PRECLINICAL STUDIES OF SUB-CELLULAR TARGETED ^{161}Tb-COMPLEXES FOR CANCER RADIOTHERANOSTICS

António Paulo

apaulo@ctn.tecnico.ulisboa.pt

C^{2}TN Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico
Universidade de Lisboa, Portugal

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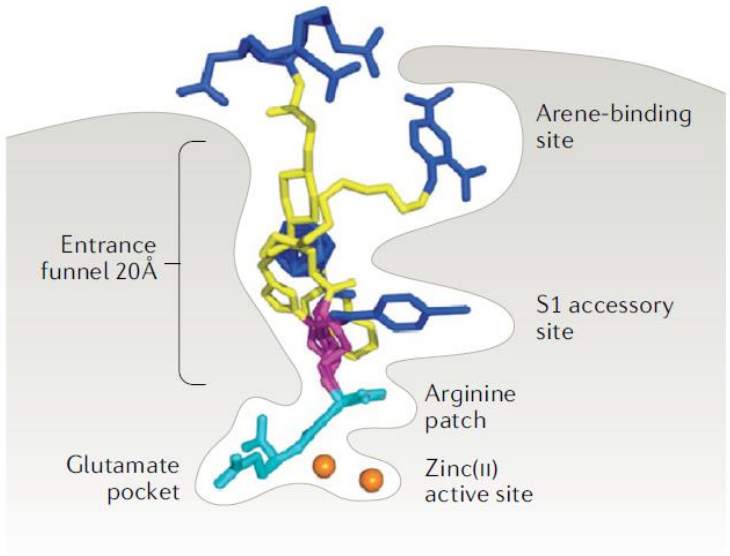
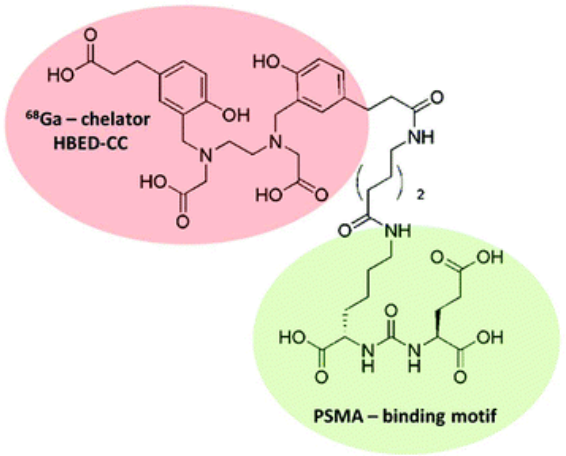
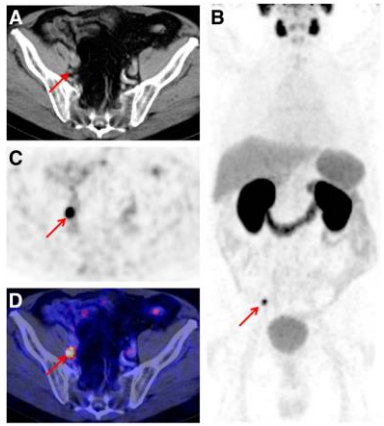
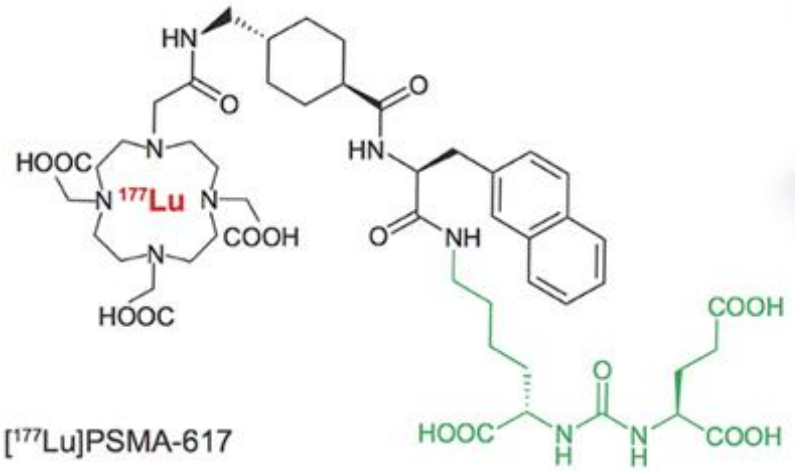
Theranostic of Prostate Cancer: PSMA Inhibitors



- Several PSMA inhibitors containing the Glu-urea-Lys unit (KuE) have been labeled with numerous radionuclides for imaging or therapy, namely with ^{68}Ga and ^{177}Lu.
- Intense research in this area enabled the development of ^{68}Ga-PSMA-11 or ^{177}Lu-PSMA-617.
- ^{177}Lu-PSMA-617 (Pluvicto^{TM}) has been recently approved by the EMA and FDA.





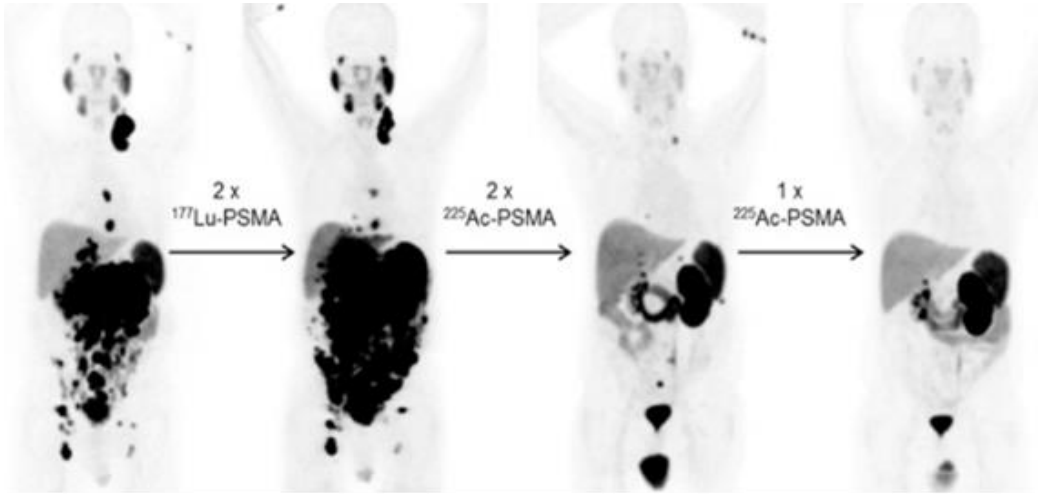


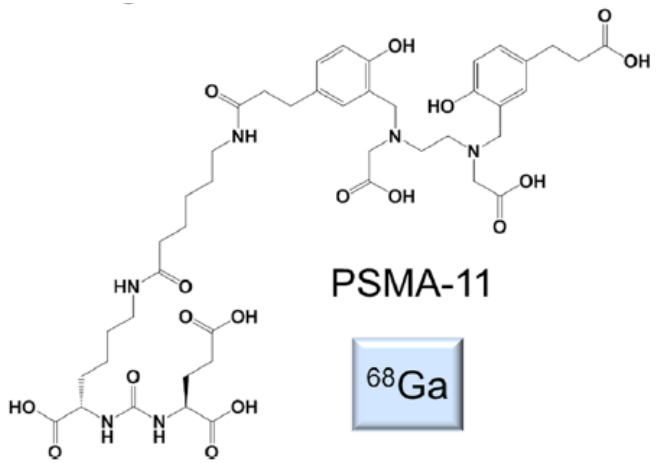
M. Eiber et al., *J Nucl Med* (2015) 56:668–674

M. Einsenhut, *Bioconjugate Chem.* (2012) 23 (4), pp 688–697

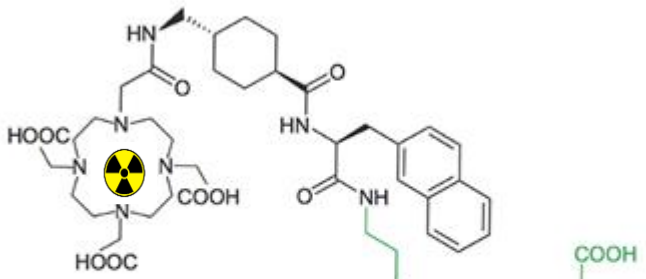
Attard, G. *et al., Lancet* (2016) 387, 70–82

PSMA Inhibitors: beta vs alpha Therapy





- Despite the good results obtained with ^{177}Lu-PSMA-617, the use of β- emitters in targeted therapy with radionuclides has some limitations: **nephrotoxicity**, **resistance to β-radiation** and **low efficacy to eradicate small lesions** .
- Therapy with α-emitters may be an alternative: promising clinical and preclinical results for ^{225}Ac-PSMA-617.
- **Alpha Emitters Limitations**: Challenging production, demanding radiochemistry, in *vivo stability* issues.



