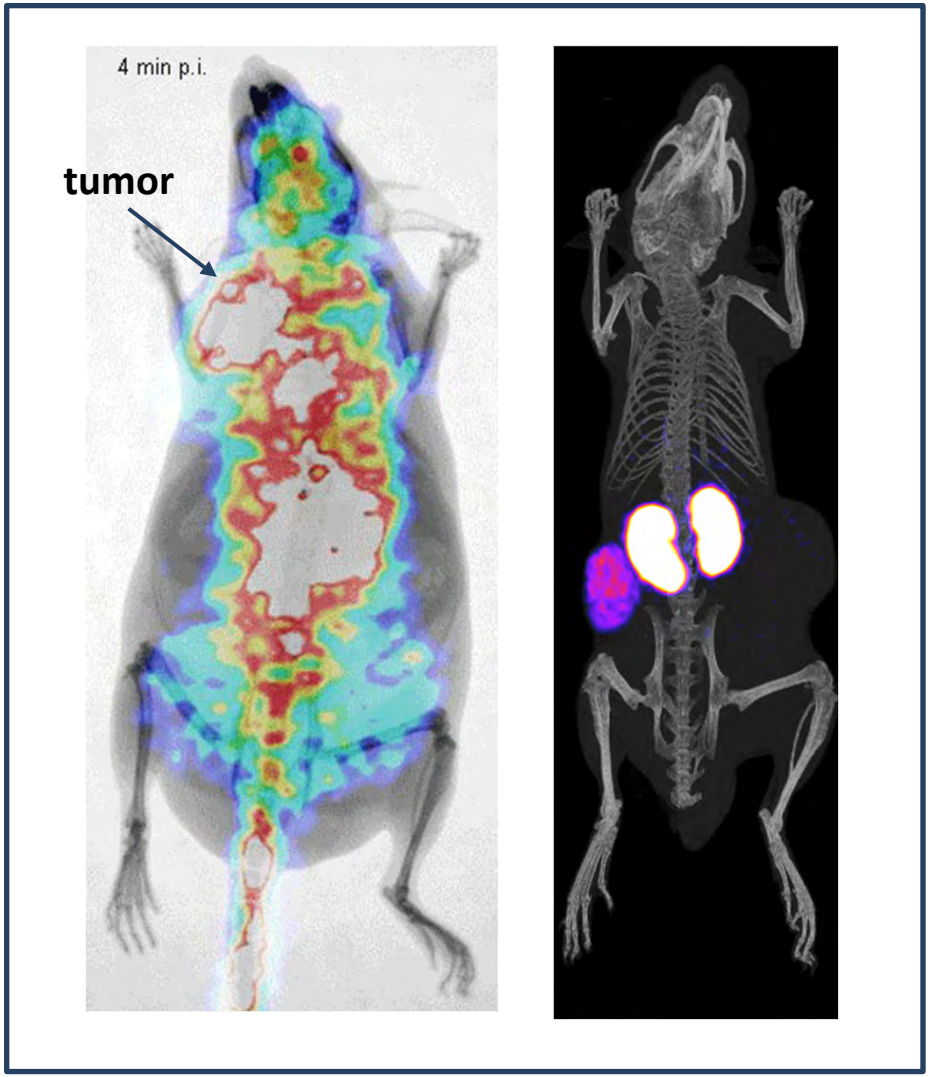
Tumor imaging

Tumor bearing mouse i.v. injected with 100 uL. 1mCi [^{99m}Tc]Tc-peptide:

i. Dynamic imaging for the first 2 hrs p.i.

ii. 3D SPECT imaging @ 4 hrs p.i.



