Selective oncological theragnostic based on radioactively labeled exosomes

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Experimental Medicine and Surgery

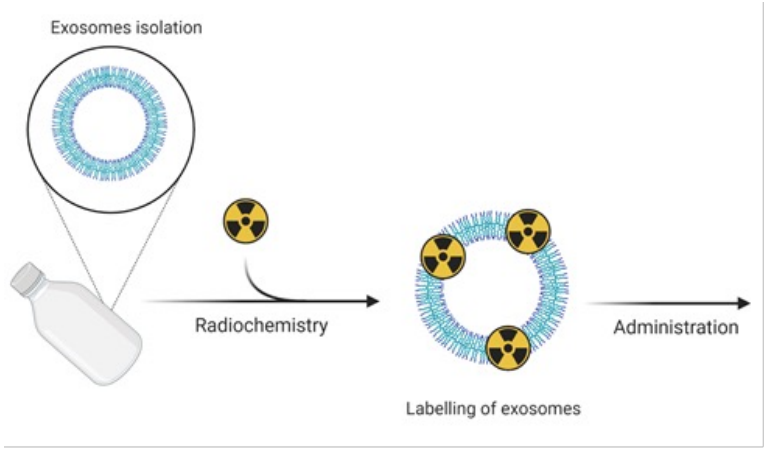
Bioengineering Dept.

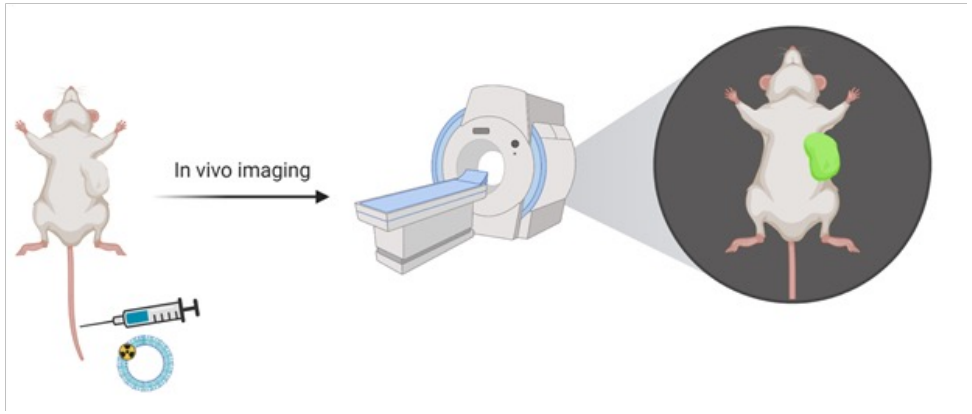
CNIC

Molecular Probes Lab

PRISMAP project

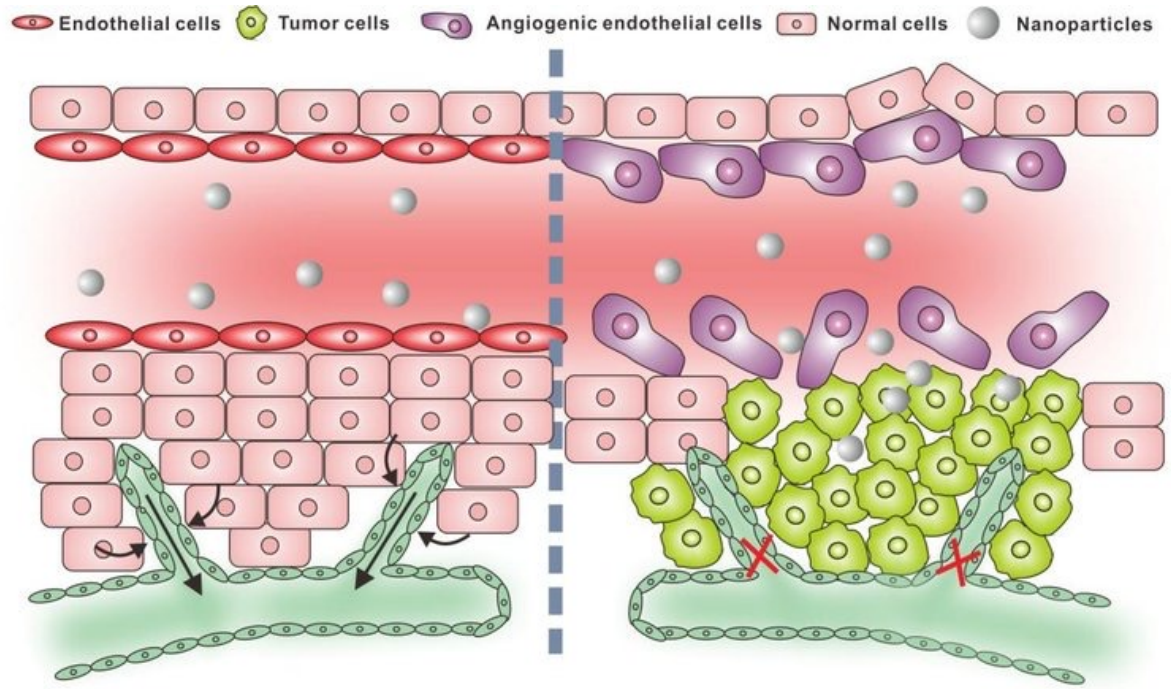
Development of new radiotheragnostic agents based on natural nanoparticles (exosomes) radioactively labeled with the novel therapeutic and diagnostic isotopes Terbium 161.





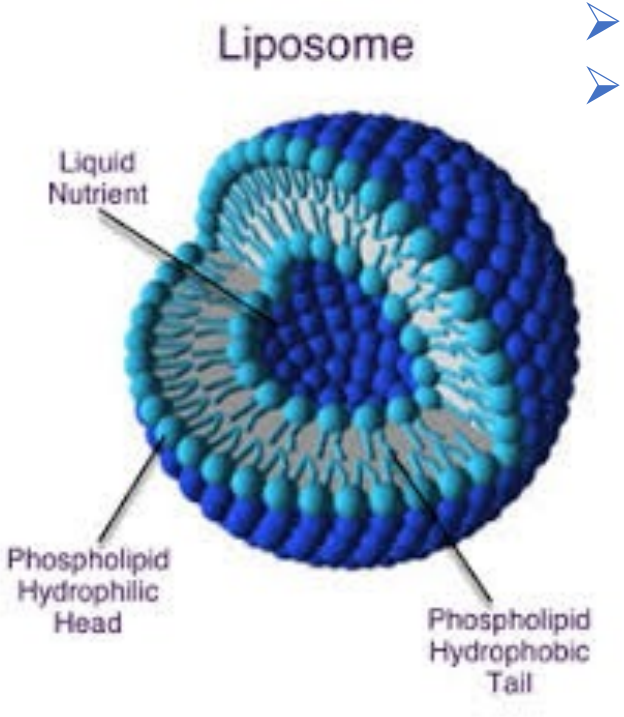
Nanoparticles theragnostics

- Large surface-to-volume ratios
- An arsenal of surface functionalization possibilities (functional groups)
- Capacity to carry large amounts of cargo
- Enhance permeability retention effect (EPR)



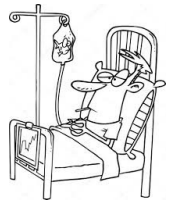
Liposomes and nanotechnology

STRUCTURE AND APPLICATIONS



- Drug cargo
- Superficial modification

FROM BENCH TO BEDSIDE



Pubmed: liposomes + preclinic +

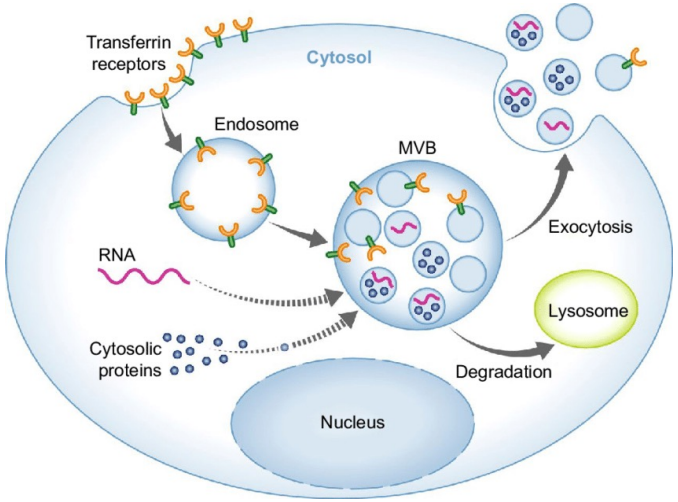
- Diagnostic : 12.873
- Therapy: 22.131

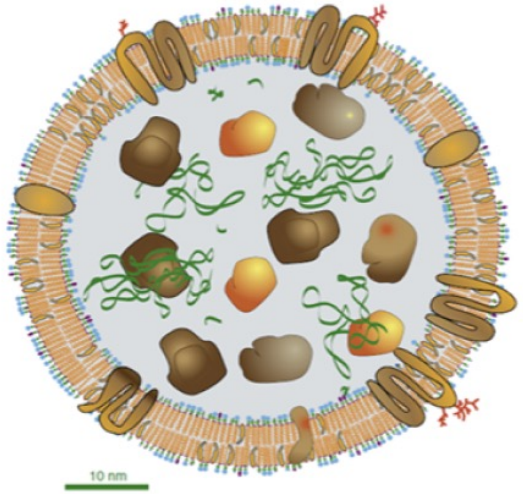
Table 1. Clinically used liposome-based products.