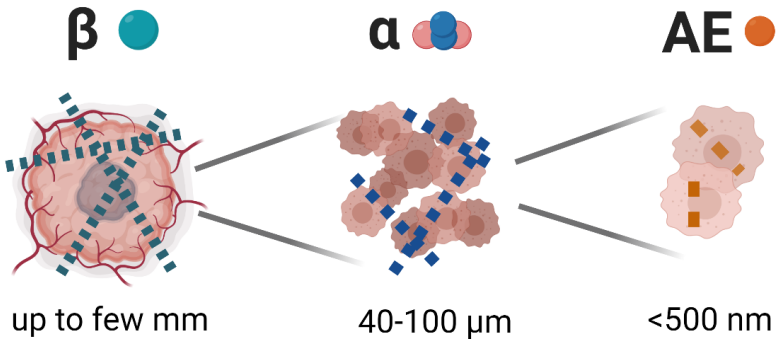
[^{177}Lu]PSMA-617

[^{225}Ac]PSMA-617

Auger Electron Therapy: Pros and Cons

- Short range (less than the cell diameter) and high LET of Auger electrons (AE) are favourable features for targeted radionuclide therapy (TRT), namely to eradicate **small tumors**, **metastases** and even non-imageable **small clusters of tumor cells**.

  Bolcaen et al., *J Nucl Med*. 2023;64(9):1344-1351.



- Biological Effects (e.g,. induction of cell death) are strongly dependent on the subcellular localization of AE emitters and distance to radiosensitive targets (**e.g., cell membrane**, **nucleus** or **mitochondria**).

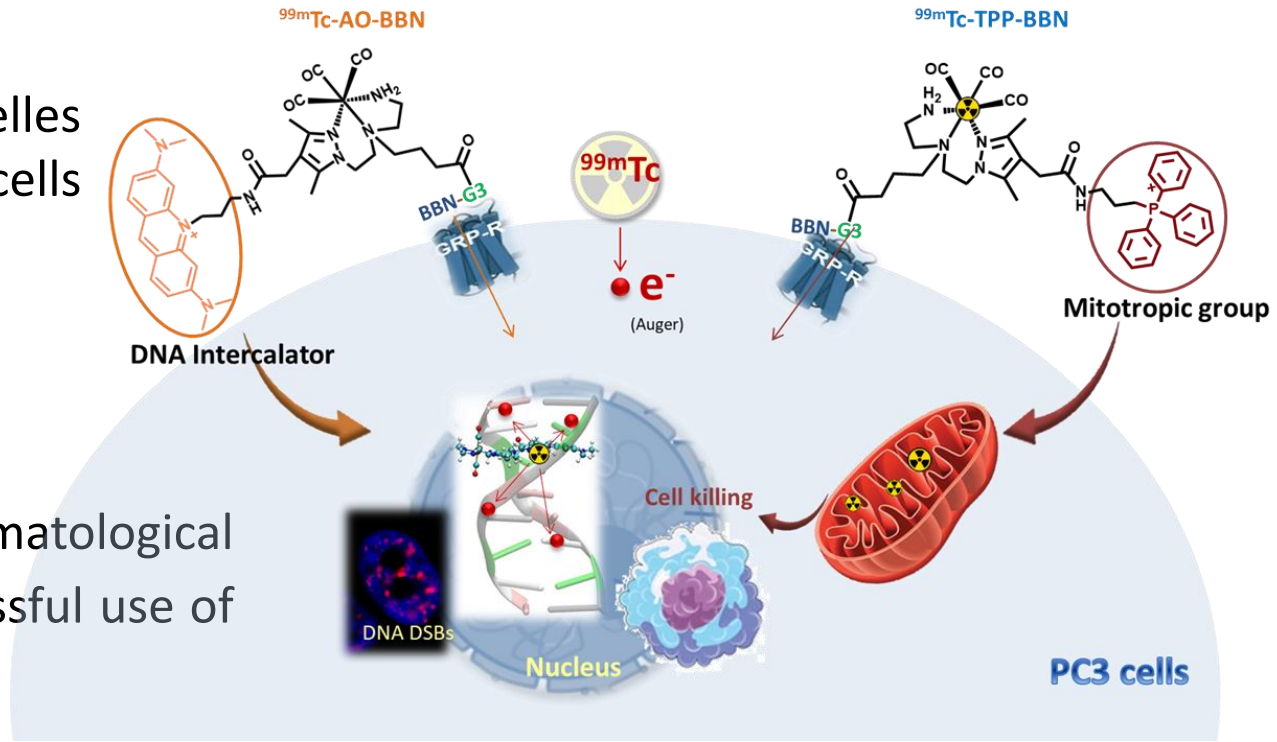
- Many Auger emitters correspond to commercially available radionuclides in use for diagnostic (e.g. **^{123}I, ^{67}Ga, ^{99m}Tc or ^{111}In**): **Theranostic potential.**

- New and more suitable Auger emitters start to be available (e.g., **^{161}Tb, ^{195m}Pt, ^{197}Hg, ^{103m}Rh, ^{165}Er**).

- A great challenge is to avoid undue irradiation of non-target tissues by γ-photons often emitted by AE emitting radionuclides.

Organelle-Targeted AE-Emitting Radioconjugates

- Specific delivery of AE emitters to radiosensitive organelles (**cell membrane**, **nucleus** or **mitochondria**) of cancer cells might enhance radiotherapeutic effects at lower doses.



- Minimization of undesired side effects (e.g. hematological toxicity, kidney damage or cardiotoxicity), for a successful use of AE emitters in TRT.

Fernandes et al., *Int J Mol Sci*. 2022;23(13):7238

- **Challenge**: impact of organelle-specific moieties in the PKs and biodistribution of the radioconjugates.

^{161}Tb *vs* ^{177}Lu

Radionuclide	^{177}Lu	^{161}Tb
Half-life (day)	6.647	6.906
Type of decay (%)	β^- (100%)	β^- (100%)
β particles mean energy (keV)	133.3	154.3
Daughter	^{177}Hf (stable)	^{161}Dy (stable)
CE (keV per decay)	13.52	39.28
CE energy range in keV (weighted average energy)[a]	6.2 – 206.3 (87)	3.3 – 98.3 (28)
AE (keV per decay)	1.13	8.94
AE energy range in keV (weighted average energy)[a]	0.01 – 61.7 (1)	0.018 – 50.9 (0.8)

Hindié et al., *EJNMMI Physics* **2020**, 7:33

- **^{161}Tb** associates the traditional advantages of a medium-energy β^- emission spectrum with the additional benefit of a high localised dose provided by **conversion and Auger electrons (AE)**.
- Very encouraging results for **^{161}Tb-PSMA-617** (PSMA inhibitor) and **^{161}Tb-DOTA-LM3** (SST2-antagonist) undergoing clinical trials.

