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Development of bispecific PSMA/GRPr targeting radioligands with optimized pharmacokinetics for PET imaging of prostate cancer

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50 Years – Research for A Life Without Cancer

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A novel class of bispecific PSMA/GRPR targeting radioligands with optimized pharmacokinetics for improved PET imaging of prostate cancer

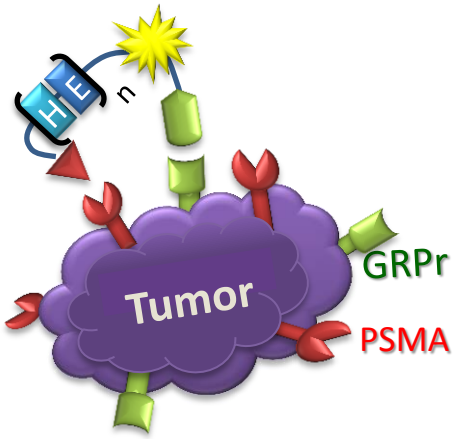
Graphical Abstract

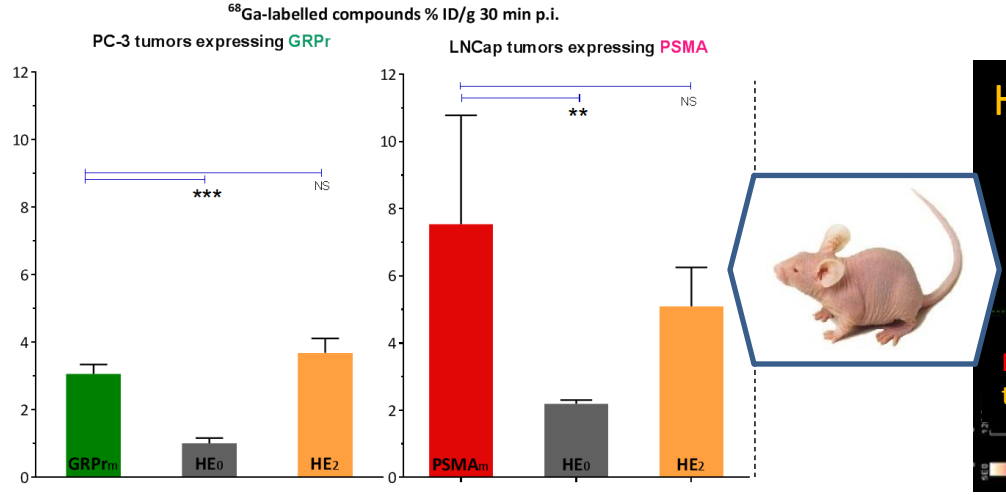
PSMA pharmacophore Glu-ureido-Lys

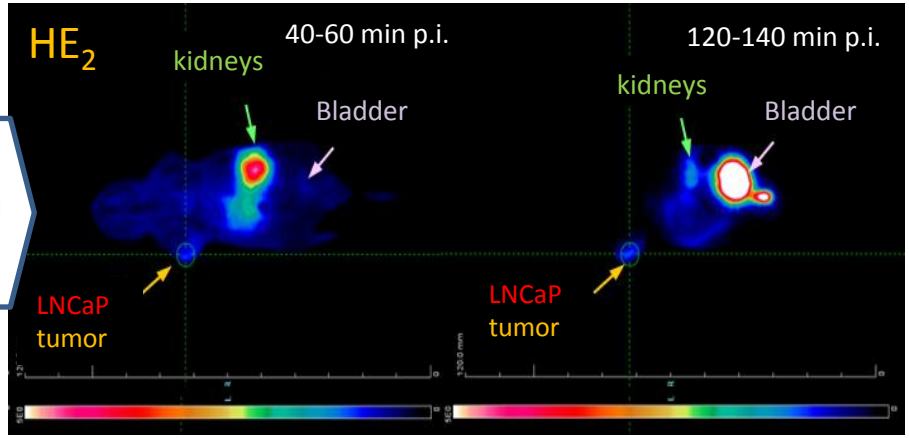
HE_n: Pharmacokinetic spacer His (H), Glu (E), (n = 0-3)