SN	Clinical Products (Approval Year)	Administration	Active Agent	Lipid/Lipid:Drug Molar Ratio	Indication	Company
1.	Doxil® (1995)	i.v.	Doxorubicin	HSPC:Cholesterol:PEG 2000-DSPE (56:39:5 molar ratio)	Ovarian, breast cancer, Kaposi's sarcoma	Sequus Pharmaceuticals
2.	DaunoXome® (1996)	i.v.	Daunorubicin	DSPC and Cholesterol (2:1 molar ratio)	AIDS-related Kaposi's sarcoma	NeXstar Pharmaceuticals
3.	Depocyt® (1999)	Spinal	Cytarabine/Ara-C	DOPC, DPPG, Cholesterol and Triolein	Neoplastic meningitis	SkyPharma Inc.
4.	Myocet® (2000)	i.v.	Doxorubicin	EPC:Cholesterol (55:45 molar ratio)	Combination therapy with cyclophosphamide in metastatic breast cancer	Elan Pharmaceuticals
5.	Mepact® (2004)	i.v.	Mifamurtide	DOPS:POPC (3:7 molar ratio)	High-grade, resectable, non-metastatic osteosarcoma	Takeda Pharmaceutical Limited
6.	Marqibo® (2012)	i.v.	Vincristine	SM:Cholesterol (60:40 molar ratio)	Acute lymphoblastic leukaemia	Talon Therapeutics, Inc.
7.	Onivyde™ (2015)	i.v.	Irinotecan	DSPC:MPEG-2000:DSPE (3:2:0.015 molar ratio)	Combination therapy with fluorouracil and leucovorin in metastatic adenocarcinoma of the pancreas	Merrimack Pharmaceuticals Inc.
8.	Abelcet® (1995)	i.v.	Amphotericin B	DMPC:DMPG (7:3 molar ratio)	Invasive severe fungal infections	Sigma-Tau Pharmaceuticals
9.	Ambisome® (1997)	i.v.	Amphotericin B	HSPC:DSPG:Cholesterol:Amphotericin B (2:0.8:1:0.4 molar ratio)	Presumed fungal infections	Astellas Pharma
10.	Amphotec® (1996)	i.v.	Amphotericin B	Cholesteryl sulphate:Amphotericin B (1:1 molar ratio)	Severe fungal infections	Ben Venue Laboratories Inc.
11.	Visudyne® (2000)	i.v.	Verteporphin	Verteporphin:DMPC and EPG (1:8 molar ratio)	Choroidal neovascularisation	Novartis
12.	DepoDur™ (2004)	Epidural	Morphine sulfate	DOPC, DPPG, Cholesterol and Triolein	Pain management	SkyPharma Inc.
13.	Exparel® (2011)	i.v.	Bupivacaine	DEPC, DPPG, Cholesterol and Tricaprylin	Pain management	Pacira Pharmaceuticals, Inc.
14.	Epaxal® (1993)	i.m.	Inactivated hepatitis A virus (strain RGSB)	DOPC:DOPE (75:25 molar ratio)	Hepatitis A	Crucell, Berna Biotech
15.	Inflexal® V (1997)	i.m.	Inactivated hemaglutinine of Influenza virus strains A and B	DOPC:DOPE (75:25 molar ratio)	Influenza	Crucell, Berna Biotech

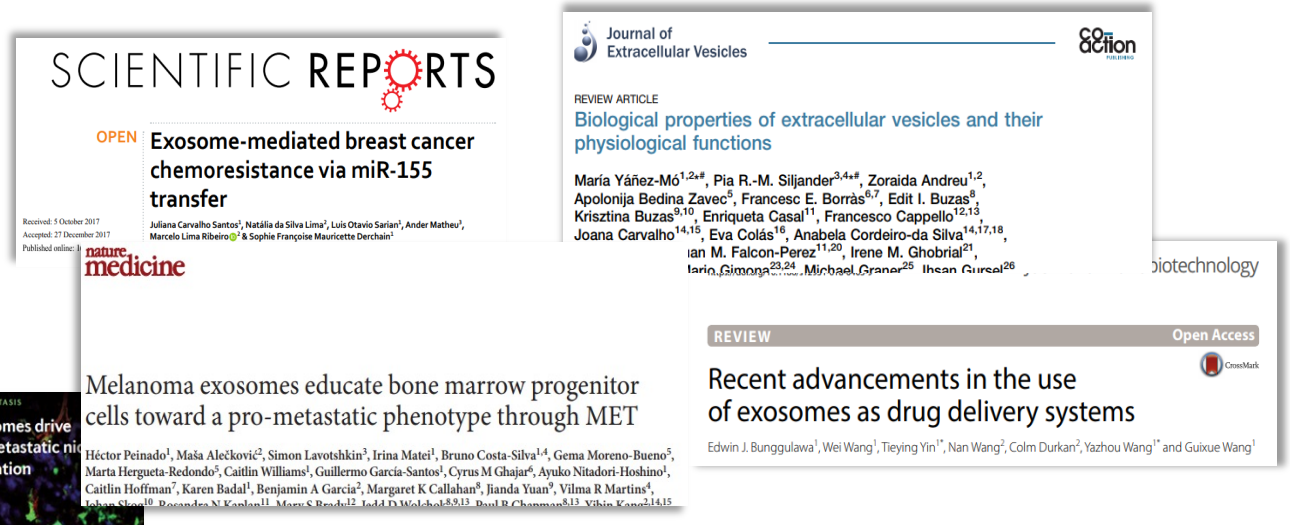
i.v. (intravenous); i.m. (intramuscular); HSPC (hydrogenated soy phosphatidylcholine); PEG (polyethylene glycol); DSPE (distearoyl-sn-glycero-phosphoethanolamine); DSPC (distearoylphosphatidylcholine); DOPC (dioleoylphosphatidylcholine); DPPG (dipalmitoylphosphatidylglycerol); EPC (egg phosphatidylcholine); DOPS (dioleoylphosphatidylserine); POPC (palmitoyloleoylphosphatidylcholine); SM (sphingomyelin); MPEG (methoxy polyethylene glycol); DMPC (dimyristoyl phosphatidylcholine); DMPG (dimyristoyl phosphatidylglycerol); DSPG (distearoylphosphatidylglycerol); DEPC (dierucoylphosphatidylcholine); DOPE (dioleoly-sn-glycero-phophoethanolamine).

Exosomes (SEVs) : natural liposomes





- Extracelullar vesicles endosomal origin
- nanometric size: 50 – 150 nm
- Extracellular communication.
- ***Structure: lipid bilayer (liposomes)***



Biological functions:

- Cell-cell communication
- Disease progression
- Tumor evolution

Natural NPs with biological function

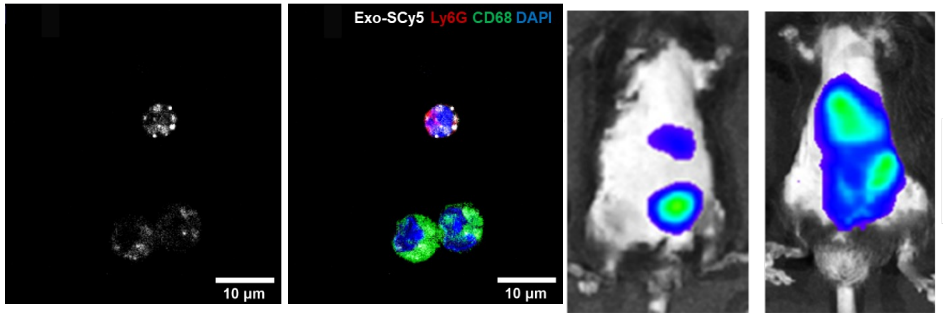
Goat milk exosomes as natural liposomes

Advantages

- Vs synthetic nanoparticles (liposomes): Biocompatibility, biodegradability
- Vs other exosomes:
  - *Economic*
  - *Scalable*
  - *Robust in degrading conditions (Pieters, B.C., et al., 2015)*
  - *Non-immunogenicity and cross-species tolerance (Munagala, R. et al., 2016)*
  - *Resistant*

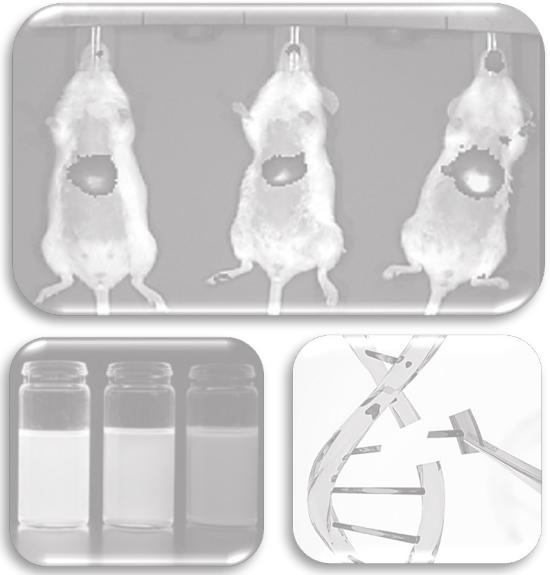
Applications:

- **Drug delivery**: structure like liposome
- **Early detection**: biomarkers

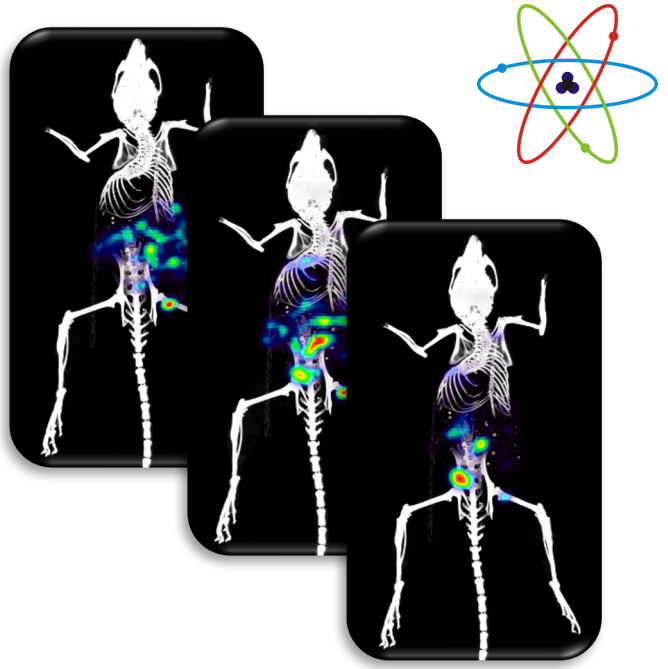


MOLECULAR IMAGING

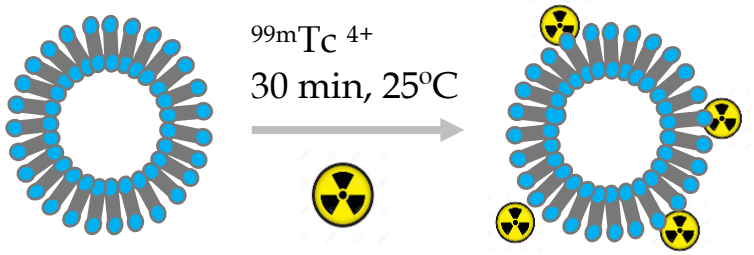
Optical imaging



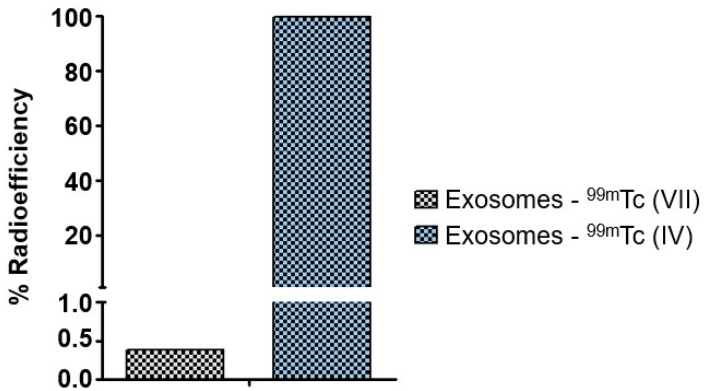
Nuclear imaging



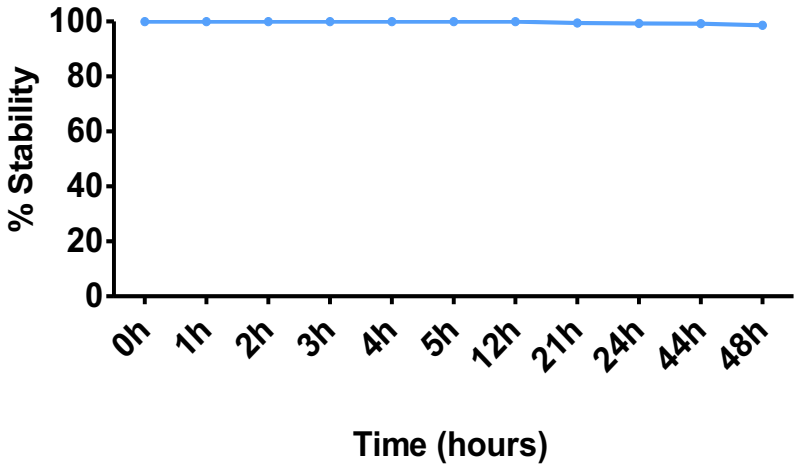
A.- Radiosynthesis



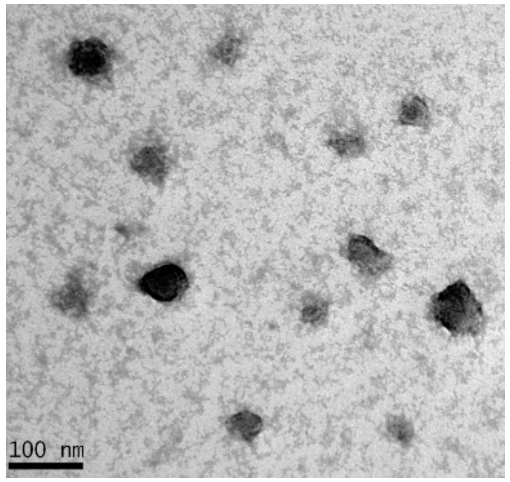
B.- Passive incorporation



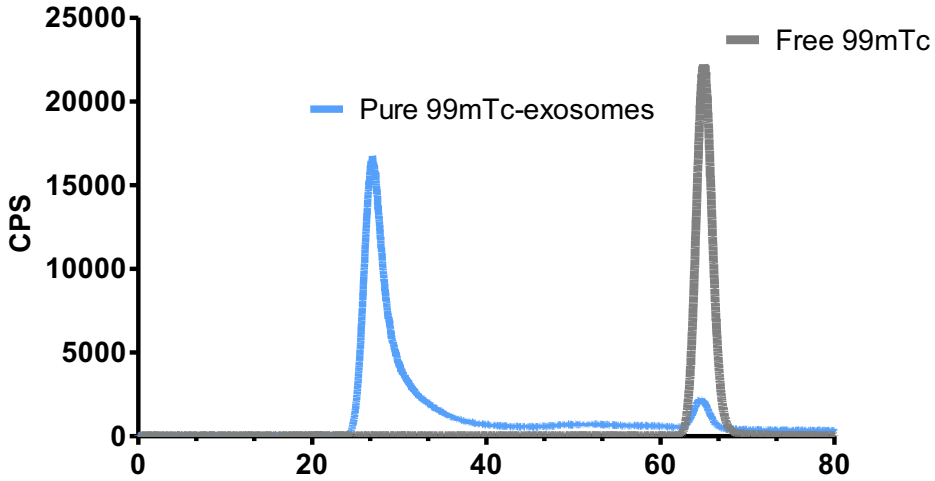
C.- Stability (in vitro)



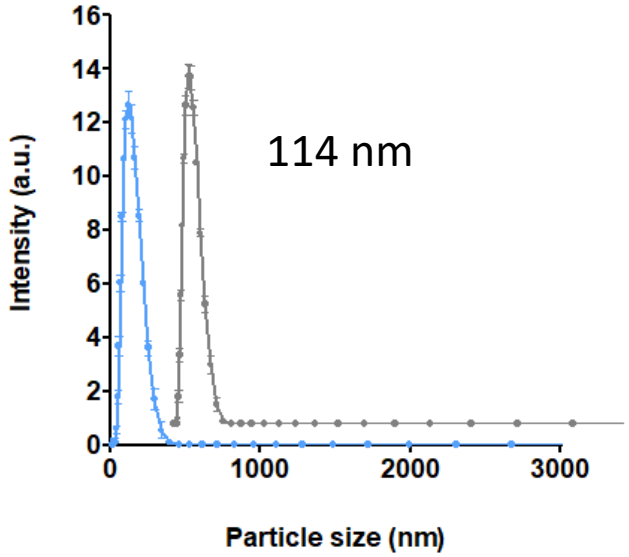
D.- TEM (microscopy)



E.- RadioHPLC (purity)

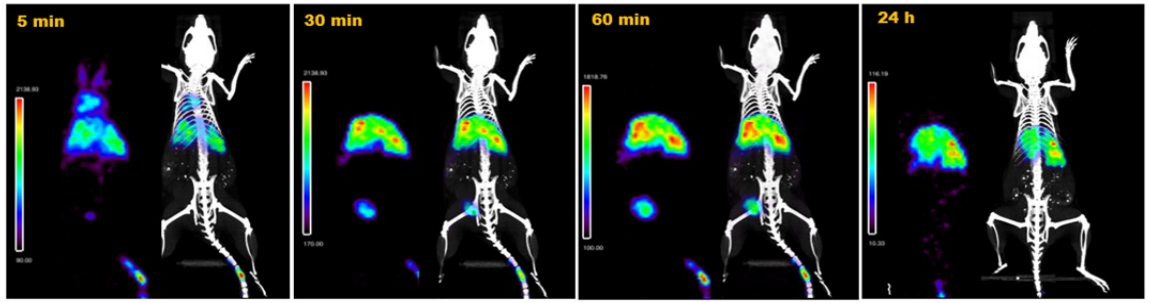


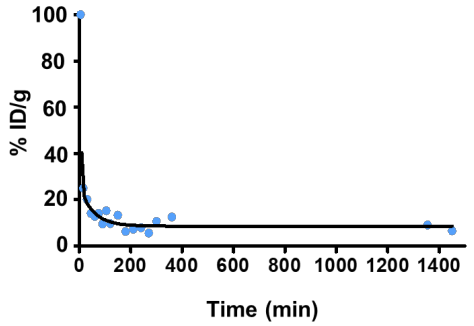
F.- DLS (size)

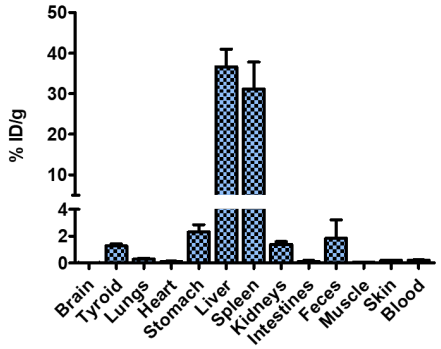


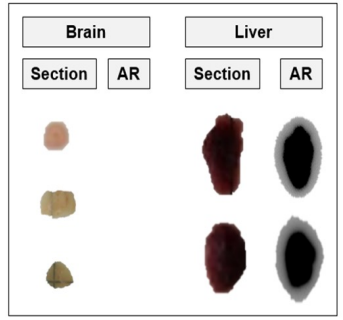
M. I. González,…, B. Salinas, Nanomaterials. 2020.