Van Laere et al., *Theranostics*. 2024;14(4):1720-1743

“Dual-Targeted” ^{161}Tb complexes

esults with ^{99m}Tc-BBN radioconjugates, we have d

P6 Day 2

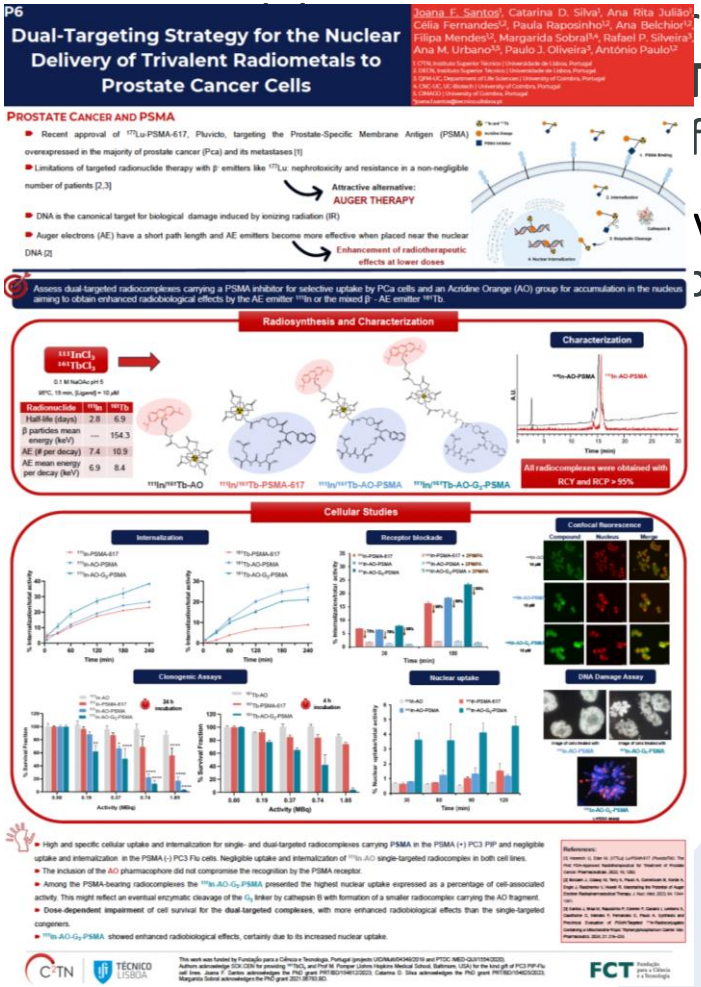
P5 Day 2

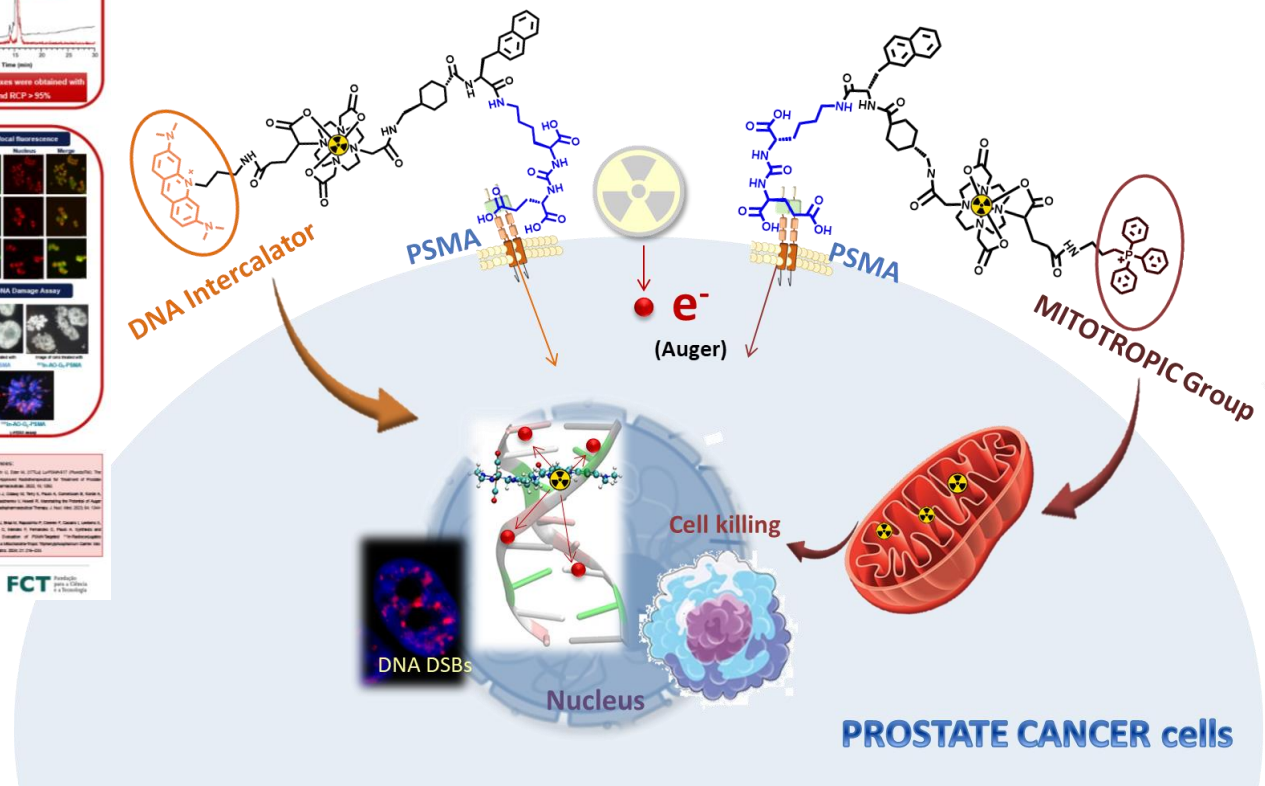
AO pharmacophores to promote the a

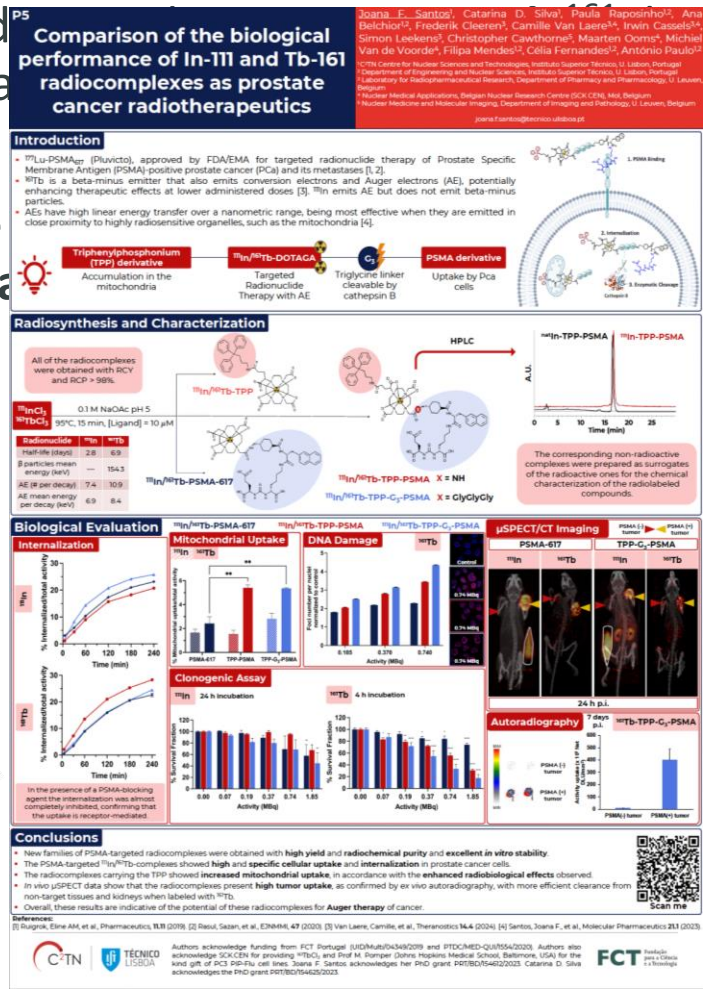
f Prostate cancer cells.

