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Developing ^{134}Ce-Labeled Radiopharmaceuticals as Imaging Surrogates for ^{225}Ac Compounds

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DOE IP Virtual Seminar Series – Ce-134

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Radiosynthesis and QC

Labeling of macropa- and DOTA-containing compounds

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Anja Wacker, Ph.D.

Radiosynthesis and QC

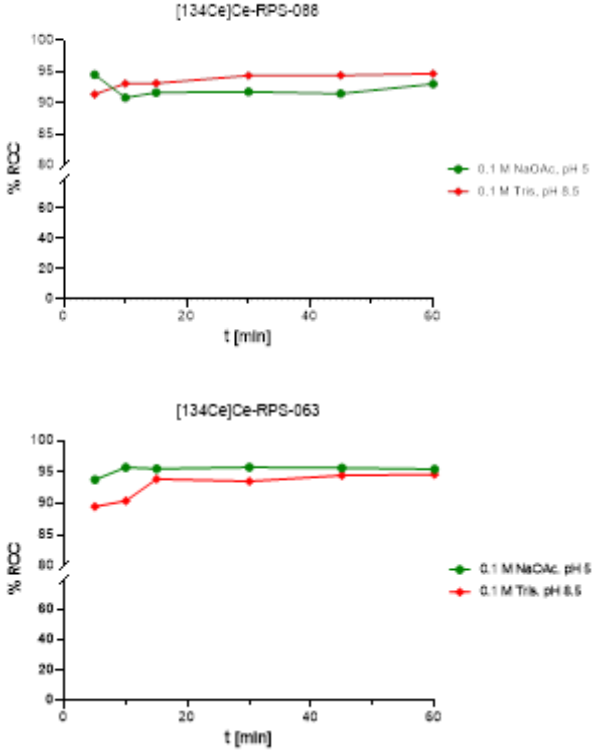
Labeling of macropa- and DOTA-containing compounds

Labeling of macropa and DOTA with Ce-134

- We compared the labeling of RPS-088, a small molecule containing macropa, and RPS-063, a small molecule containing DOTA with Ce-134 (provided in 0.1 M HCl)
- The mass of precursor was 0.5 nmol, and total reaction volume was 55 µL. The reaction was sampled from 5 min to 60 min

Precursor	Buffer	Temperature
RPS-088	0.1 M Tris, pH 8.5	25 °C
RPS-088	0.1 M NaOAc, pH 5	25 °C
RPS-063	0.1 M Tris, pH 8.5	95 °C
RPS-063	0.1 M NaOAc, pH 5	95 °C

Macropa complexes > 90% $[^{134}Ce]Ce^{3+}$ within 5 min at 25 °C, while DOTA complexes > 90% within 5 min at 95 °C



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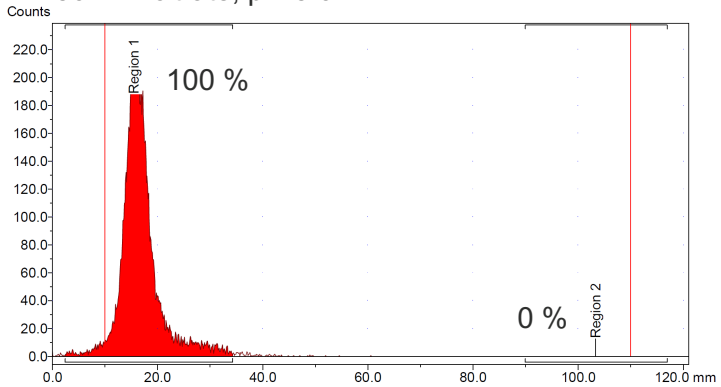
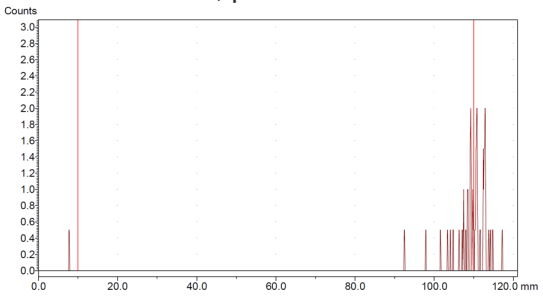
QC of the radiolabeled compounds

- RCP was evaluated by radio-iTLC using two mobile phases: 50 mM citrate, pH 5 and 1 M NH_4OAc (pH 5.5):DMF = 1:1
- TLC plates were scanned 60 min after development
- In 50 mM citrate, [^{134}Ce]Ce-RPS-088 remains at the application point and unbound Ce-134 migrates with the solvent front