Intravenous administration

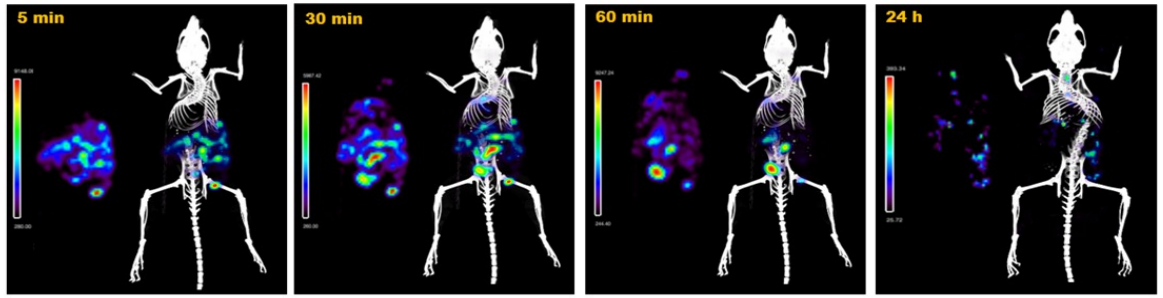


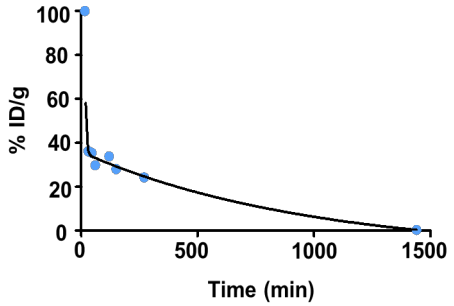


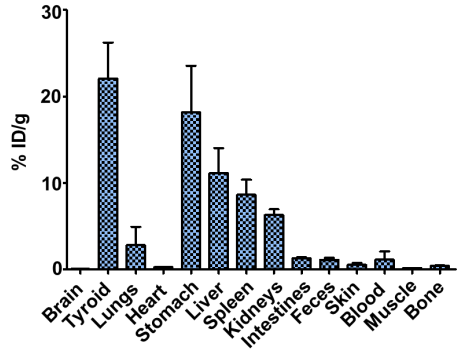


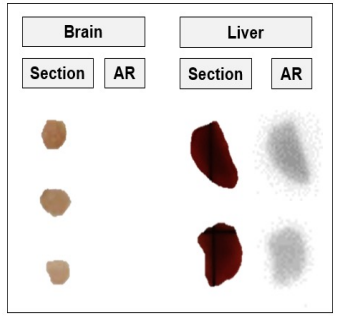


Intraperitoneal administration

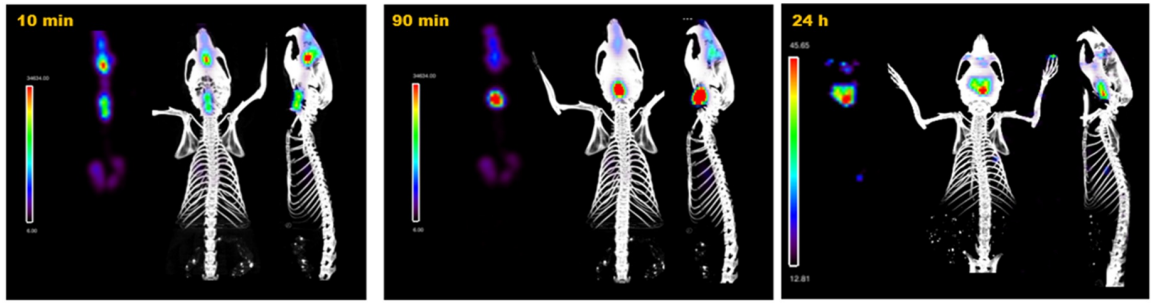


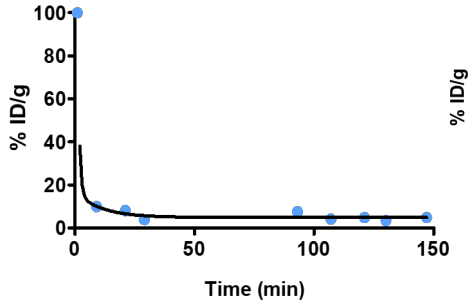


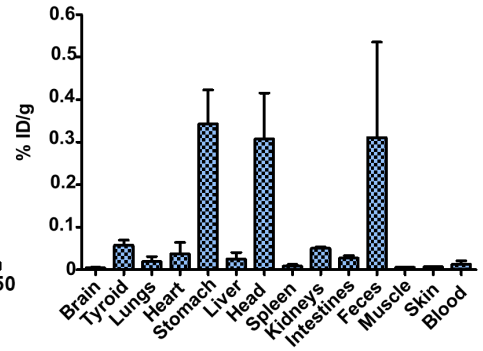


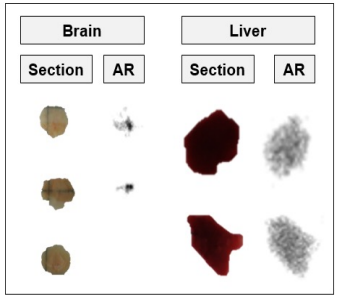


Intranasal administration





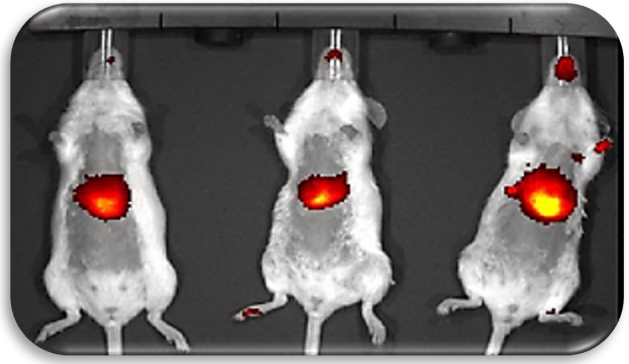




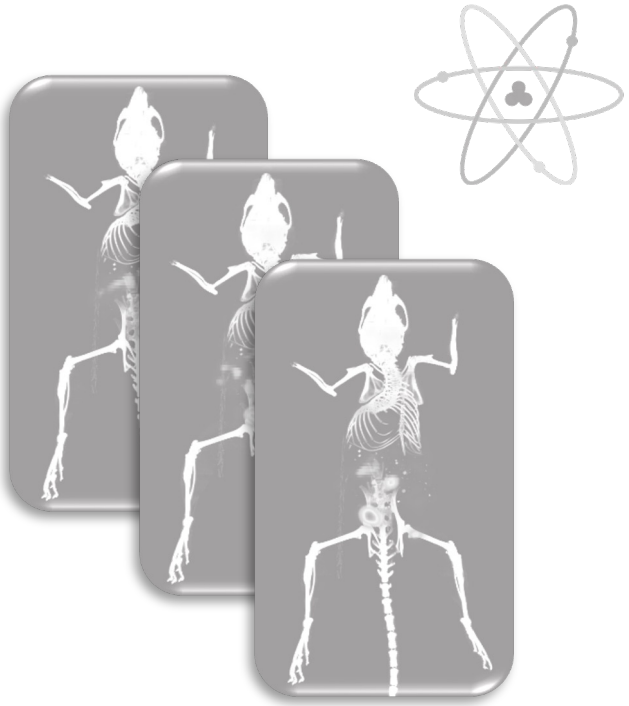
M. I. González,…, B. Salinas, Nanomaterials. 2020.