HBED-CC chelator for ^{68}Ga

GRPr Pharmacophore BN analogue
$H_2N\text{-}PEG_2\text{-}[D\text{-}Tyr^6, \beta\text{-}Ala^{11}, Thi^{13}, Nle^{14}]BN(6–14)$







Abstract: A series of novel low-molecular weight bispecific radioligands were developed, which were able to target the prostate-specific membrane antigen (PSMA) and the gastrin releasing peptide receptor (GRPr), both expressed on prostate cancer cells. These bispecific radiotracers combined the peptidomimetic urea-based pseudo-irreversible inhibitor of PSMA: Glu-ureido-Lys with the bombesin (BN) analogue: H_2N-PEG_2-[D-Tyr^6, β-Ala^{11}, Thi^{13}, Nle^{14}]BN(6–14), which binds to GRPr with high affinity and specificity. The two pharmacophores were linked together through the chelating agent HBED-CC and spacers made of positively charged His (H) and negatively charged Glu (E): -$(HE)_n$-, (n=0-3) amino acids. The positron emitter ^{68}Ga ($t_{1/2}$ = 68 min, β^+ 88 %, $E_{\beta+}$ max. 1.9 MeV) was used for the radiolabelling of the bispecific radioligands and preliminary pharmacological data were collected from *in vitro* assays on prostate cancer cell lines (PC-3, AR42J, LNCaP) and *in vivo* experiments in normal and tumor bearing mice (biodistribution and small animal PET imaging studies). The new bispecific ligands *in vitro* showed binding affinities, which essentially matched the ones of the respective monomers, while *in vivo* they were able to target both PSMA (LNCaP) and GRPr (PC-3) positive tumors. In addition the charged -$(HE)_n$-, (n=1-3), linkers improved the tracer's pharmacokinetics by significantly reducing the normal organ uptake (i.e. kidney and spleen) and by increasing the tumor to-background ratio. In conclusion, the bispecific (PSMA and GRPr) targeting ligands, developed in this study could be considered as novel radiotracer candidates for more sensitive PET/CT-imaging of prostate cancer (PCa) in future clinical application.

Keywords: ^{68}Ga, PET-prostate cancer diagnosis, PSMA/ GRPr bispecific radioligands, low-molecular weight heterodimer

Introduction

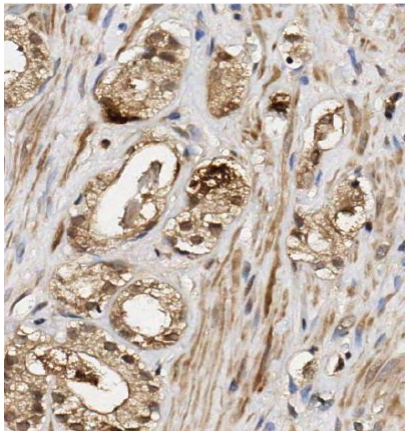
- ***Prostate-specific membrane antigen (PSMA)***

Membrane-bound protein overexpressed in 95-100% of human prostate cancer (PCa) cases.

Frequently PSMA (+) cases contain large areas with PSMA (-) cells !

- ***Gastrin releasing peptide receptors (GRPrs)***

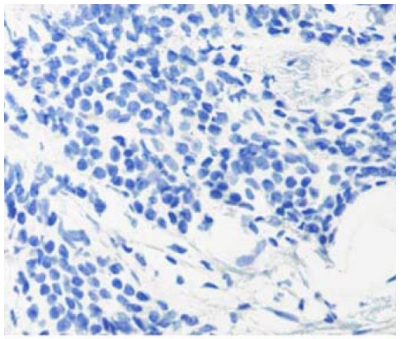
Membrane-bound protein overexpressed in 84-100% PCa cases, including small cell lung and pancreatic cancers[1,2]