[^{134}Ce]Ce-RPS-088
50 mM citrate, pH 5.0



free Ce-134
50 mM citrate, pH 5.0



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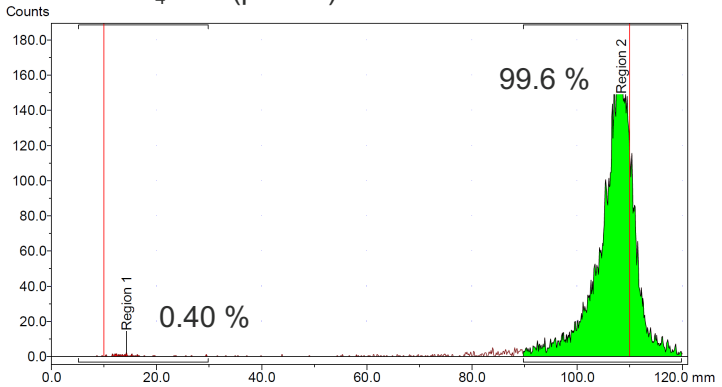
[^{134}Ce]Ce-RPS-088

1 M NH_4OAc (pH 5.5)/DMF 1:1



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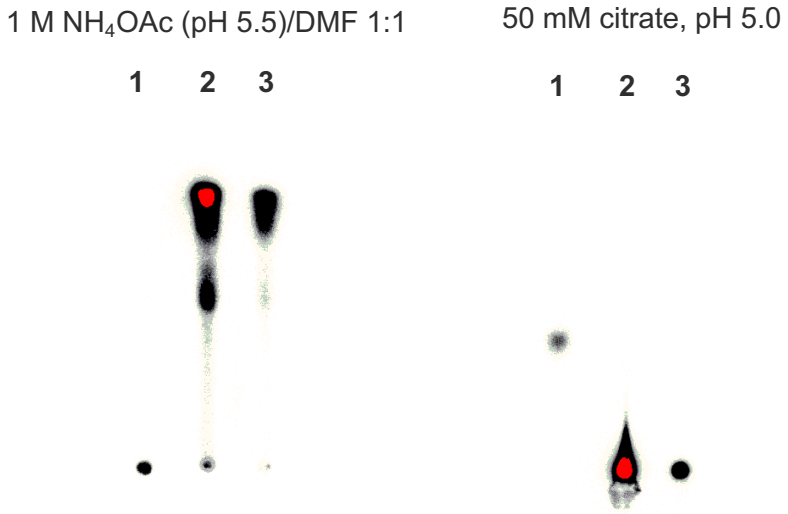
- In 1 M NH_4OAc:DMF = 1:1, unbound Ce-134 remains at the application point and [^{134}Ce]Ce-RPS-088 migrates with the solvent point
- The same results are obtained when [^{225}Ac]Ac-RPS-088 is run in the mobile phases and evaluated 2 h after plate development



1. Free [^{225}Ac]Ac^{3+}

2. [^{225}Ac]Ac-RPS-088, crude

3. [^{225}Ac]Ac-RPS-088, purified

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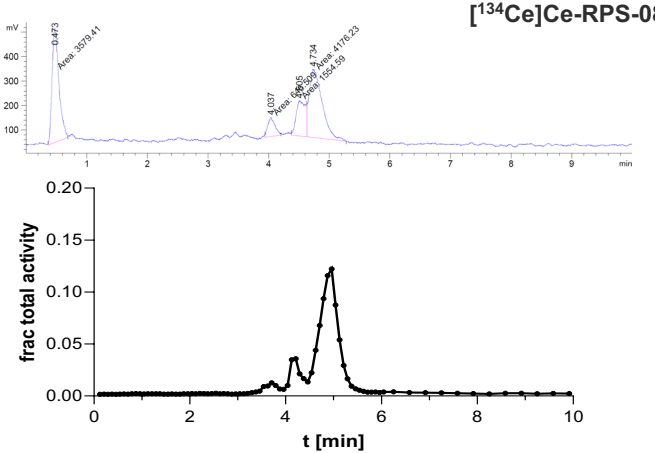
QC of the radiolabeled compounds