MOLECULAR IMAGING

Optical imaging

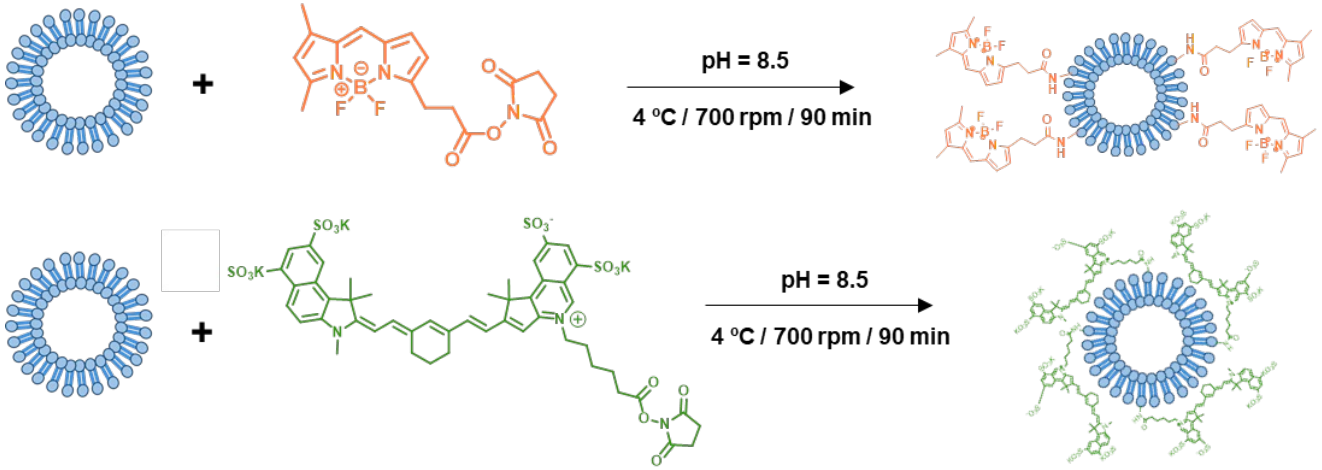


Nuclear imaging



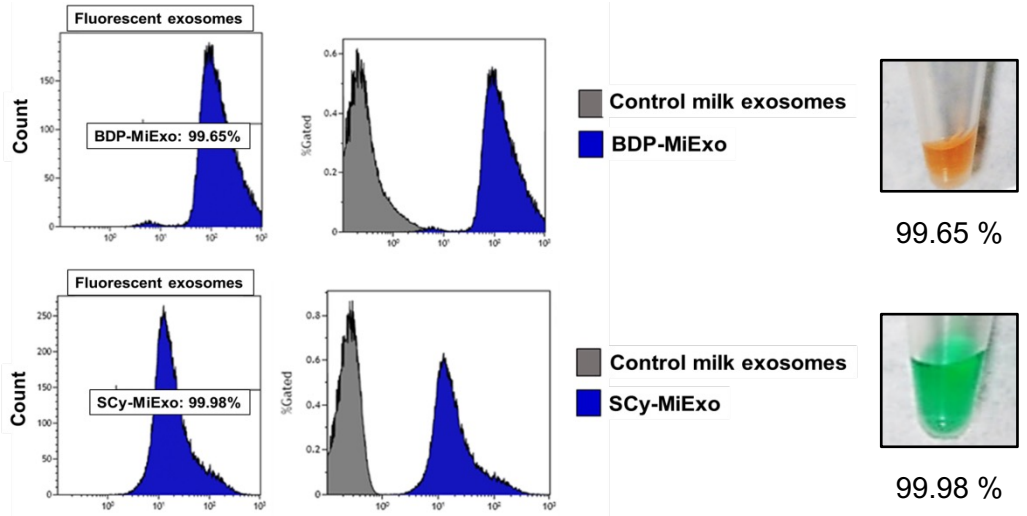
Covalent labelling

A.- Reaction

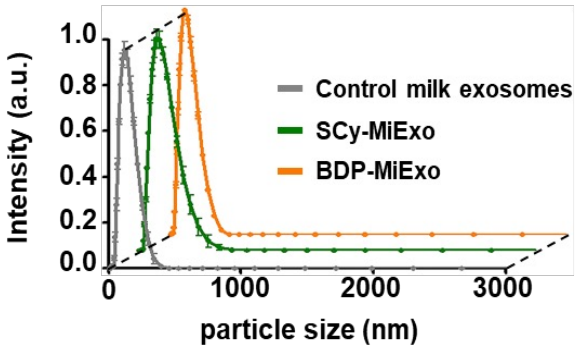


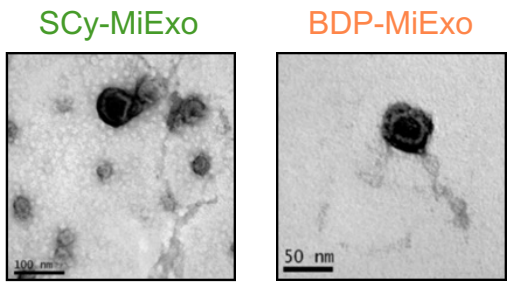
✓ Milk Exosomes ✓ Tumor Exosomes

B.- Flow citometry and yield of labelling

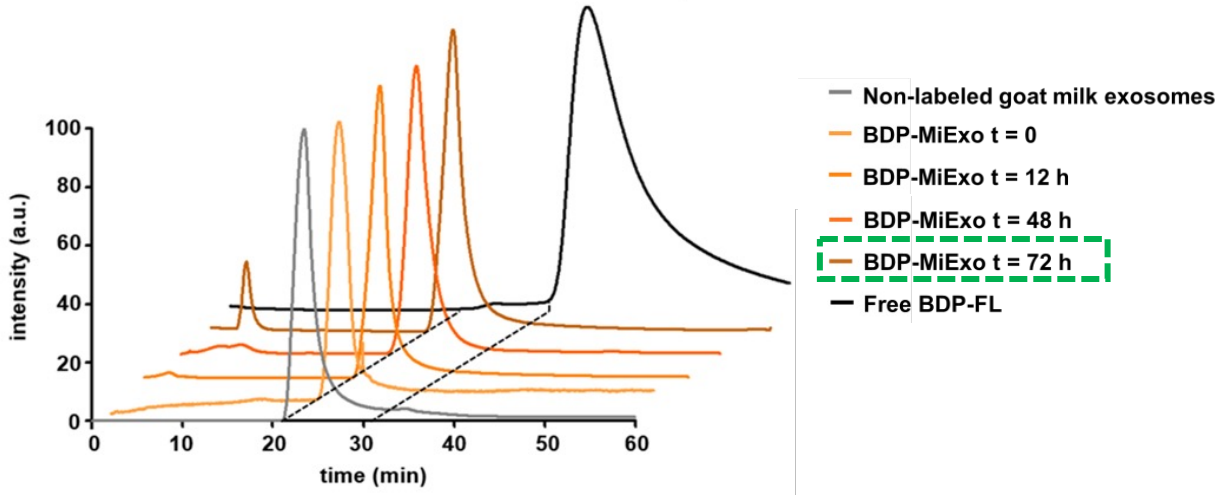


C.- Physicochemial characterization





D.- In vitro stability (PBS):

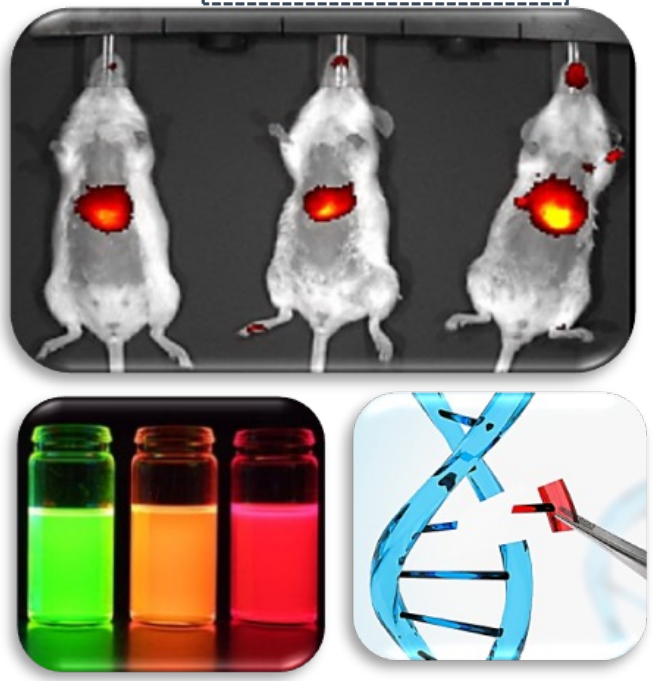


González, M.I, … , B Salinas. Biomedicines, 2021

MOLECULAR IMAGING

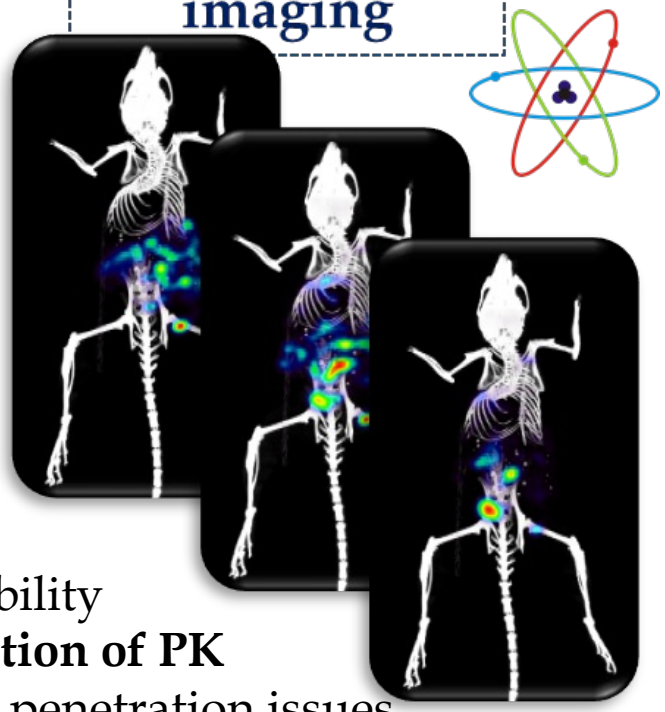
Dual probe

Optical imaging



- High sensitibity
- **Visualization (in vitro, histology)**
- Non-ionizing radiation

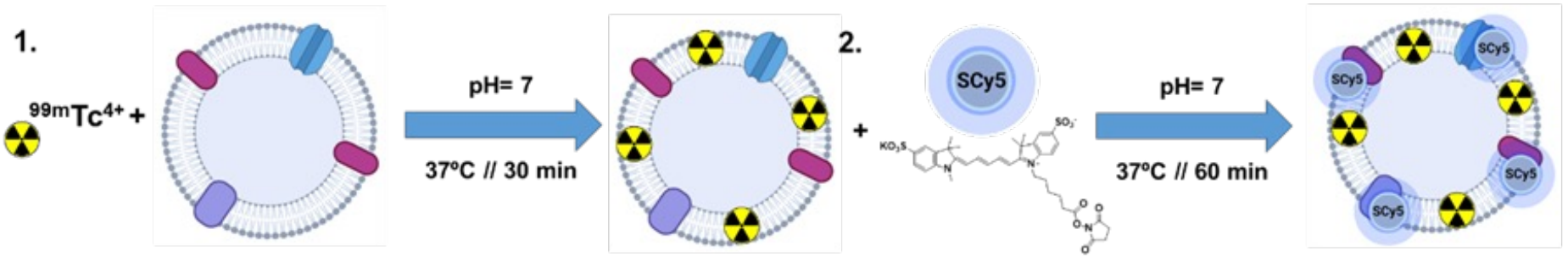
Nuclear imaging



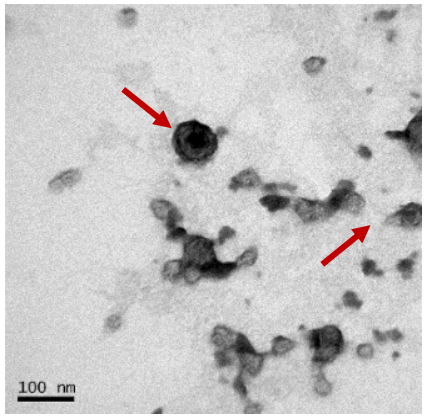
- High sensibility
- **Quantification of PK**
- Non depth penetration issues