vantage of AEs emitted by ^{161}Tb to obtain **selective**

pared with single-targeted congeners to **improve thera**

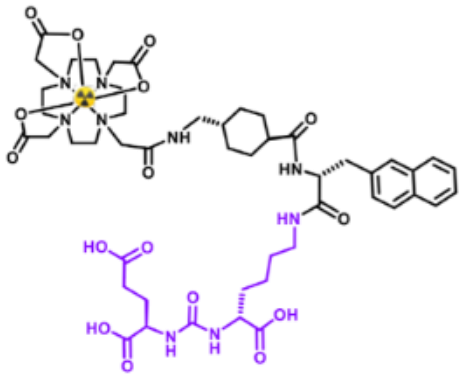




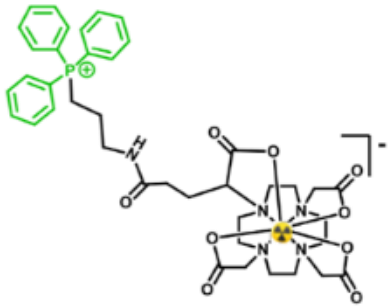


^{161}Tb complexes Carrying Mitotropic (TPP) Units

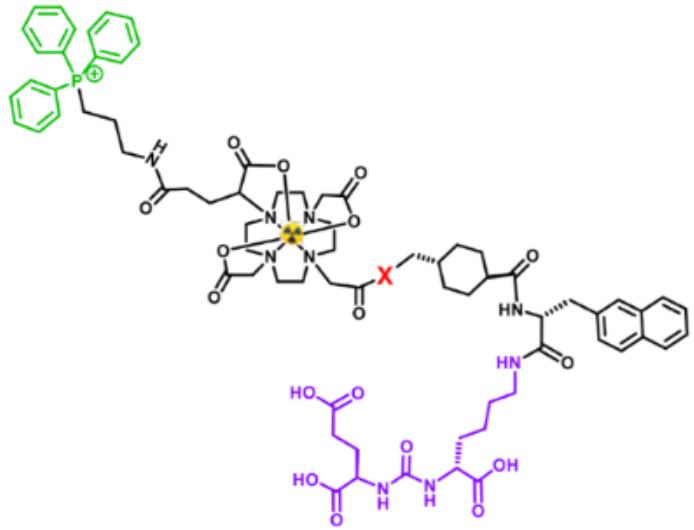
❑ Synthesized and Evaluated Complexes



[^{161}Tb]Tb-PSMA-617

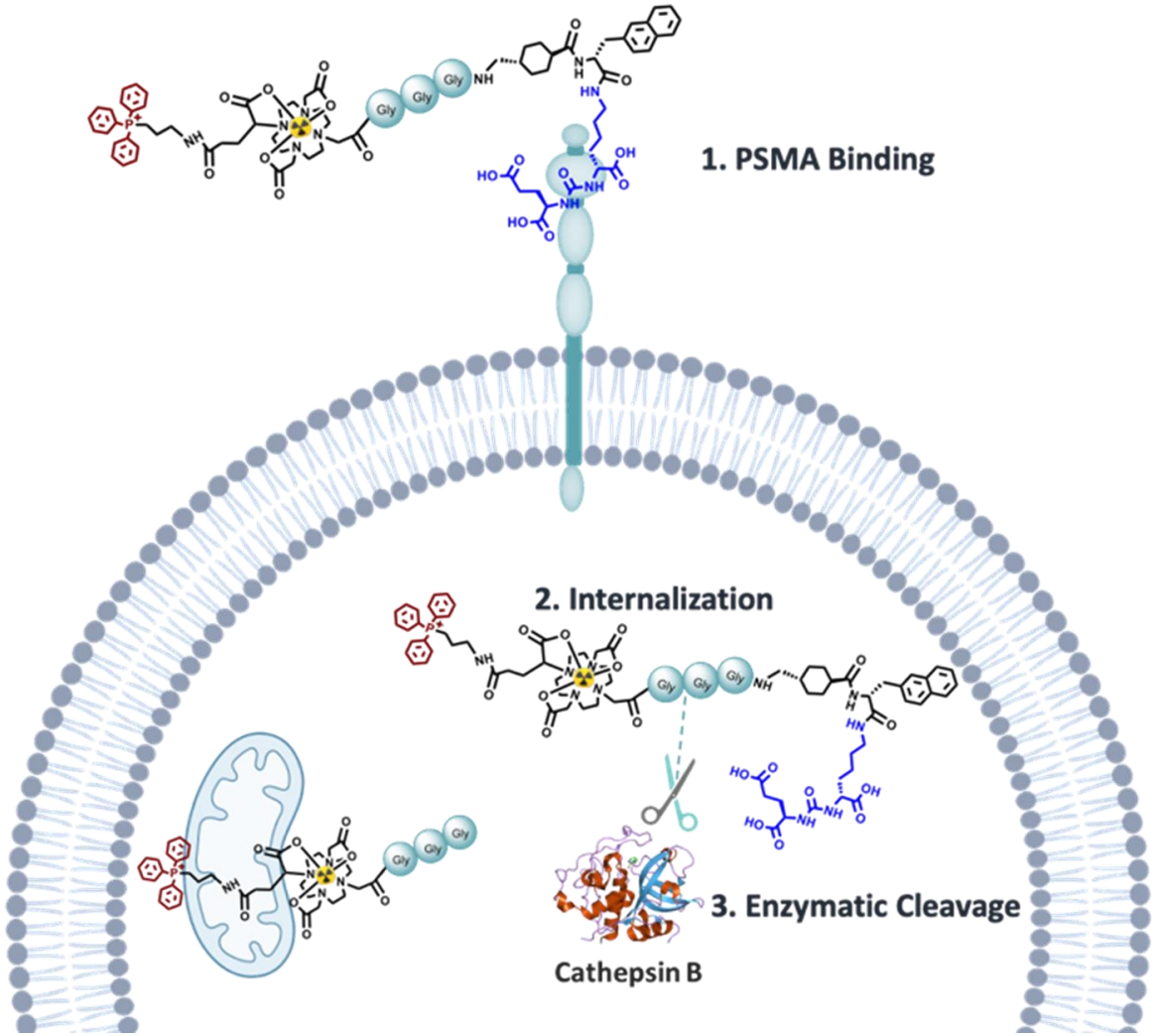


[^{161}Tb]Tb-TPP

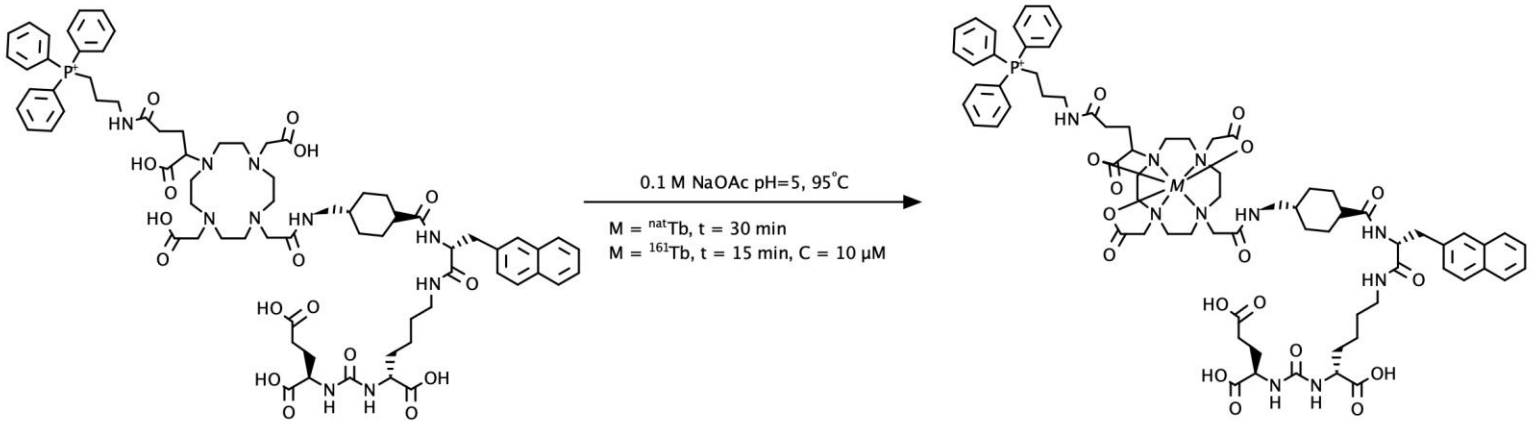


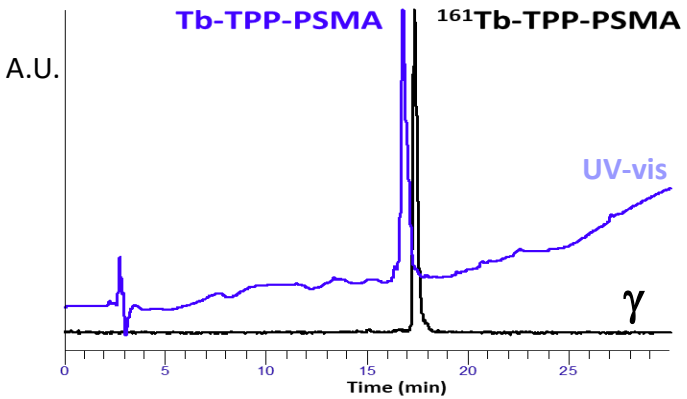
X = NH, [^{161}Tb]Tb-TPP-PSMA

X = G_3, [^{161}Tb]Tb-TPP-G_3-PSMA



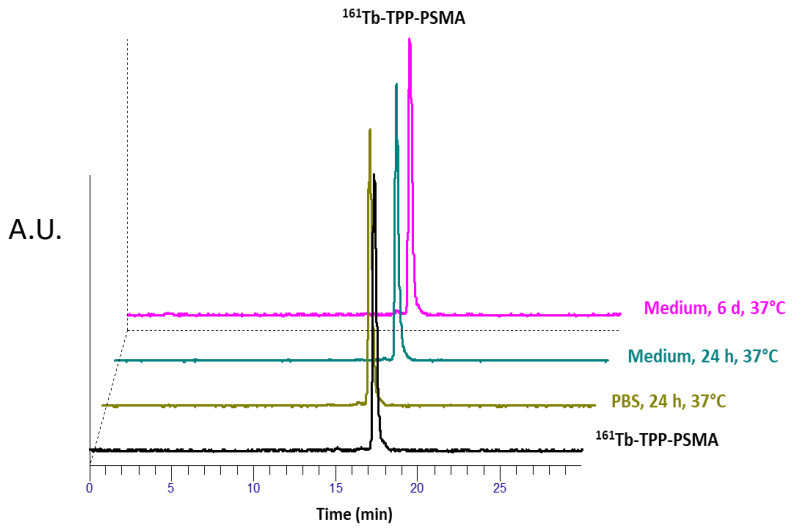
Radiolabeling/ In vitro Stability Studies





- ✓ Non-radioactive congeners characterized by ESI-MS analysis.
- ✓ HPLC co-injection runs of ^{nat}Tb and ^{161}Tb complexes confirmed the chemical identity of the radioactive ones.