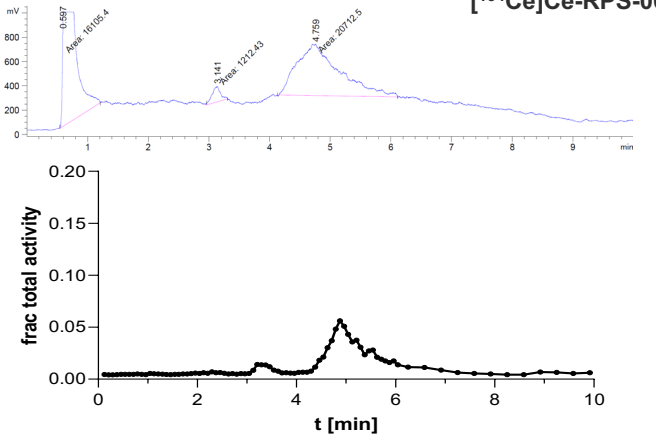
- RCP determination by HPLC is also possible through fraction collection
- Fractions are spotted on a grid and imaged by phosphorimager 1 h after collection
- For small molecules, this allows radiochemical identity to be determined simultaneously

The sizeable peak at the injection front disappears upon gamma counting, indicating that it is due to La-134

[^{134}Ce]Ce-RPS-088



[^{134}Ce]Ce-RPS-063



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In vitro experience

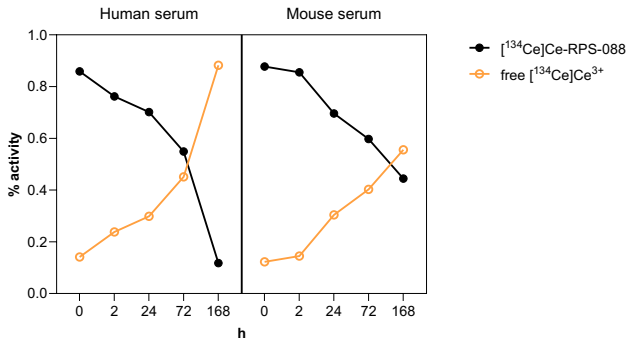
Stability studies and challenge experiments

Serum stability in human and mouse

- [^{134}Ce]Ce-RPS-088 was incubated at 37 °C in 100 µL human or mouse serum
- Aliquots were assayed pre- and post-precipitation of proteins up to 168 h post injection
- Stability assessed by radio-iTLC using 50 mM citrate, pH 5 mobile phase

The ^{134}Ce-macropa complex is approximately 80% stable in human and murine serum after 72 h and approximately 25% stable after 168 h

Pre-Precipitation



Post-Precipitation

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Serum stability in human and mouse

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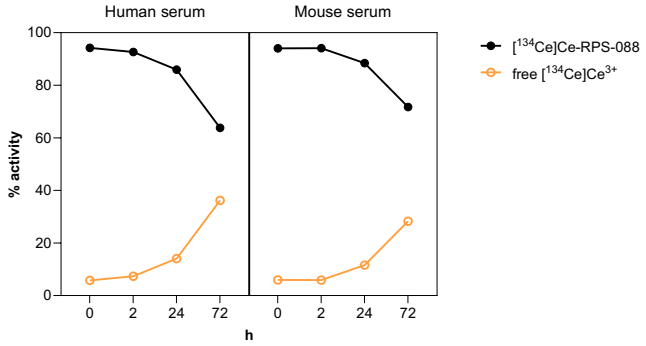
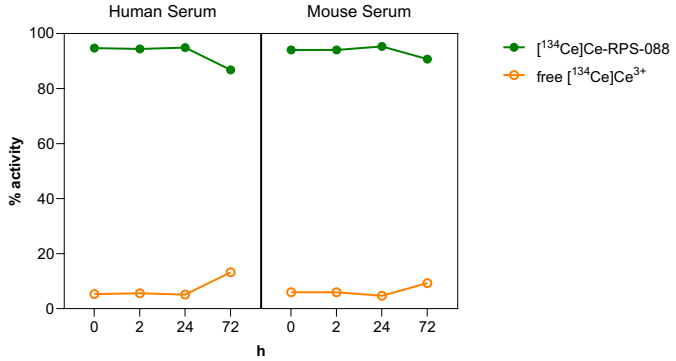
The ^{134}Ce-DOTA complex is approximately 95% stable in human and murine serum after 72 h. This complex is more stable than the ^{134}Ce-macropa complex.