Dual labelling and characterization

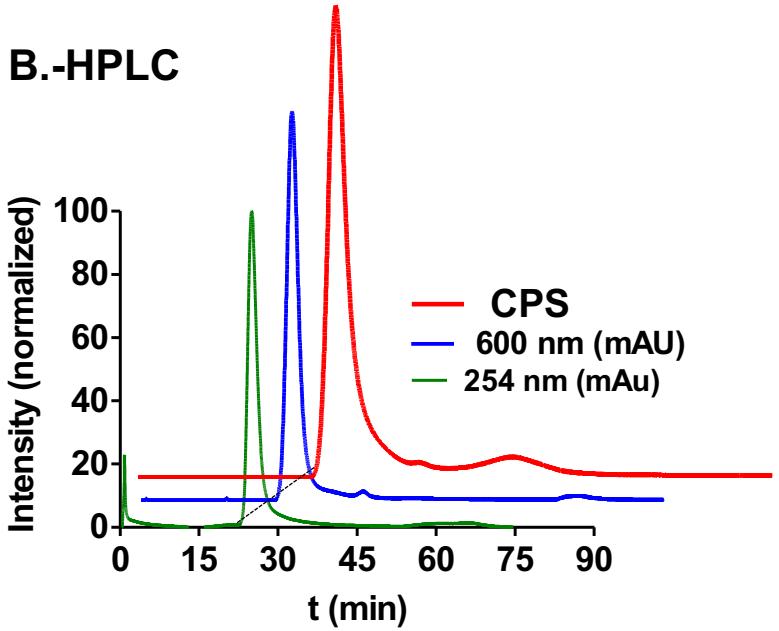
Reaction



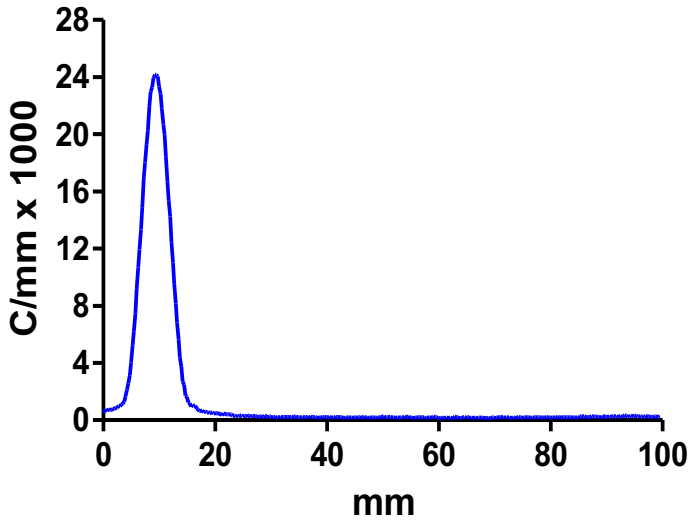
A.-TEM



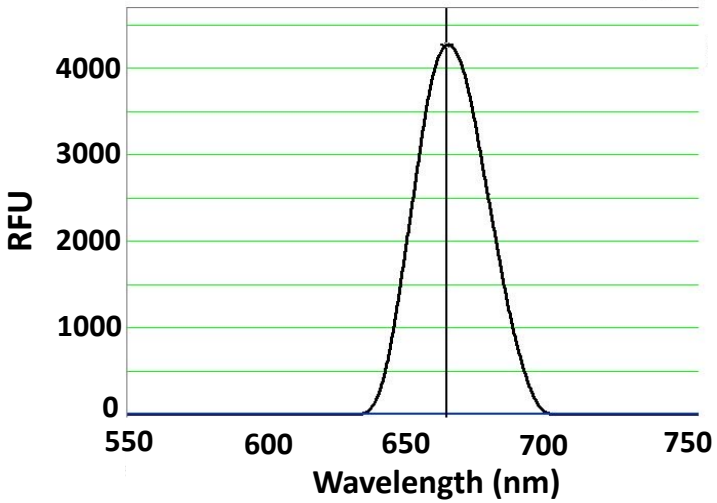
B.-HPLC



C.-iTLC

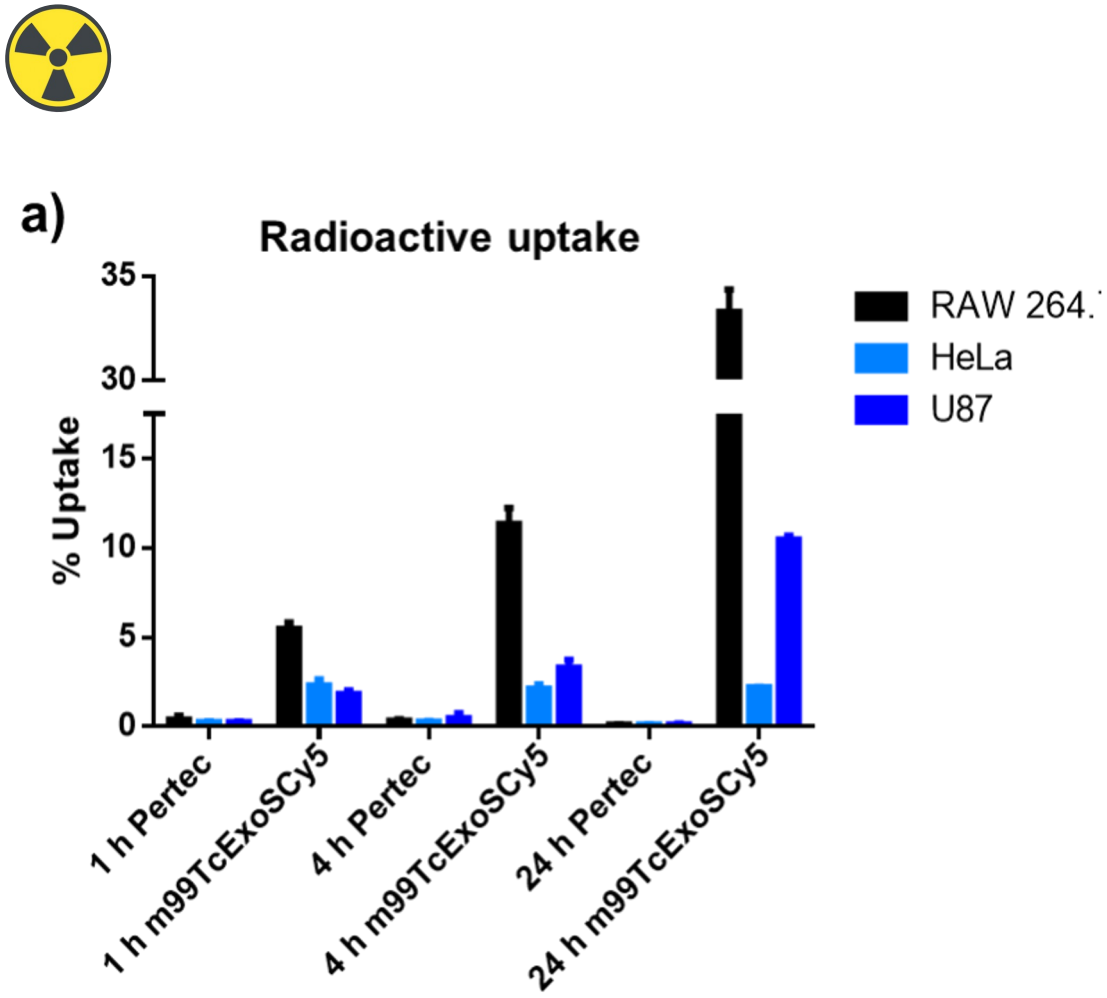


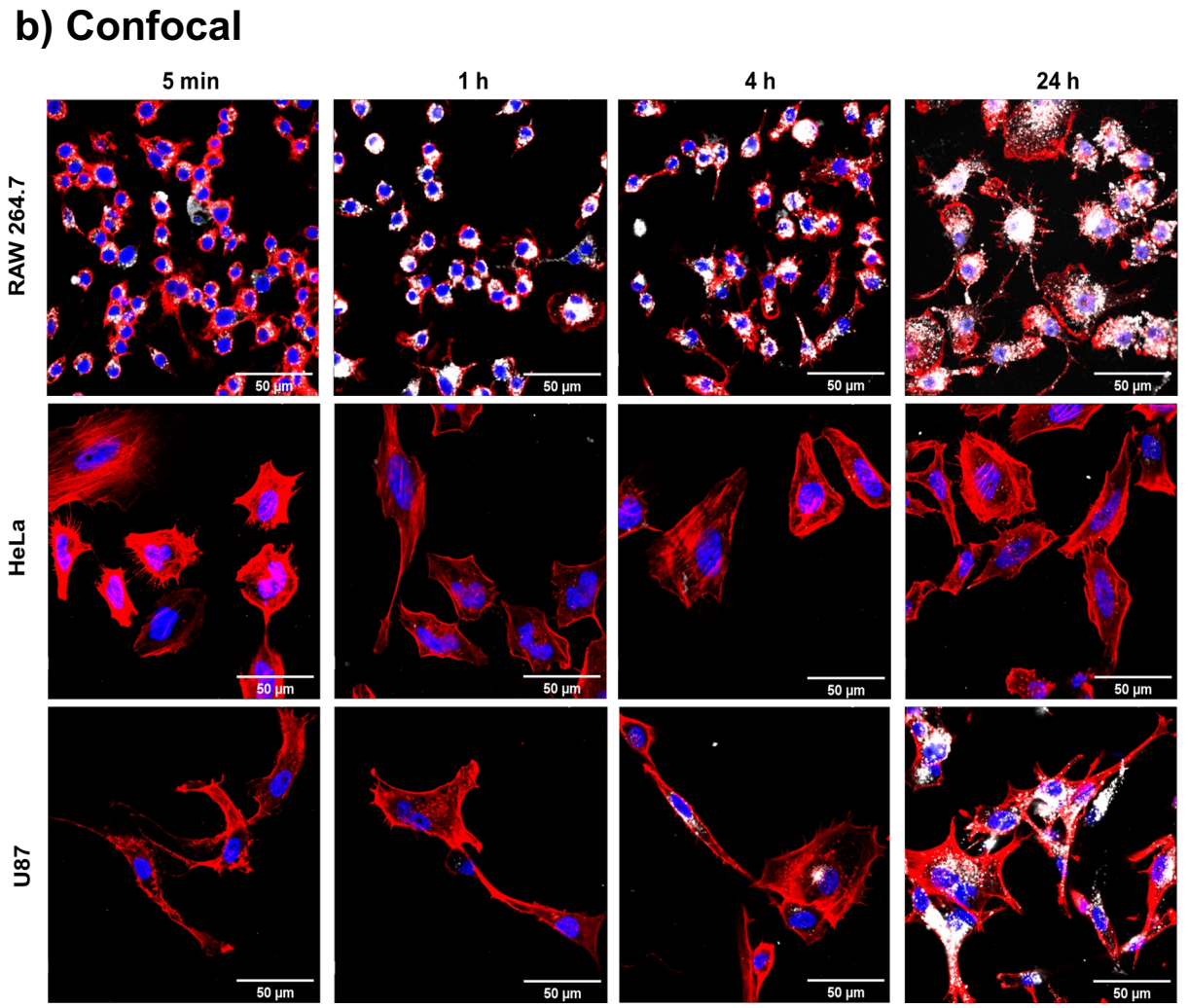
D.-Fluorescence



D Herreros,, …, B Salinas. submitted, 2022

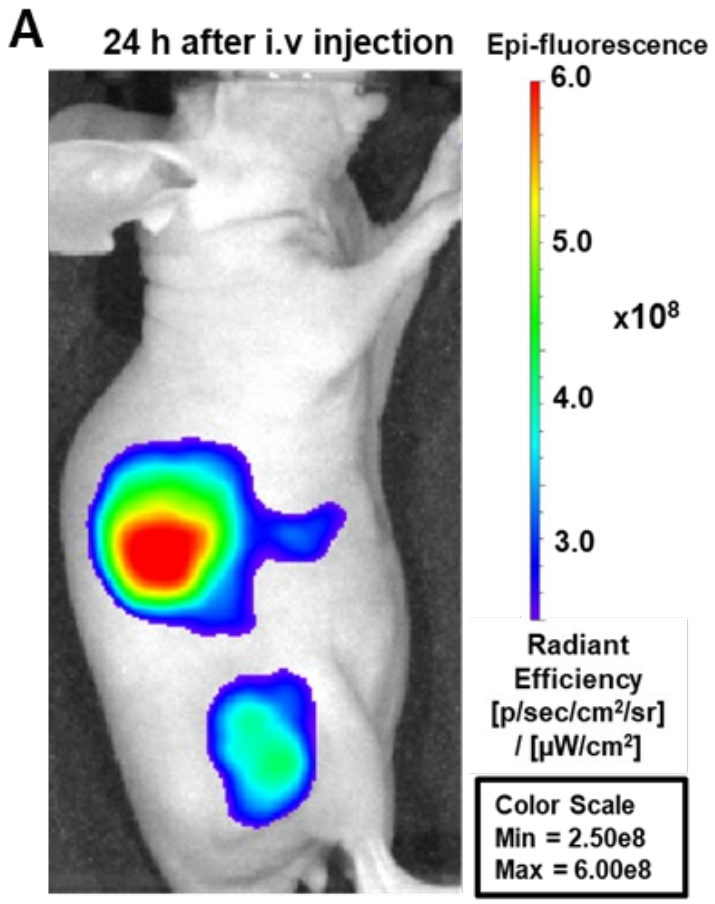
In vitro assessment (Radioactive and optical)