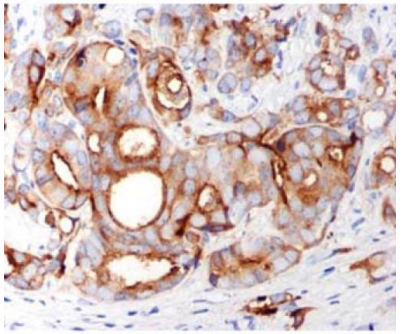
GRPR +

Prostate cancer/ stromal cells

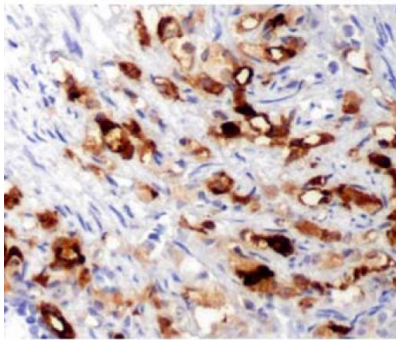
[2] Rybalov et al *Int.J.Mol.Sc. 2014*



PSMA -



PSMA +



PSMA -/+

Prostate cancer/ stromal cells

[1]Mannweiler et al. *Pathol. Oncol. Res.* 2009

Aims of this study

Synthesis of *multimeric ligands* with binding affinity for both receptors GRPr/PSMA

HE spacers: *incorporation of PK modification spacer*

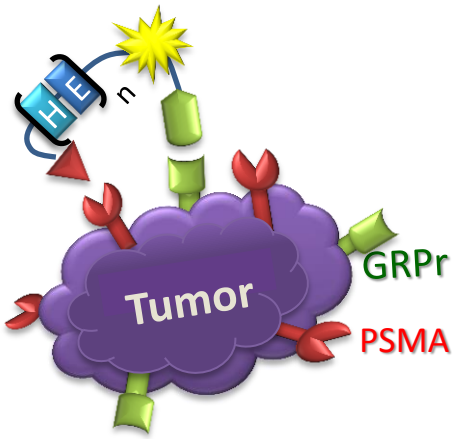
- **Comparison** with monomers
- **PK Improvement ->** high **tumor/normal tissue contrast ratios** without losing affinity and specificity.
- **Insight** for the design of new Radioligands in the future.
- **Selection** of the optimal tracer.

PSMA pharmacophore Glu-ureido-Lys

HE_n: Pharmacokinetic spacer His (H), Glu (E), (n = 0-3)

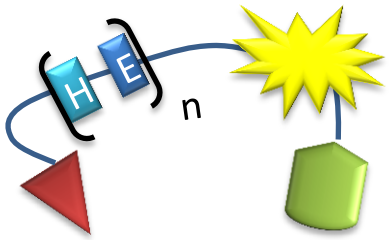
HBED-CC chelator for ^{68}Ga

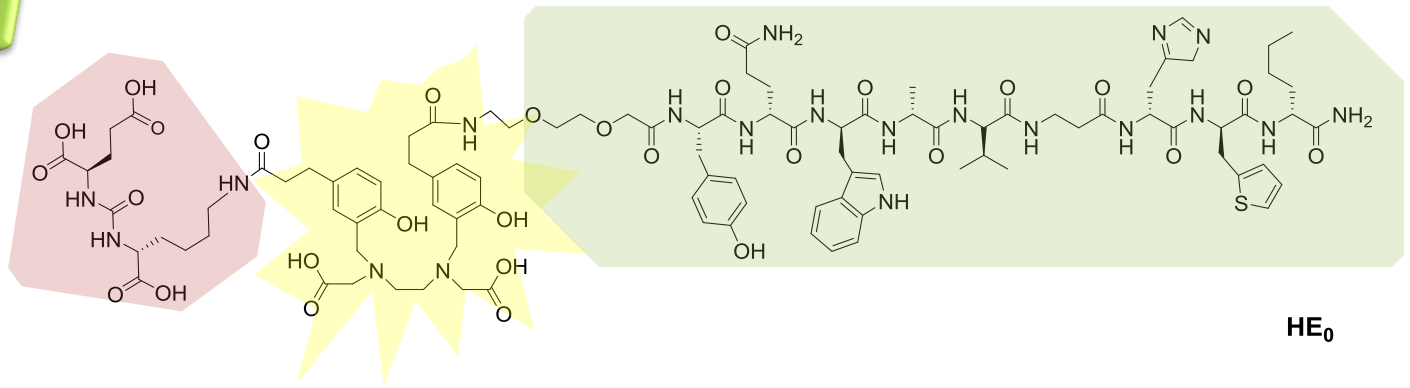
GRPr Pharmacophore BN analogue
H_2N-PEG_2-[D-Tyr^6, β-Ala^{11}, Thi^{13}, Nle^{14}]BN(6–14)