Pre-Precipitation



Post-Precipitation



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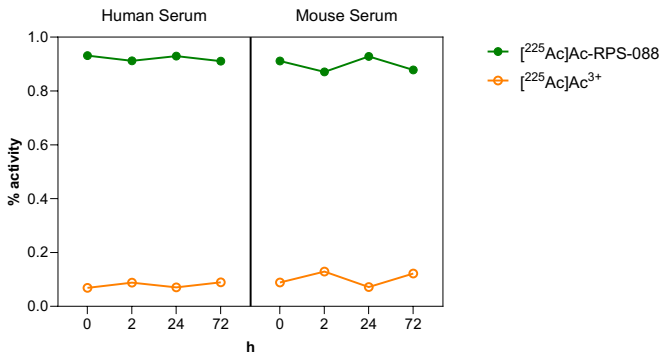
Serum stability in human and mouse – comparison to Ac-225

- [^{225}Ac]Ac-RPS-063 and [^{225}Ac]Ac-RPS-088 were incubated at 37 °C in 100 µL human or mouse serum
- Aliquots were assayed pre- and post-precipitation of proteins up to 72 h post injection
- Stability assessed by radio-iTLC using 50 mM citrate, pH 5 mobile phase

As expected, the ^{225}Ac-macropa complex is significantly more stable than the ^{225}Ac-DOTA complex. This is the inverse of the ^{134}Ce complex stabilities

[^{225}Ac]Ac-RPS-088



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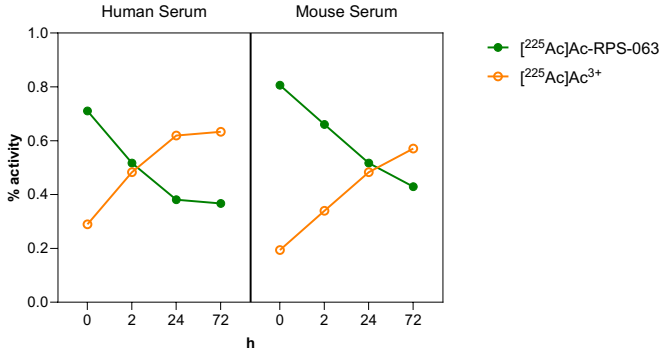

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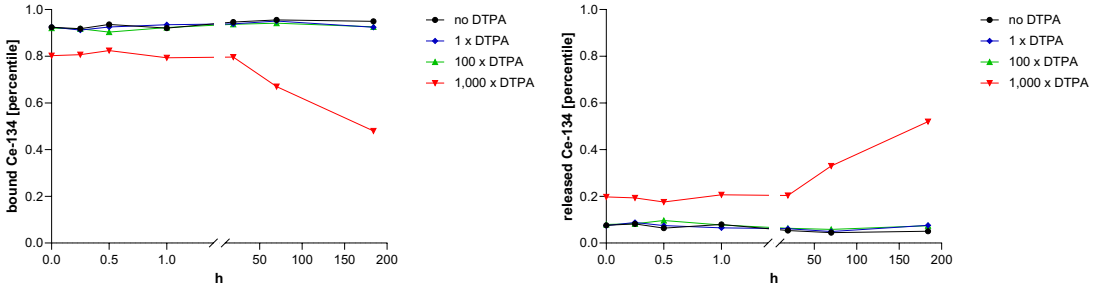
DTPA challenge experiment

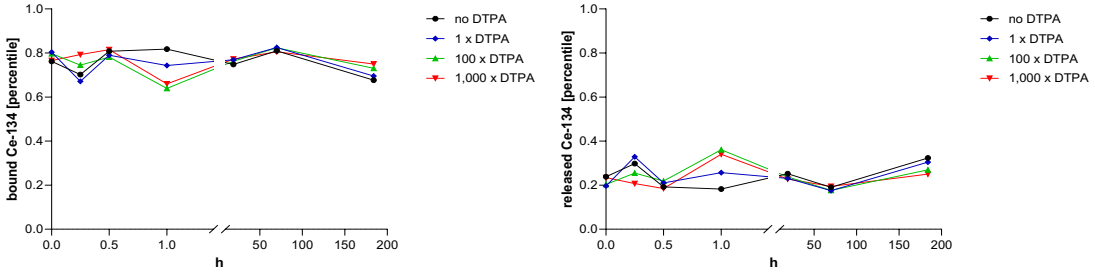
- [^{225}Ac]Ac-RPS-063 and [^{225}Ac]Ac-RPS-088 were incubated at 37 °C with 0, 1, 100, and 1000 molar equivalents of DTPA
- Aliquots were assayed pre- and post-precipitation of proteins up to 168 h post injection
- Stability assessed by radio-iTLC using 50 mM citrate, pH 5 mobile phase