Imaging examples (II): Lungs

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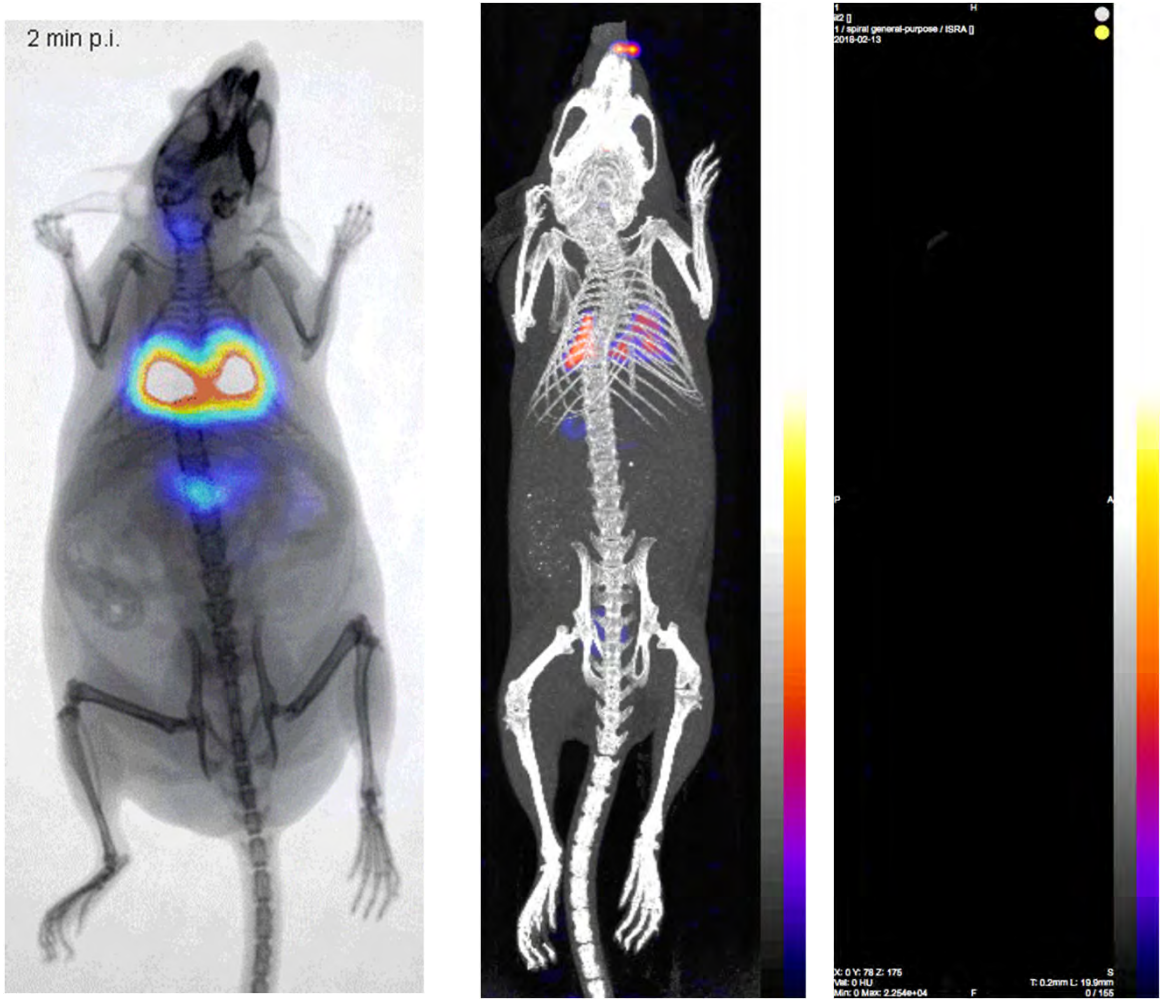
Lung imaging

Normal mouse and intra-tracheal administration:

a) Dynamic imaging: 1 hr p.i.

b) Imaging @ 1 hrs p.i.



Molecular Screening Applications

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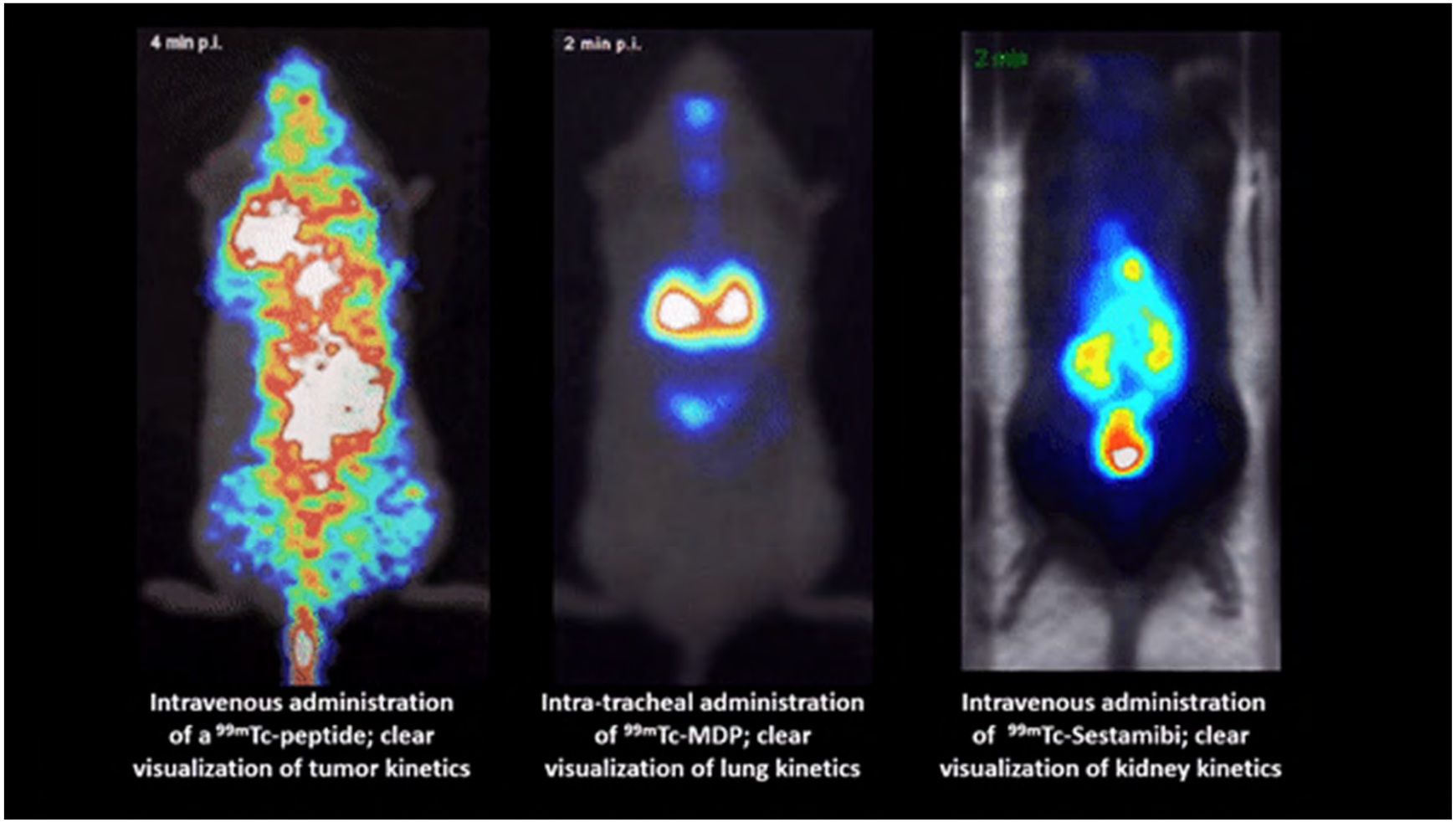