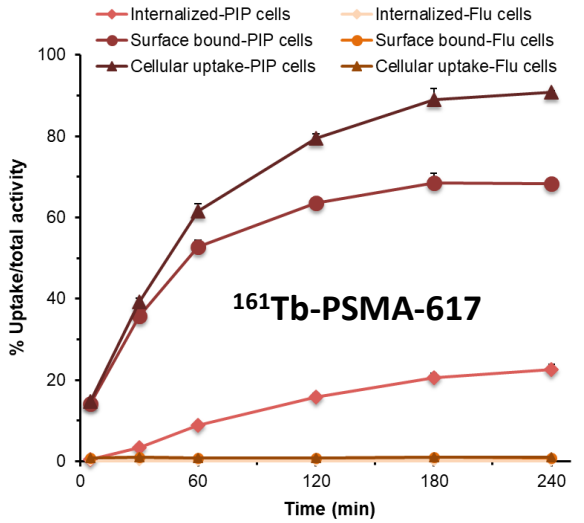
In vitro stability in PBS and cell culture medium

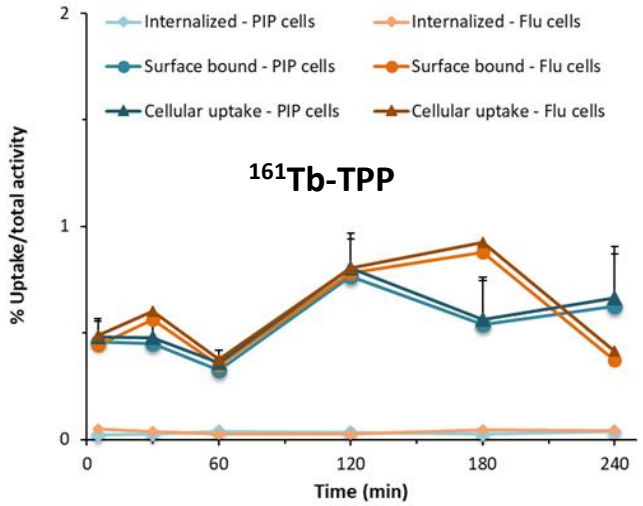


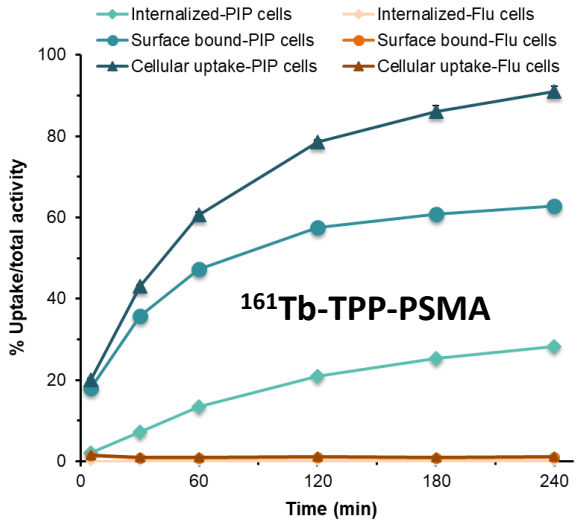
- ✓ High RCY/RCP
- ✓ High Specific Activity
- ✓ High In Vitro Stability

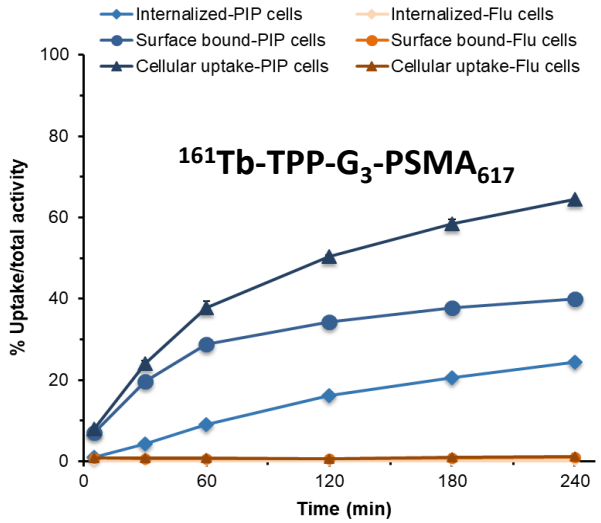
Cellular Studies: Uptake, Internalization and Blockade Assays

❑ Uptake and internalization in PC3 PIP cells (PSMA+) and PC3 flu cells (PSMA-)