The ^{134}Ce-macropa complex is stable up to 100x DTPA while the ^{134}Ce-DOTA complex is stable at all concentrations of DTPA

[^{134}Ce]Ce-RPS-088



[^{134}Ce]Ce-RPS-063



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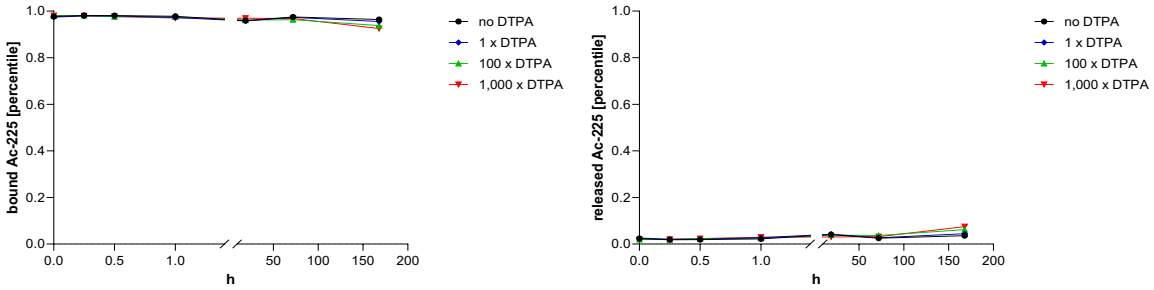
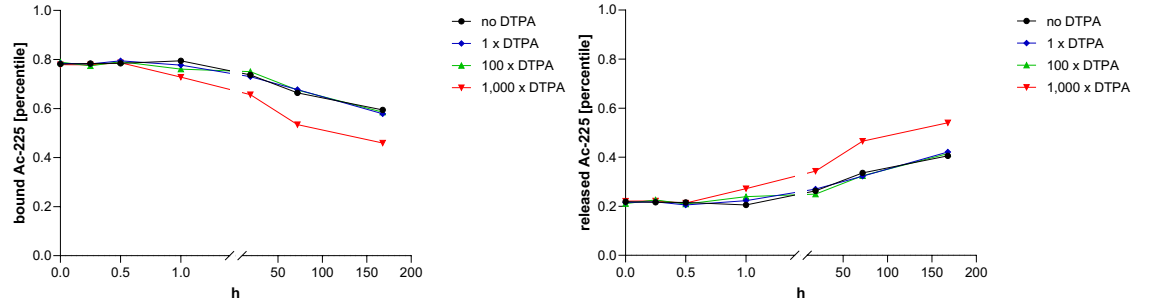
[^{225}Ac]Ac-RPS-088



[^{225}Ac]Ac-RPS-063



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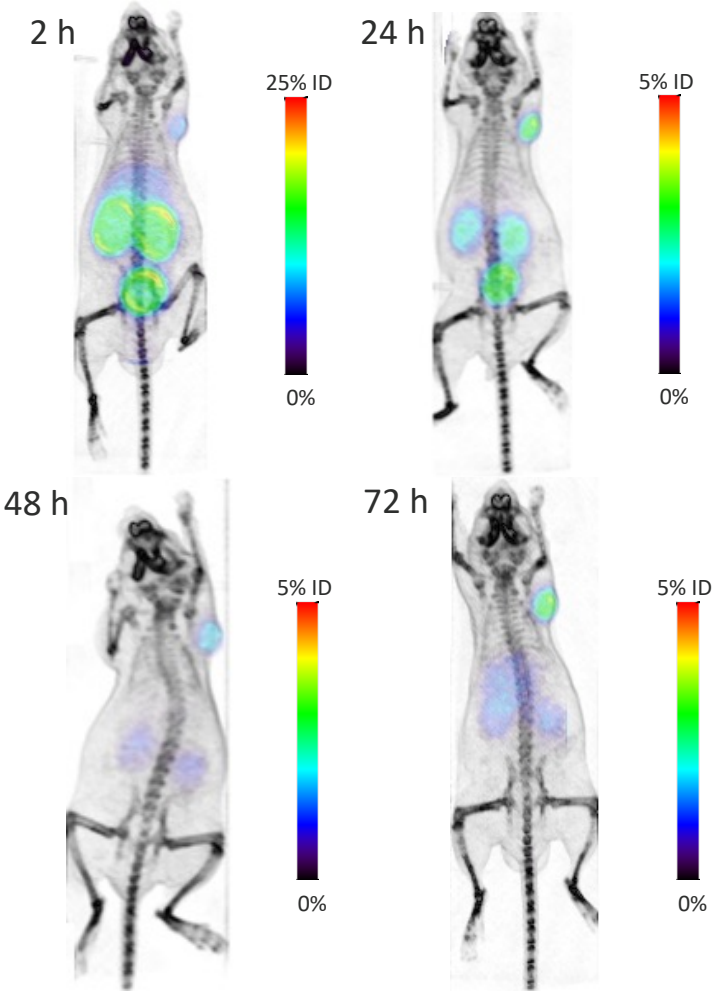
In vivo experience