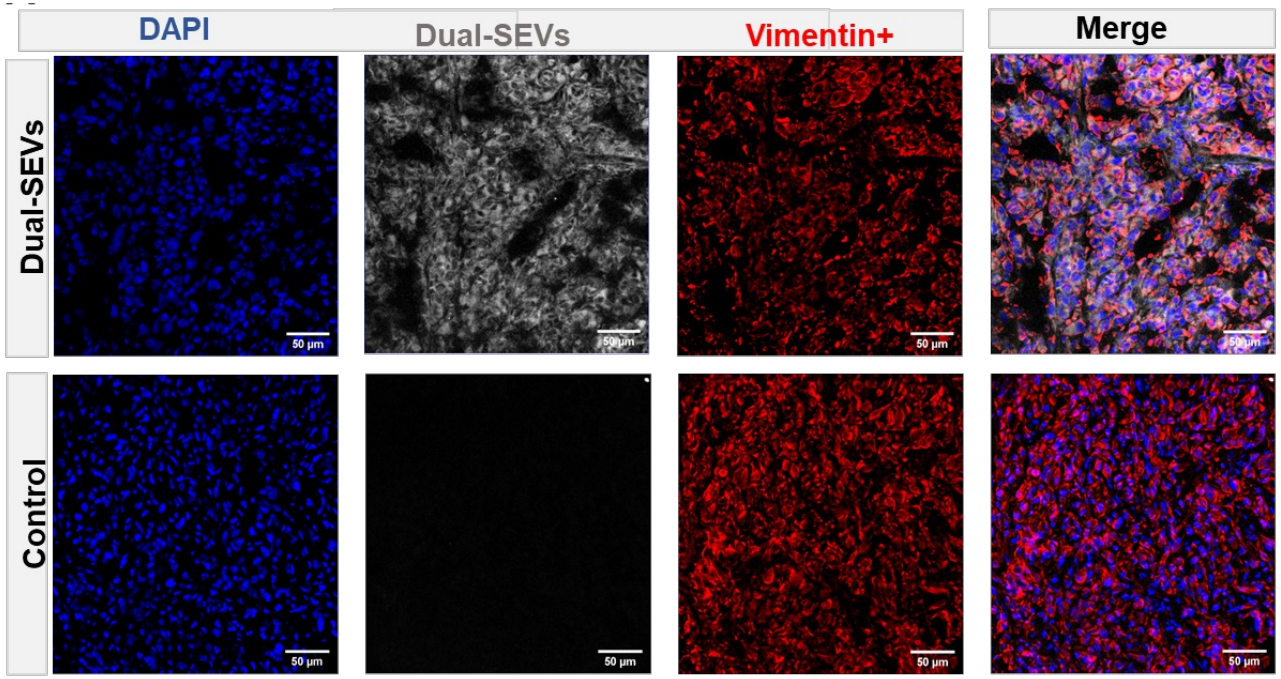




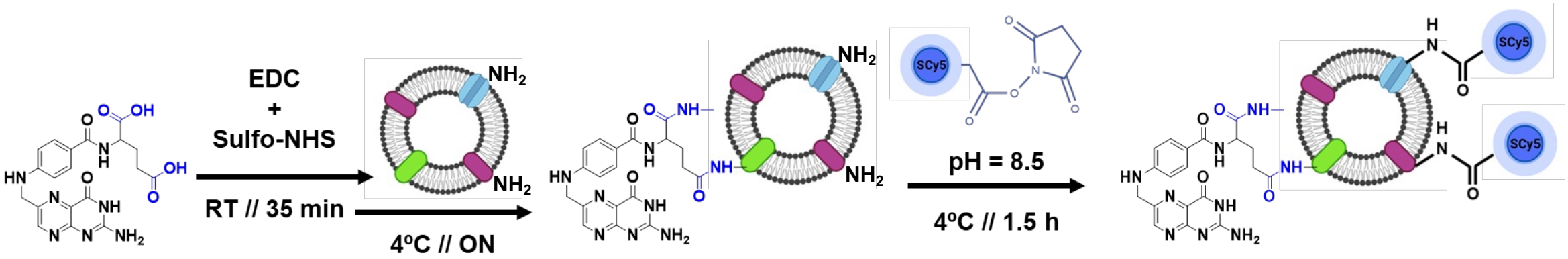
D Herreros,, …, B Salinas. submitted, 2022