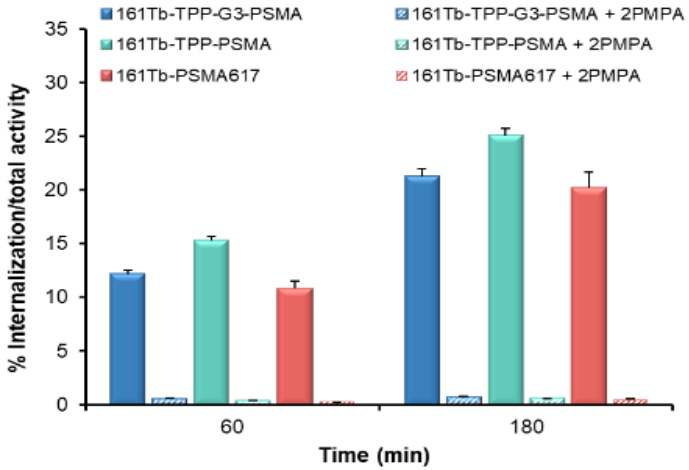


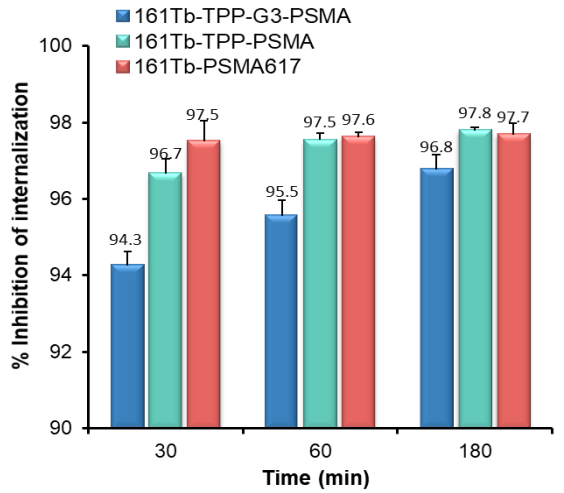






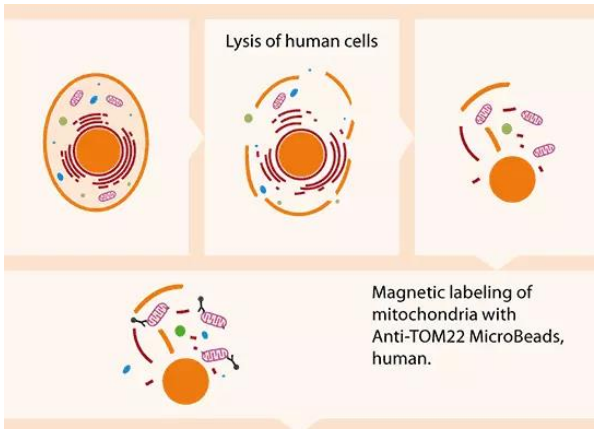
❑ PSMA-blocking study in PC3 PIP cells

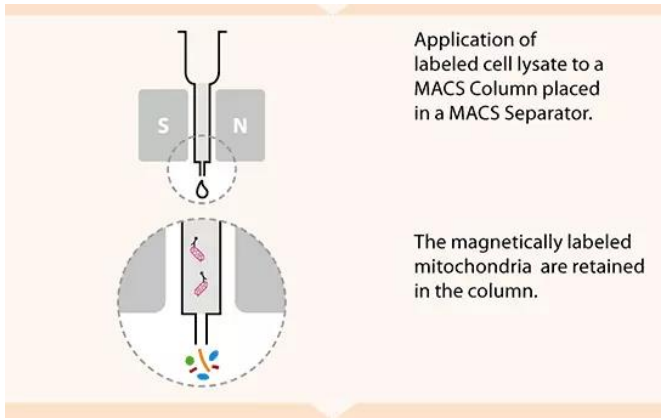


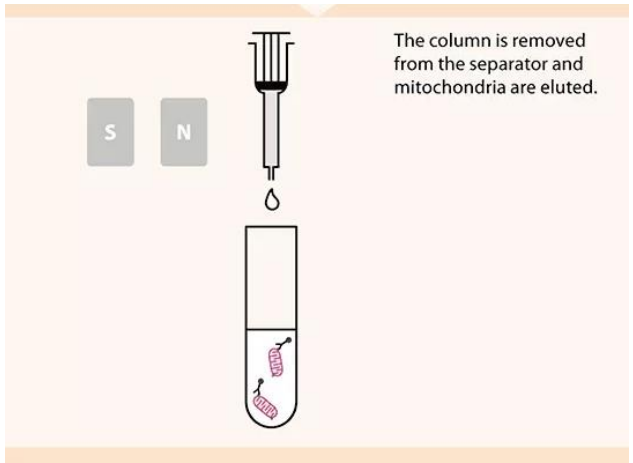


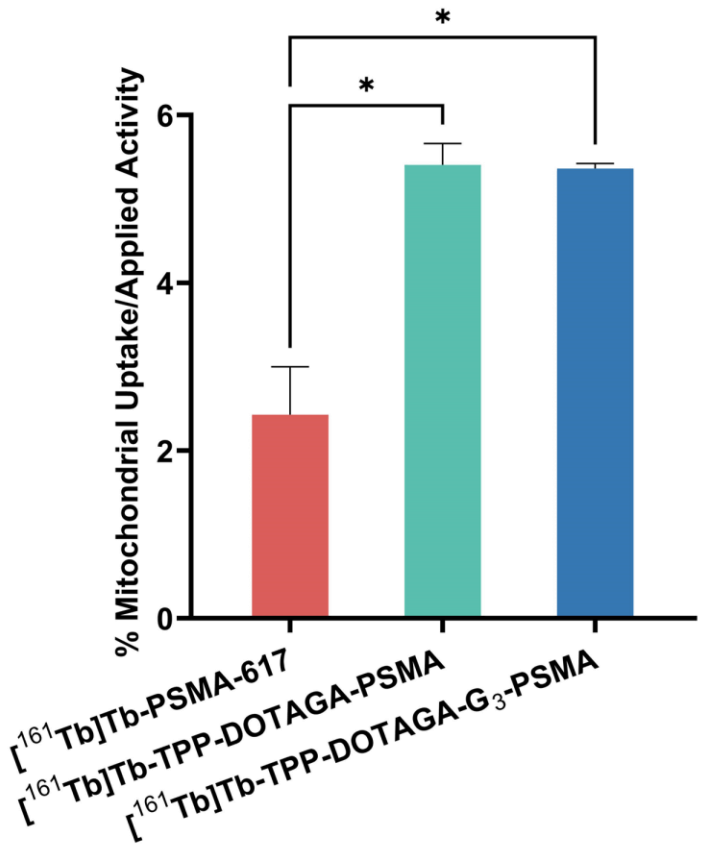
Cellular Studies: Mitochondrial uptake

- **Mitochondrial Uptake** 1 h
  PC3-PIP cells
  Mitochondria Isolation Kit (using magnetic beads) (Miltenyi Biotec)



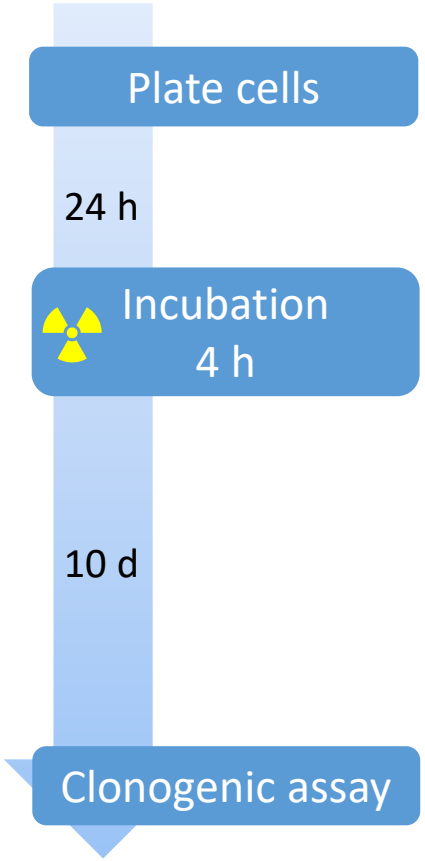


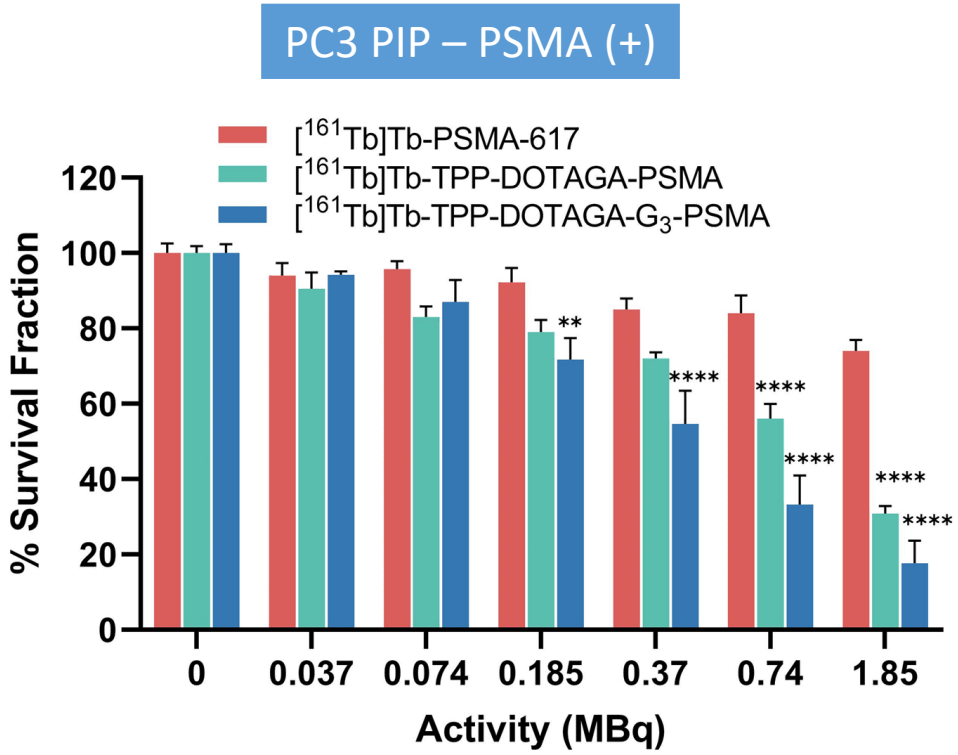


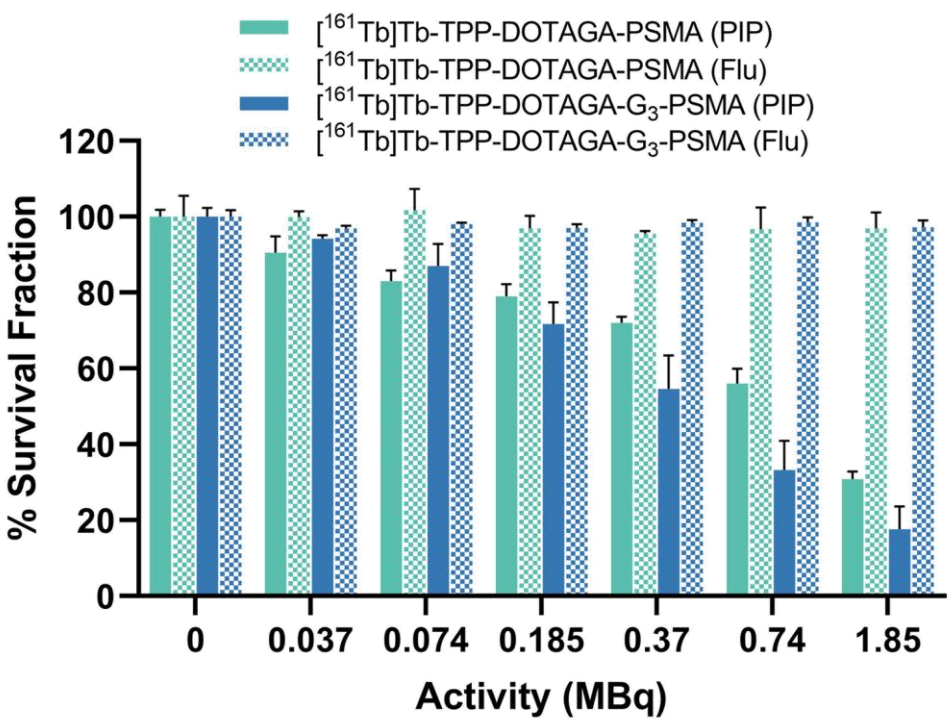


Cellular Studies: Radiobiological Effects

- **Clonogenic assay**: assess the ability of cells to divide and form colonies upon exposure to the radioconjugates.