Experiments in an LNCaP tumor model

microPET/CT imaging

- Mice were administered 4.5 MBq (120 µCi) of [^{134}Ce]Ce-RPS-088 (a macropa-containing PSMA ligand) and imaged at 2, 24, 48, and 72 h post injection
- Scan acquisition time was 30 min
- The mice were imaged with a 1% injected dose standard to enable image-based quantification of uptake

- Biodistribution was as expected, with the exception of the elevated liver uptake
- We suspect some in vivo decomplexation



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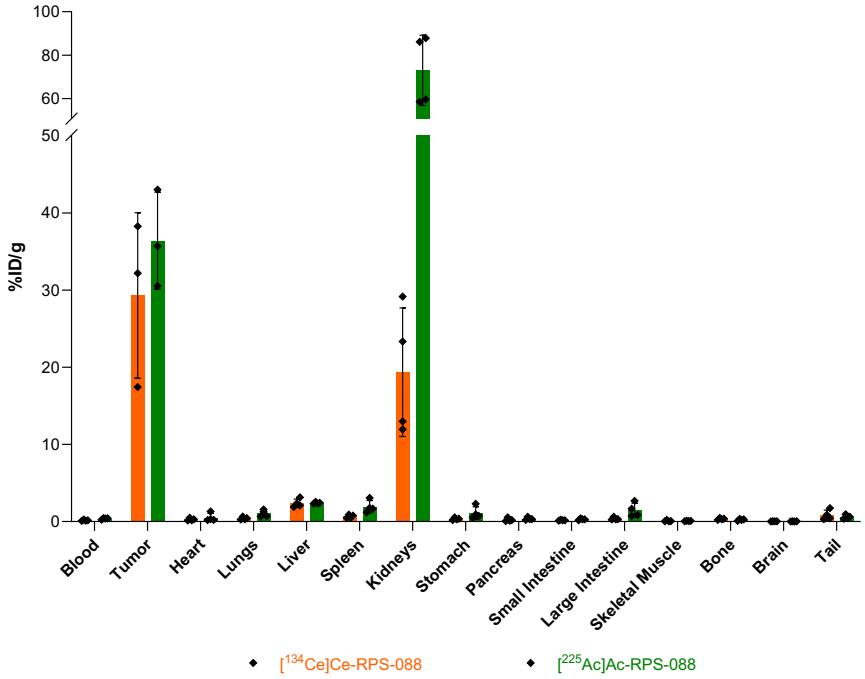
Biodistribution: Intersubject comparison with ^{225}Ac-labeled compound

- Mice were administered RPS-088 labeled with either Ce-134 (3.5 MBq) or Ac-225 (50 kBq)
- The mice were sacrificed at 24 h post injection
- Tissues were counted 24 h after collection and uptake quantified by comparison to a 1% injected dose standard

A key difference is evident in the kidneys. Our working hypothesis is that the decomplexed Ce-134/La-134 clears via the hepatobiliary pathway faster than 24 h and is not captured by this time point



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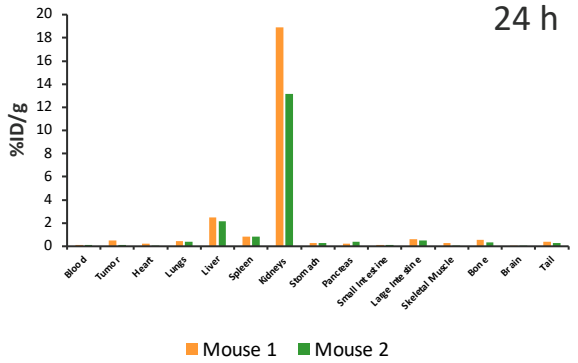
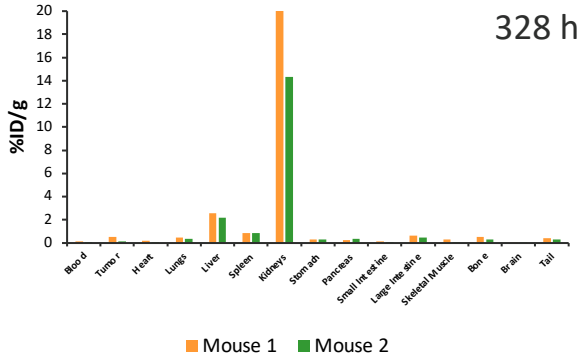
Biodistribution: Intrasubject comparison with ^{225}Ac-labeled compound