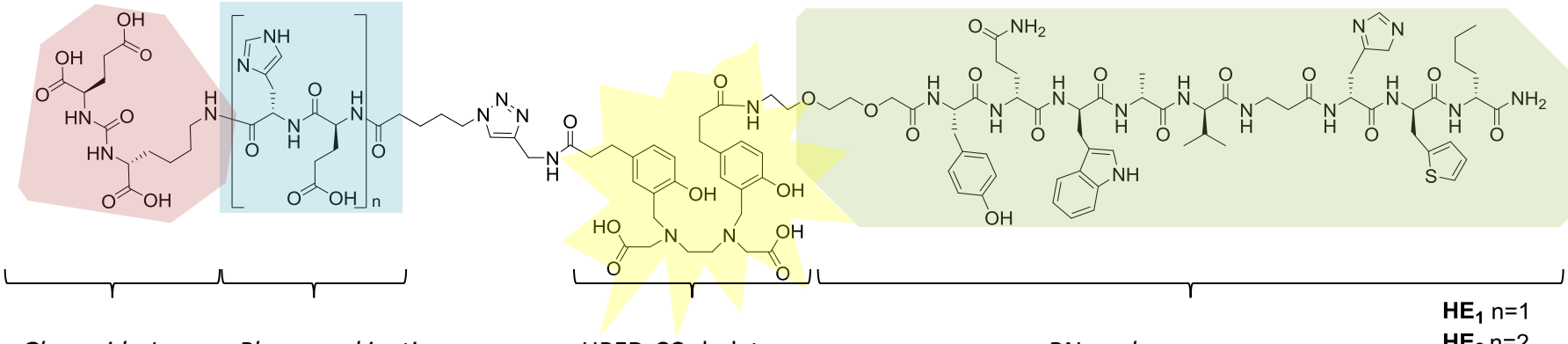
Results and discussion

Chemical structures





HE_0



HE_1 n=1
HE_2 n=2
HE_3 n=3

Glu-ureido-Lys

Pharmacokinetic spacer His (H), Glu (E)

HBED-CC chelator

BN analogue
H_2N-PEG_2-[D-Tyr^6, β-Ala^{11}, Thi^{13}, Nle^{14}]BN(6–14)

Results and discussion

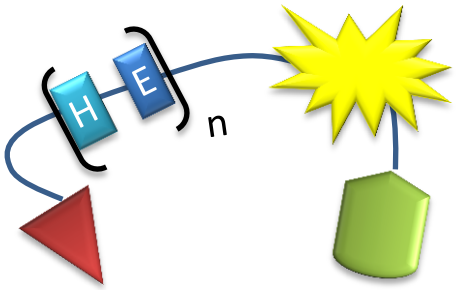
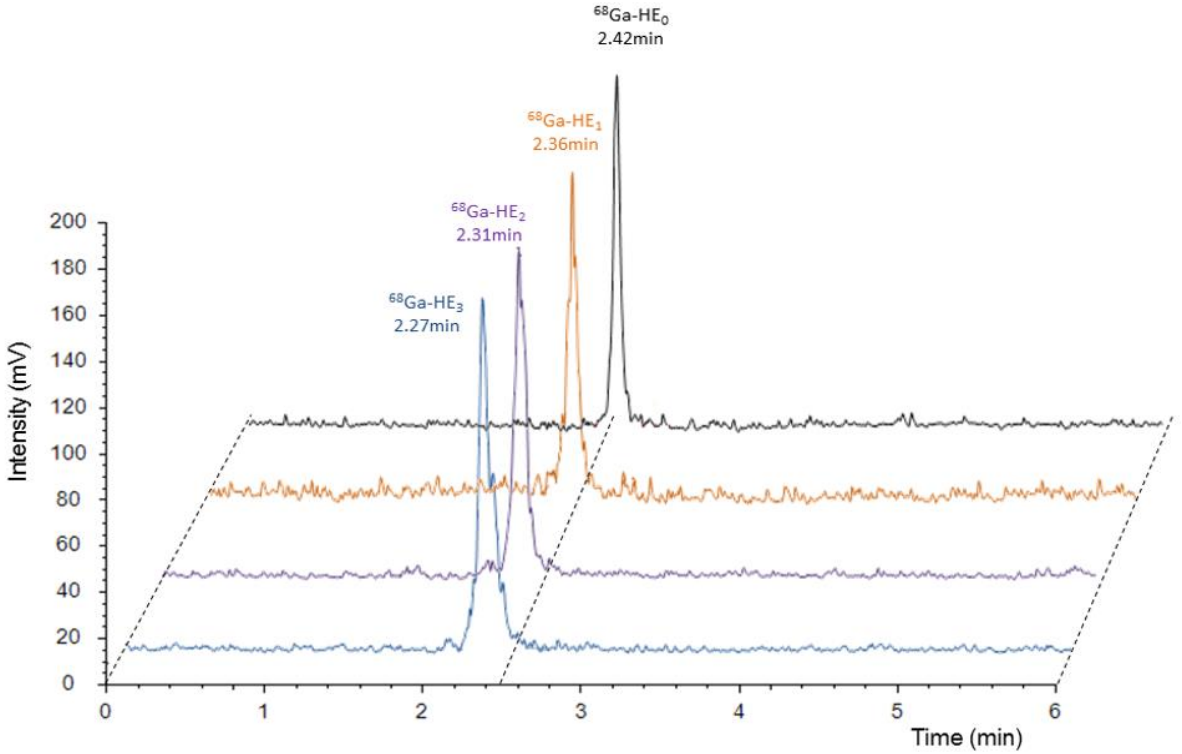