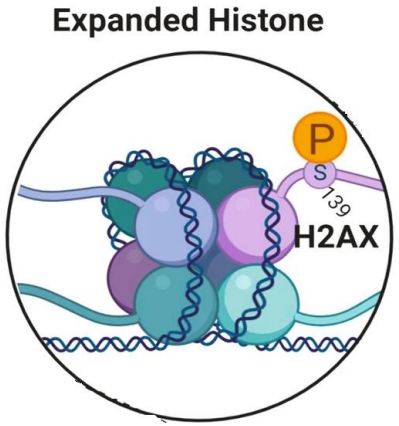




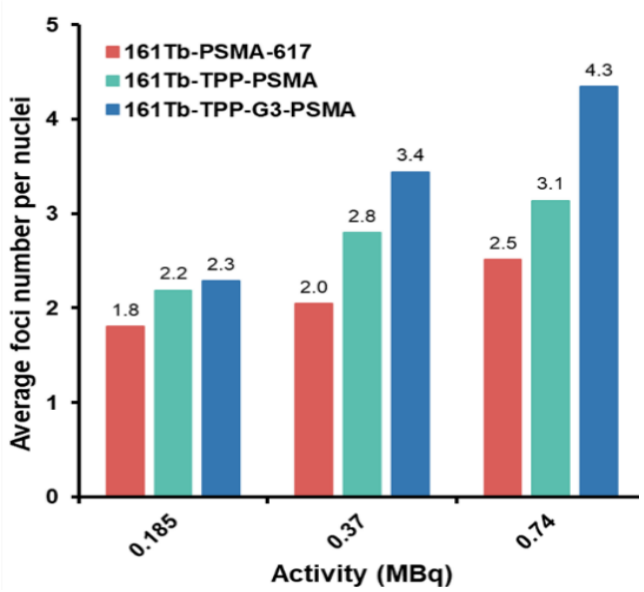
- The dual complexes **^{161}Tb-TPP-PSMA** and **^{161}Tb-TPP-G$_3$-PSMA** induces stronger radiobiological effects than **^{161}Tb-PSMA-617**, inhibiting more efficiently the proliferation of PC3-PIP cells for the highest tested activities without affecting the PC3-FLU cells.

Cellular Studies: Radiobiological Effects

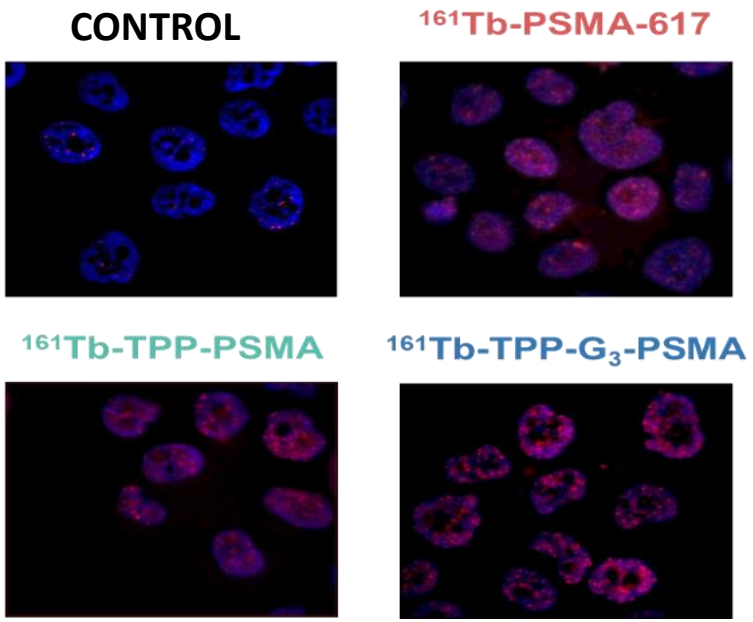
- **γ-H2AX Assay**: Quantification of individual γ–H2AX foci by fluorescence microscopy
- Phosphorylation of the Ser-139 residue of the histone variant H2AX leads to the formation of γ-H2AX foci, which is an early cellular response to the induction of DNA double-strand breaks .
- γ-H2AX foci are correlated with the induction of DNA double-strand breaks



Activity-dependent (0.185-0.74 MBq) DNA damage after 4 h incubation at 37 °C



Fluorescence images of cells exposed to 0.74 MBq for 4h



(*Cells were immunostained for γ-H2AX;*

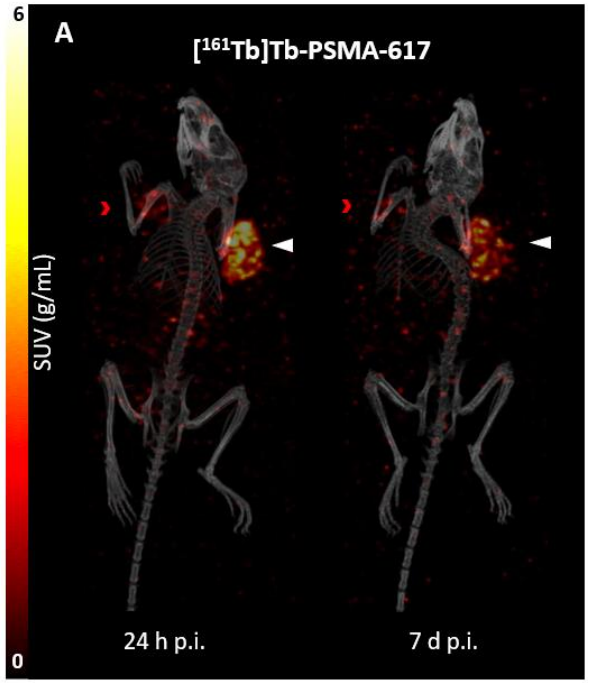
DAPI was used to visualize the nuclei)

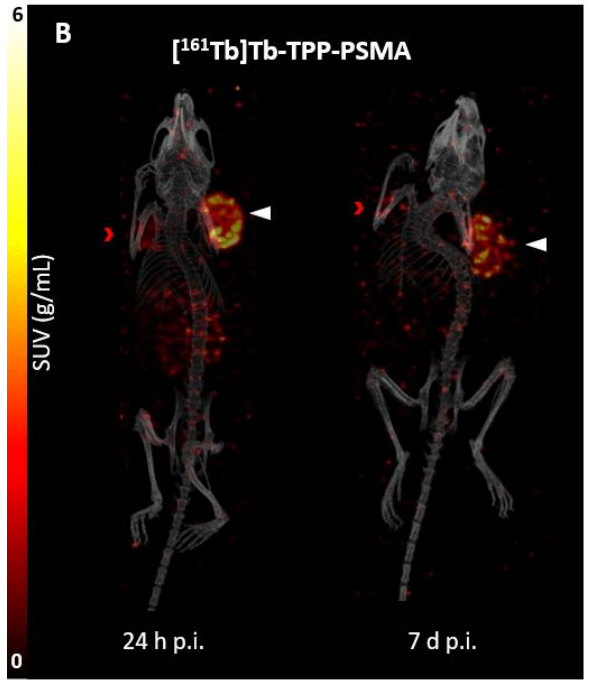
MicroSPECT Imaging Studies

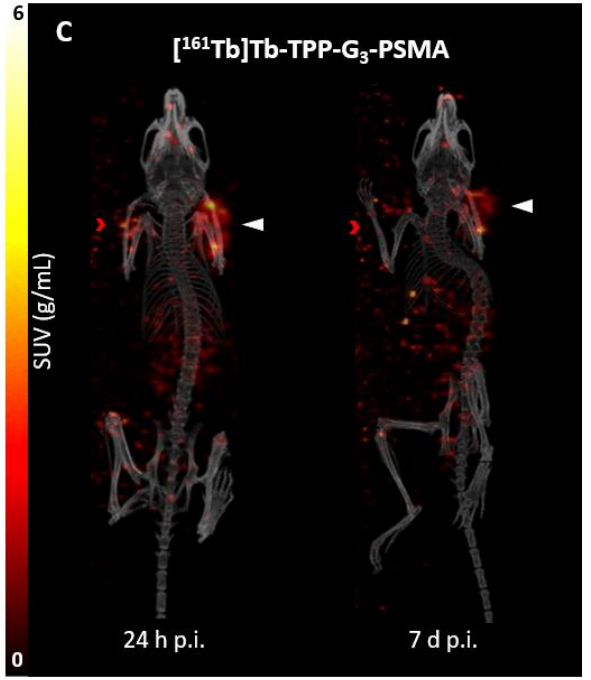
- µSPECT 24 h and 7 d p.i.