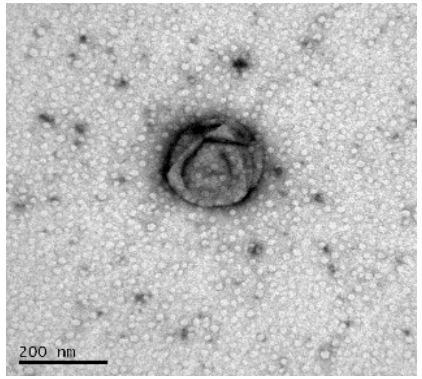
In vivo validation

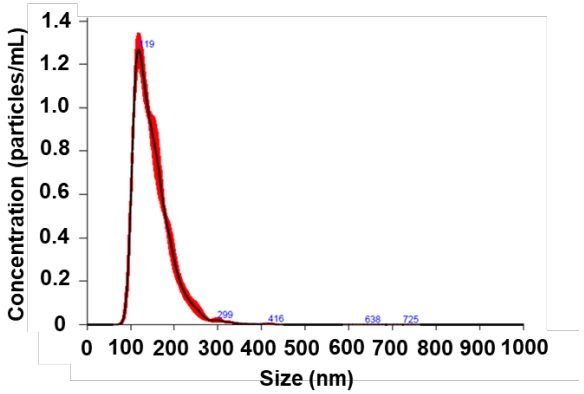


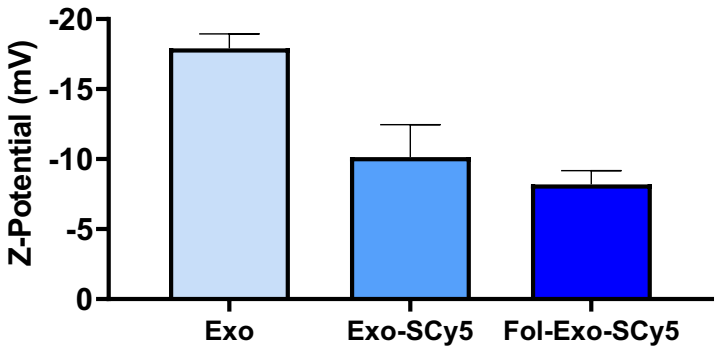


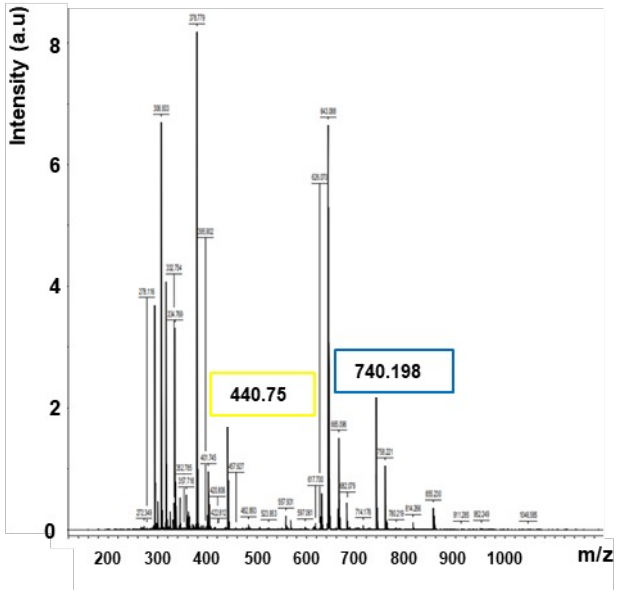
Exosome engineering_ Folic acid



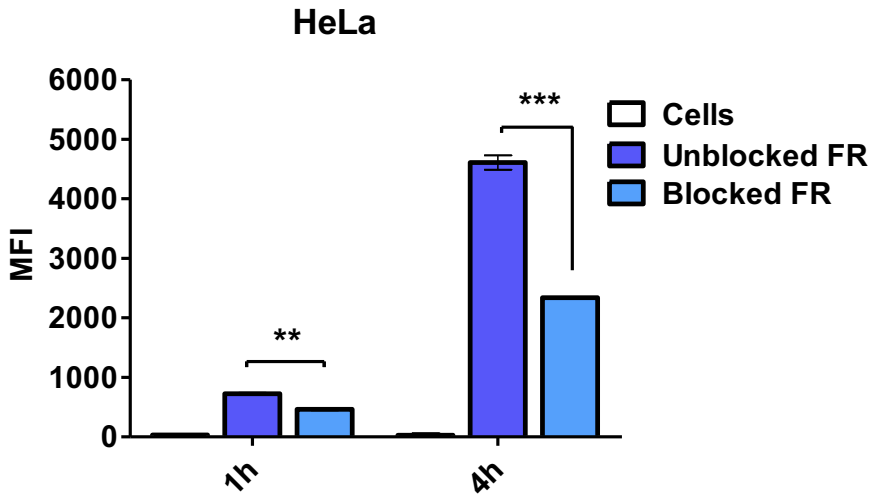


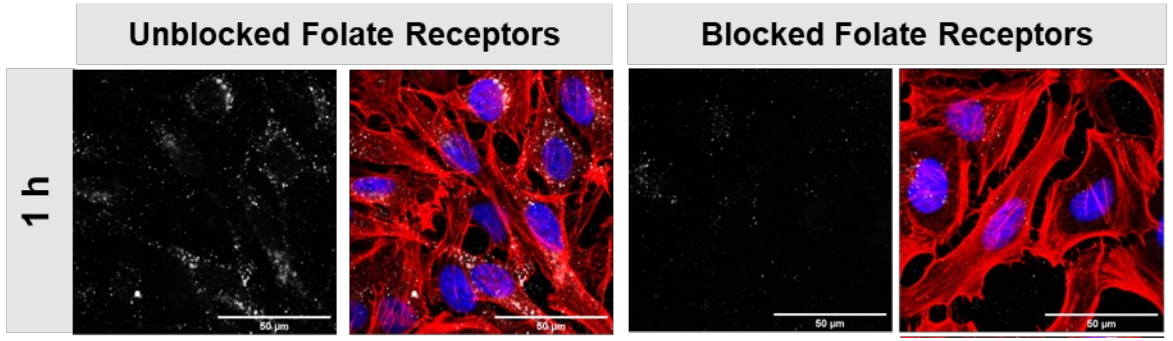




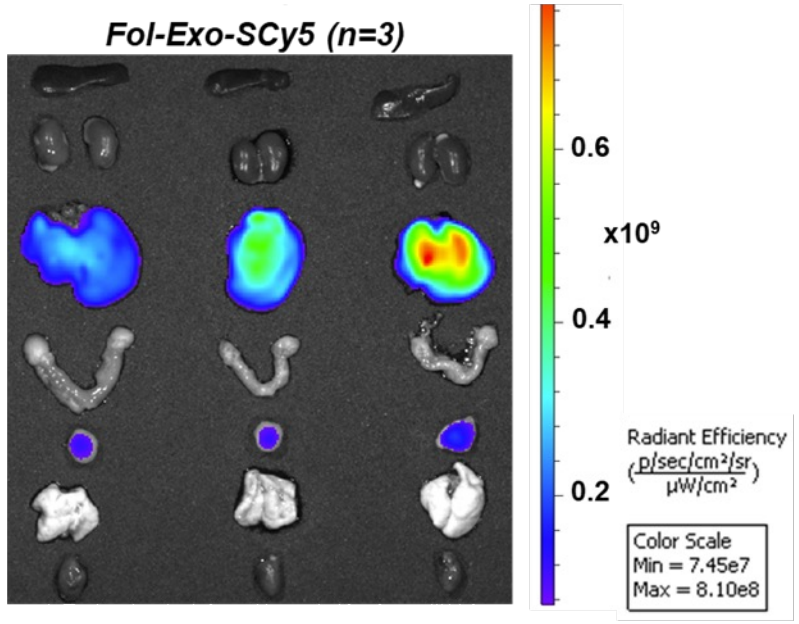


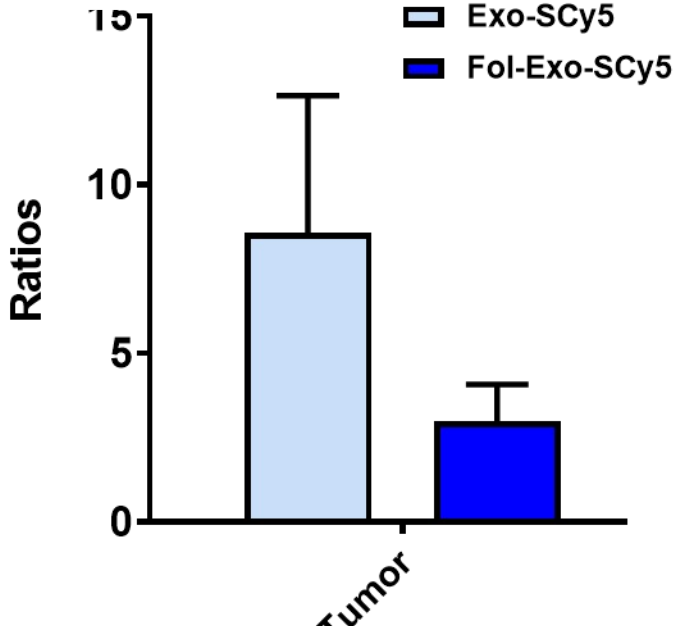
A.- In vitro evaluation





B.- In vivo validation



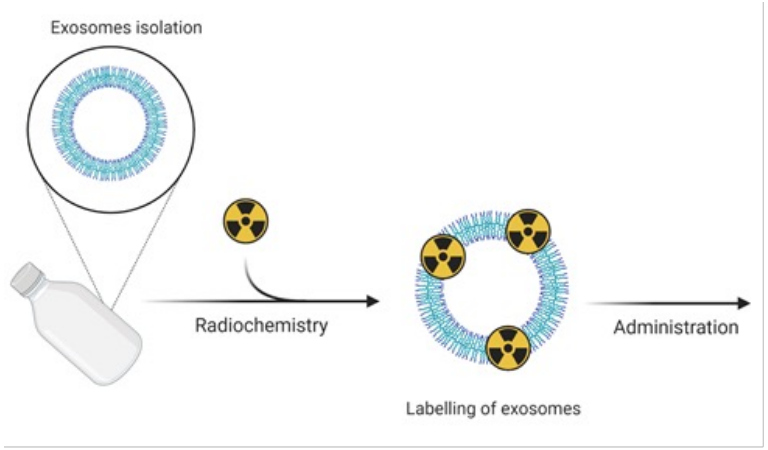


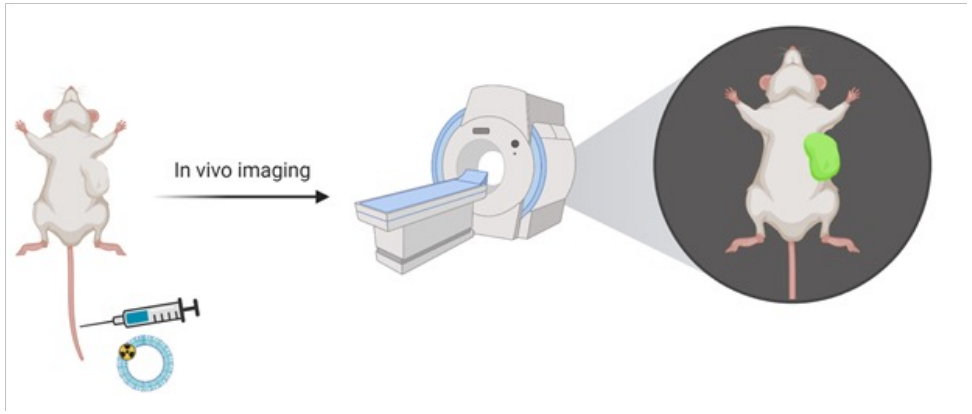
Take home message

Labelling of exosomes has allowed us to confirm not only the diagnostic capacity of the nanoparticles in tumor models but also the evaluation of the biological behavior for further use as DDS or imaging agent

PRISMAP project

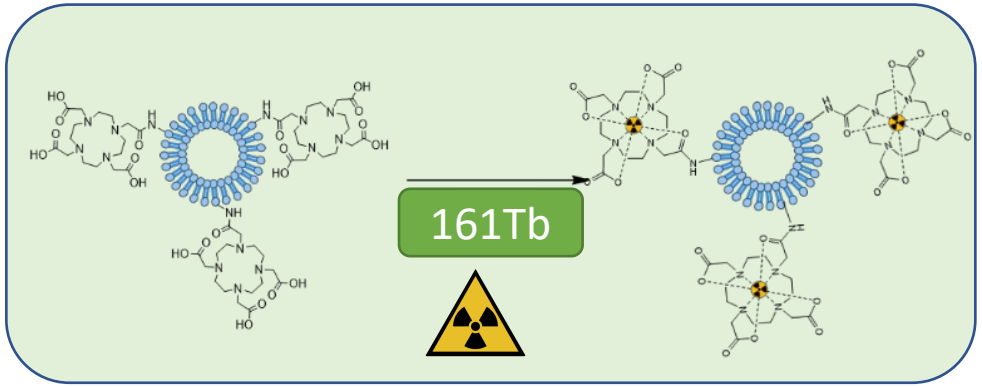
Development of new radiotheragnostic agents based on natural nanoparticles (exosomes) radioactively labeled with the novel therapeutic and diagnostic isotope Terbium 161 (161Tb).

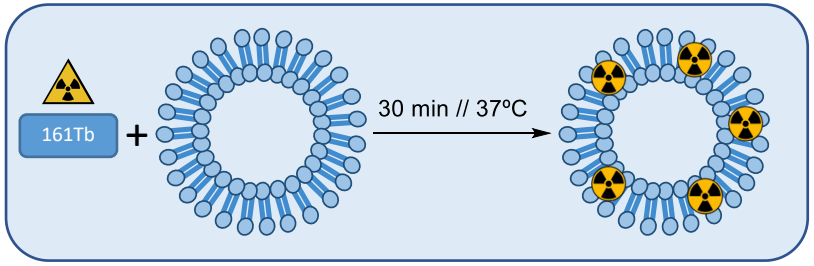




PRISMAP project

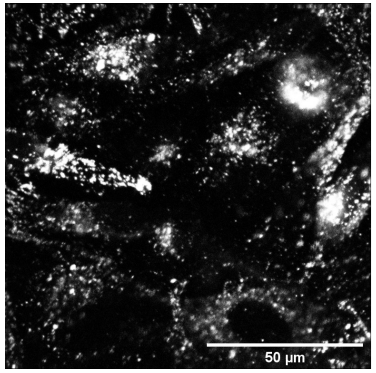
1.- Labelling ^{161}Tb

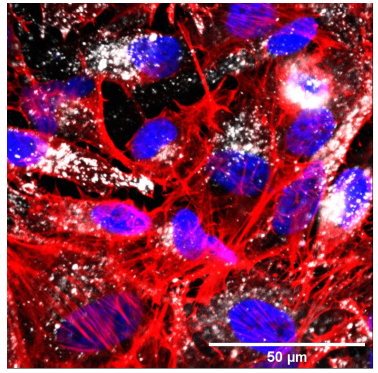




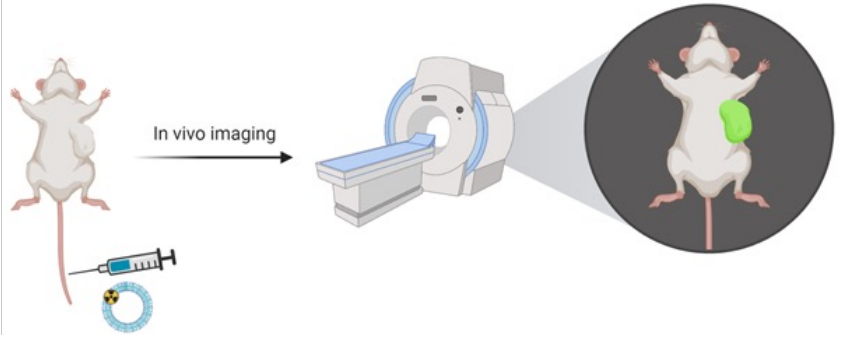
,

2.- In vitro (^{161}Tb)





3.- In vivo (^{161}Tb)



PRISMAP project

How did Prismap come into our life?

Previous studies in collaboration with TUM on 177Lu labelling of exosomes → Dr. Weber and Dr. Kossatz

What does the Prismap consortium offer us?

- Limited access to cyclotrons and new radionuclides
- New field of research in Spain
- PRISMAP: ideal platform for the development of new lines of research

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Fondo Social Europeo (FSE)
“Investing in your future”
Instituto de Salud Carlos III
Unión Europea
Comunidad de Madrid
GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN
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Invertimos en su futuro

Selective oncological theragnostic based on radioactively labeled exosomes

Beatriz Salinas Rodríguez

Molecular probes Lab
Biomedical imaging and instrumentation Group
Instituto de investigaciones Sanitarias Gregorio Marañón.

Biomedical engineering dept, Universidad Carlos III Madrid.

Advanced Imaging Unit, National Center of Cardiovascular Diseases