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Conclusions

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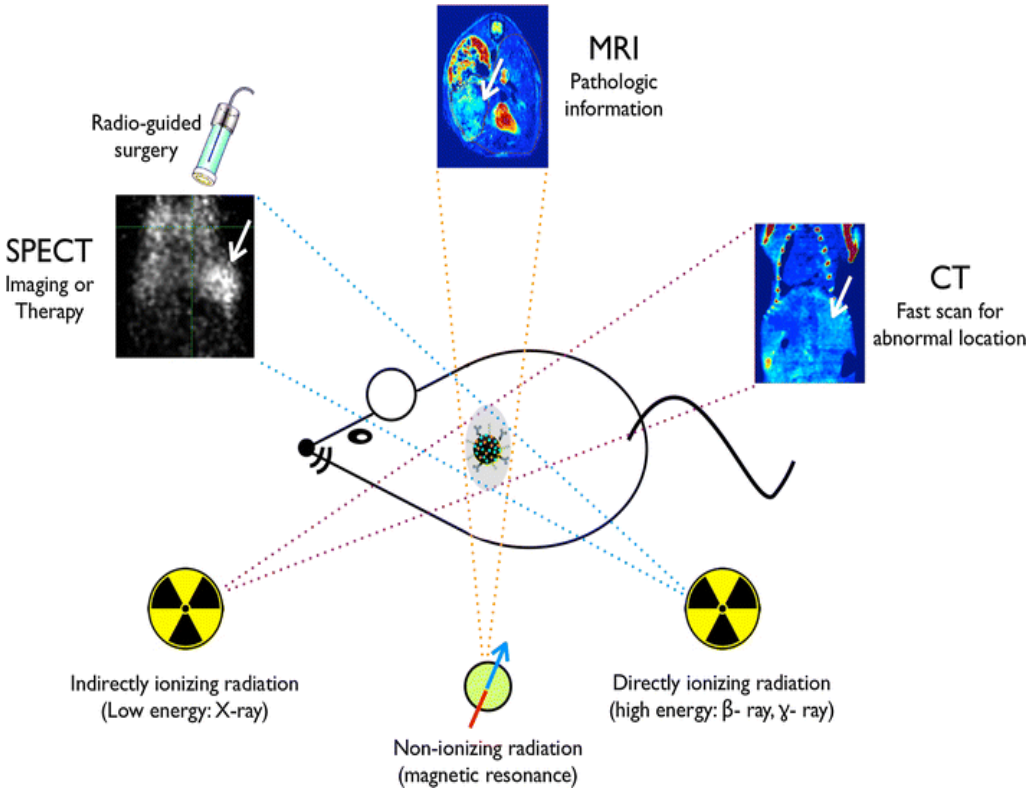
- Radiolabeling of the nanoparticles may be tricky
  - There are lot of parameters to be taken into consideration.
- Imaging technologies are powerful tools for evaluating the biodistribution of different nanoparticles.
  - They provide unique non invasive tools for repeated studies over time
- It is important to understand the advantages of each imaging technology as well as the properties of different nanoparticles



Our team…

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Team includes

- Biomedical Engineers
- Mechanical Engineers
- Physicists
- Biologists
- Radiochemists
- Software Developer
- Software Engineer
- Project Management

Thank you for your attention