Left tumor
PSMA-
(PC3 Flu)

Right tumor
PSMA+
(PC3 PIP)

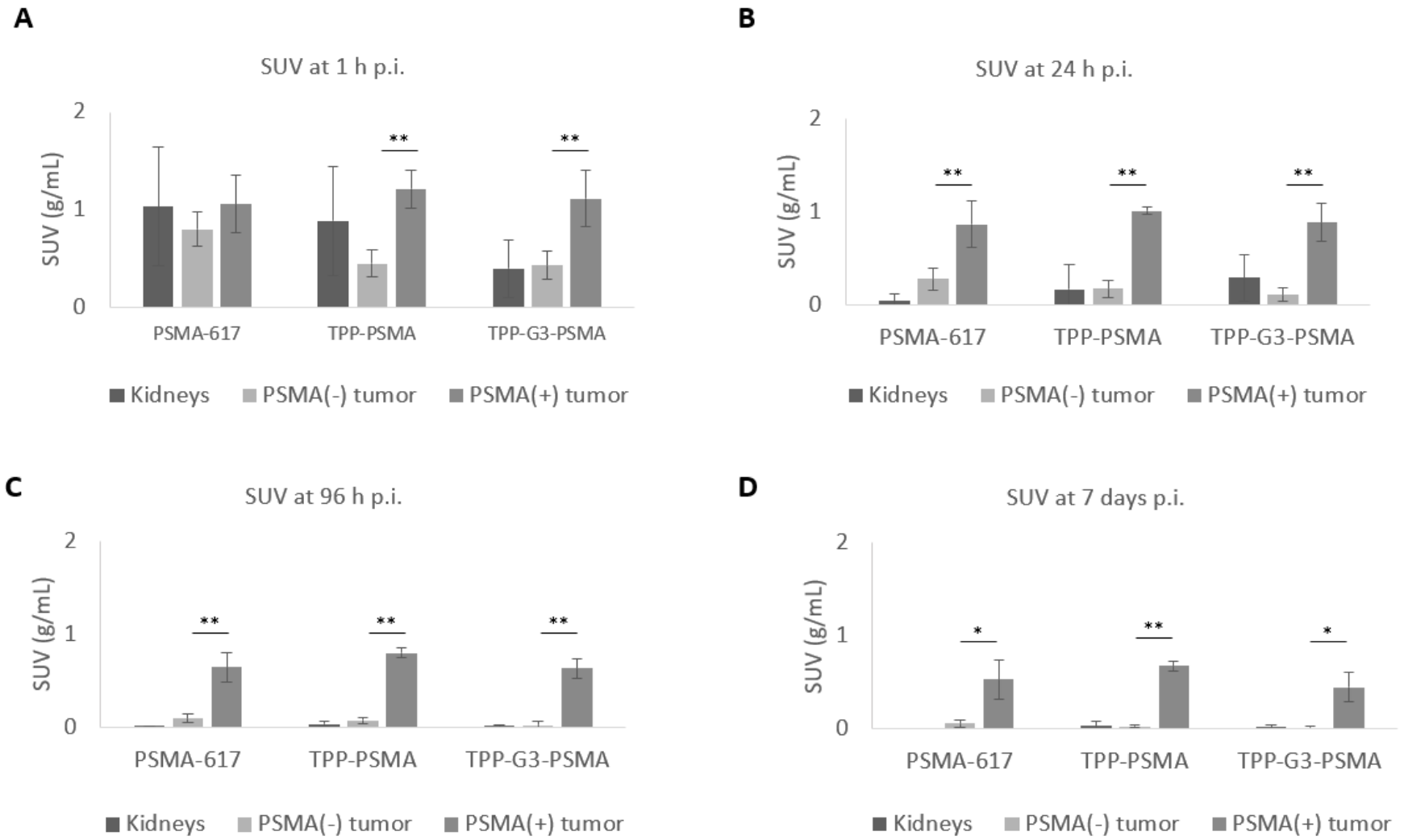






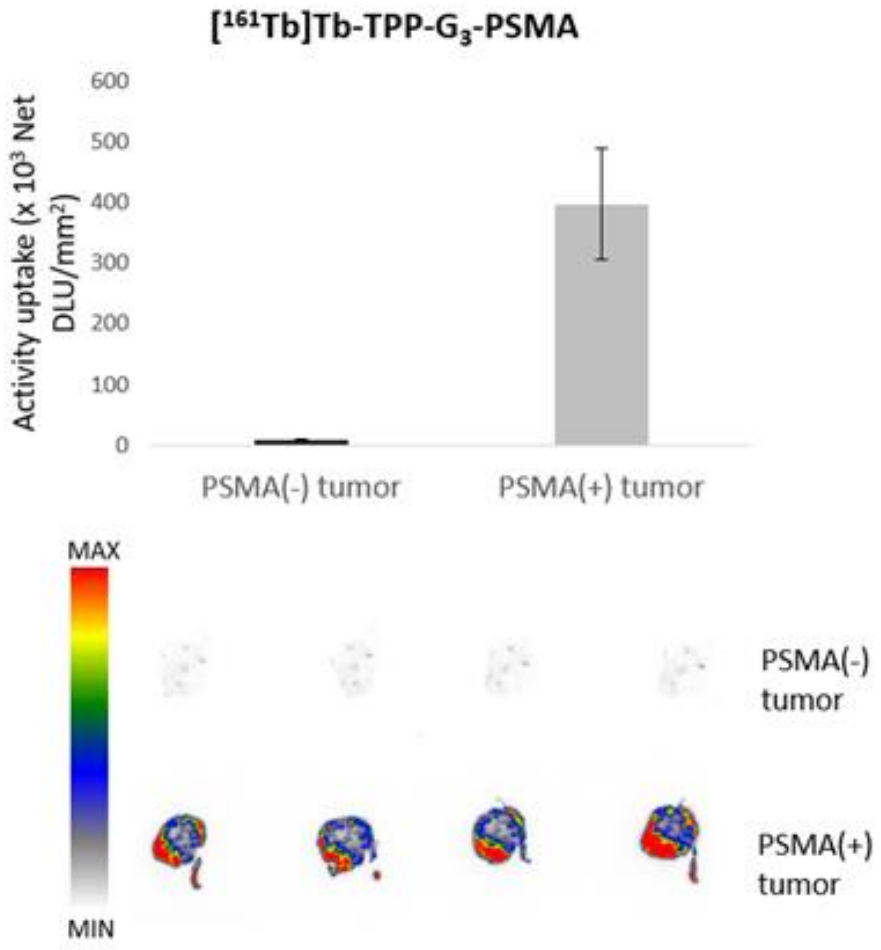
MicroSPECT Imaging Studies

- SUV values (kidneys and tumors) (1 h, 24 h, 96 h and 7 d p.i.)

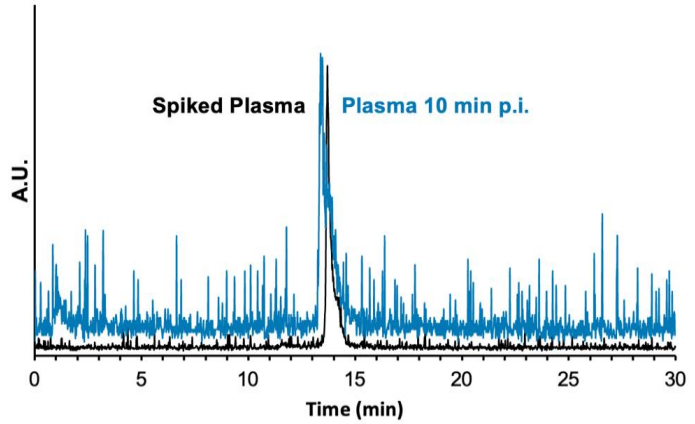


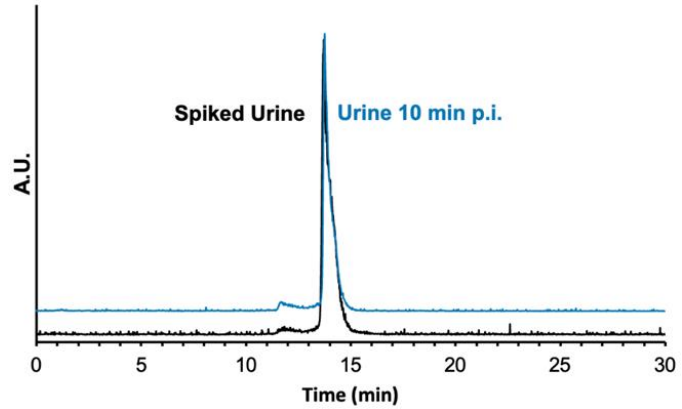
Autoradiography and Radiometabolite Studies

- **Ex vivo autoradiography data at 7 days post-injection**



- **HPLC radiochromatograms of plasma and urine samples from mice injected with [^{161}Tb]Tb-TPP-G_3-PSMA (10 min. p.i. and comparison with samples spiked with the radiocomplex)**





Conclusion and Perspectives

- TPP-containing ^{161}Tb radiocomplexes, combining PSMA and mitochondria targeting ability, significantly enhance radiocytotoxicity of ^{161}Tb in PSMA-positive prostate cancer cells, leading to increased DNA damage.

- Their excellent *in vivo* stability, successful *in vivo* tumor targeting and favorable pharmacokinetics make these TPP-containing radiocomplexes good candidates to improve the efficacy of PCa treatment based on Auger electron therapy.

- Therapeutic assays in tumor bearing mice are foreseen for the best performing **^{161}Tb-TPP-G_3-PSMA**, together with further studies to enlighten the possible role of mitochondrial damage on the observed radiobiological effects.

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Camille Van Laere

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UID/Multi/04349/2019

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PRISMAP
Medical Radionuclides

THANK YOU FOR YOUR ATTENTION!

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PRISMAP PROJECT

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