- 2 Mice were co-administered [^{225}Ac]Ac-RPS-088 (50 kBq) and [^{134}Ce]Ce-RPS-088 (1.5 MBq)
- The mice were sacrificed at 24 h post injection
- Tissues were counted 24 h after collection and again at 61 h, 85 h, 160 h, and 328 h after collection

As expected, the quantification of total tissue uptake is consistent at all time points





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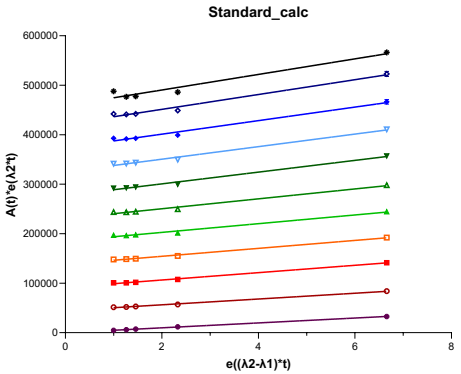
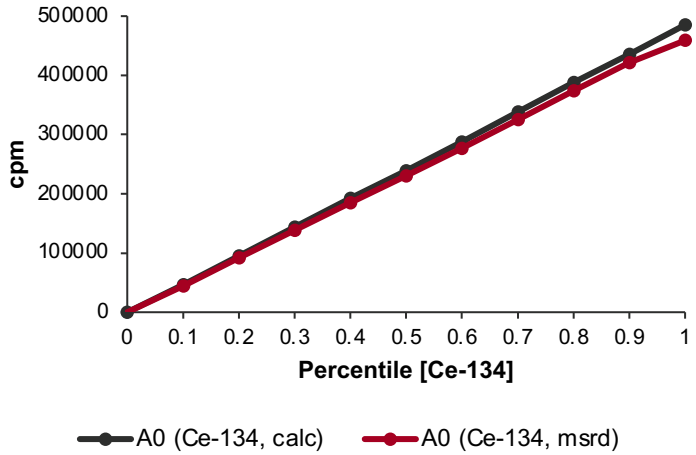
Biodistribution: Intrasubject comparison with ^{225}Ac-labeled compound

- We used a graphical method to estimate the contribution of each compound to the total tissue activity
- Standards ranging in activity from 0-100% Ce-134 were serially measured to provide data for curve fitting
- The y-intercepts of the curves give the activity of Ce-134 at t=0 (time of sacrifice)

The fit for Ce-134 is good, with a maximum error of 5.5% at higher percentages of Ce-134



Nonlin fit Table of results	A 0	B 0.1	C 0.2	D 0.3
Straight line				
Best-fit values				
Yintercept	-62.38	44617	91538	138291
Slope	4940	5848	7439	8024
95% CI (profile likelihood)				
Yintercept	-403.9 to 279.1	43658 to 45576	90156 to 92920	136823 to 139759
Slope	4836 to 5043	5557 to 6138	7021 to 7858	7579 to 8469
Goodness of Fit				
Degrees of Freedom	18	13	13	13
R squared	0.9982	0.9932	0.9913	0.9915
Sum of Squares	3873983	15660843	32493066	36686271
Sy.x	463.9	1098	1581	1680
Number of points				
# of X values	20	20	20	20
# Y values analyzed	20	15	15	15



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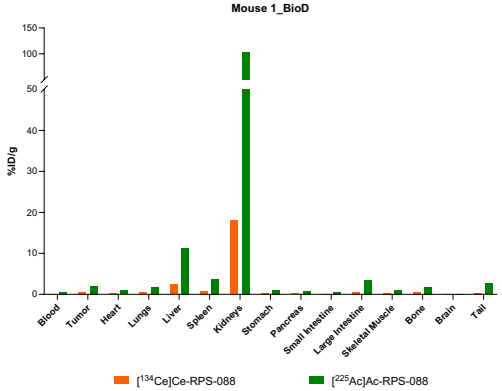
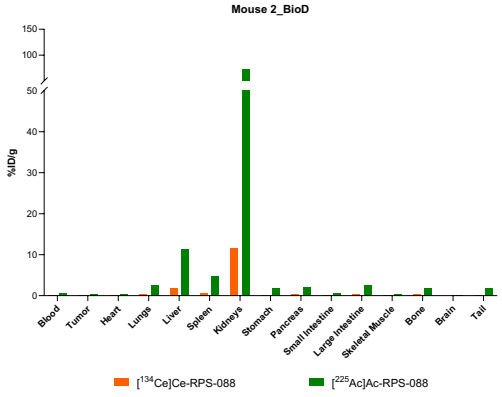
- We estimated the tissue activity due to [^{134}Ce]Ce-RPS-088 using the graphical method
- Contribution of [^{225}Ac]Ac-RPS-088 was the difference between total uptake and uptake due to [^{134}Ce]Ce-RPS-088
- Slightly higher background of [^{225}Ac]Ac-RPS-088, likely because of the 3-5.5% error in Ce-134 quantification

The tissue distribution is consistent between animals and with the results obtained in separate mice





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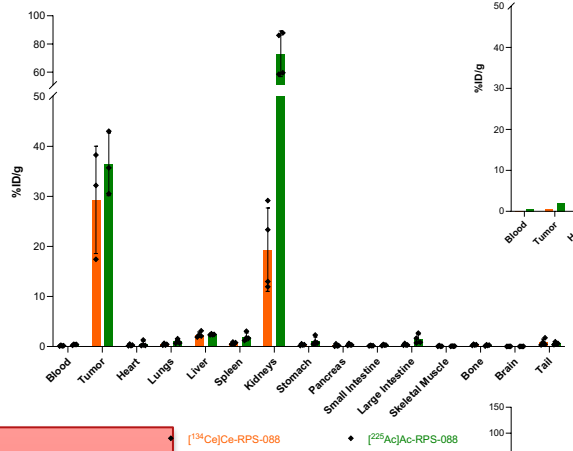

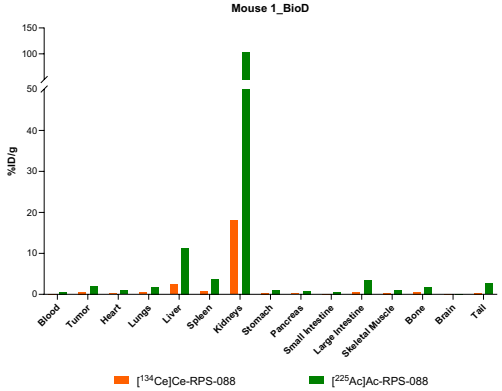

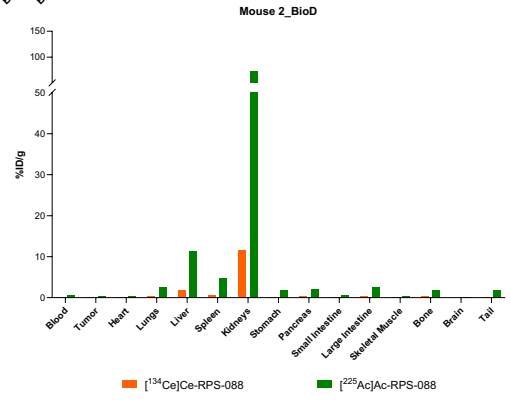

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Summary

- Both macropa and DOTA rapidly complex [^{134}Ce]Ce^{3+} at 95 °C
- The ^{134}Ce-DOTA complex is stable in serum and to DTPA challenge. ^{134}Ce-macropa decomplexes under the same conditions. This is the inverse complex stability of Ac-225
- Administration of [^{134}Ce]Ce-RPS-088 to mice results in high quality microPET/CT images. Small accumulation of signal in liver hints at instability of the complex in vivo
- In vivo decomplexation of ^{134}Ce-macropa may lead to different doses absorbed by excretory organs in comparison to ^{225}Ac-macropa compounds
- It will likely be possible to disentangle the contributions of Ce-134 and Ac-225 when co-administered to the same animal. This may enable improved dosimetry comparisons

Acknowledgments

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Alejandro Amor-Coarasa, Ph.D.

Oak Ridge National Laboratory

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