Table 1. High-resolution mass spectrometry data of the free ligands $[M+H]^+$.

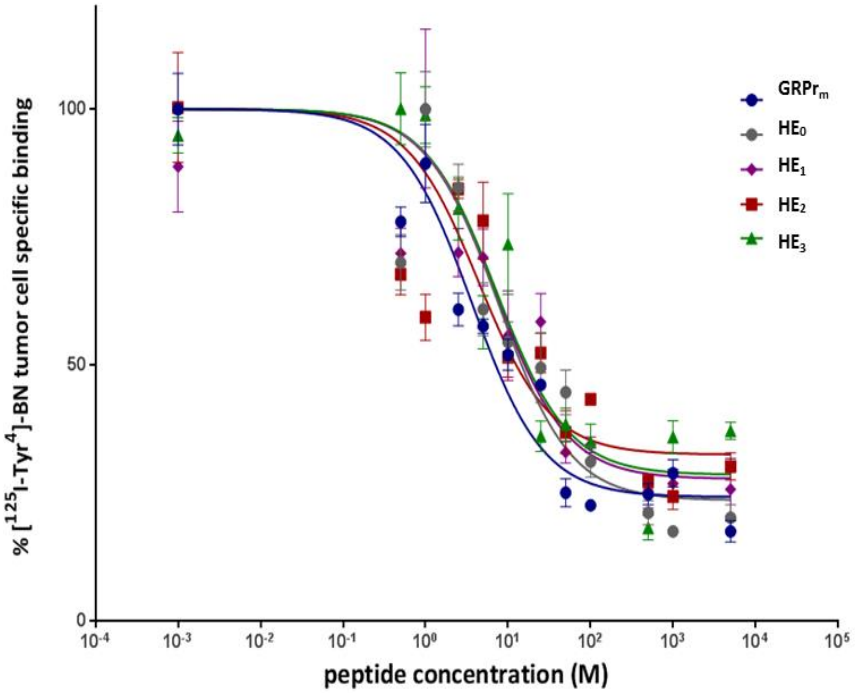
Compound	m/z calculated $[M+H]^+$	m/z experimental $[M+H]^+$
$GRPr_m$	1800.0	1800.8
PSMA-11	947.4	947.4
HE_0	2101.3	2100.5
HE_1	2547.8	2547.3
HE_2	2814.1	2814.0
HE_3	3080.3	3080.3

^{68}Ga-Radiolabeling

Comparative RP-HPLC analysis studies of the ligands **HE_n**, n=0-3, after labelling with ^{68}Ga (gamma-trace).



Results and discussion - *in vitro*



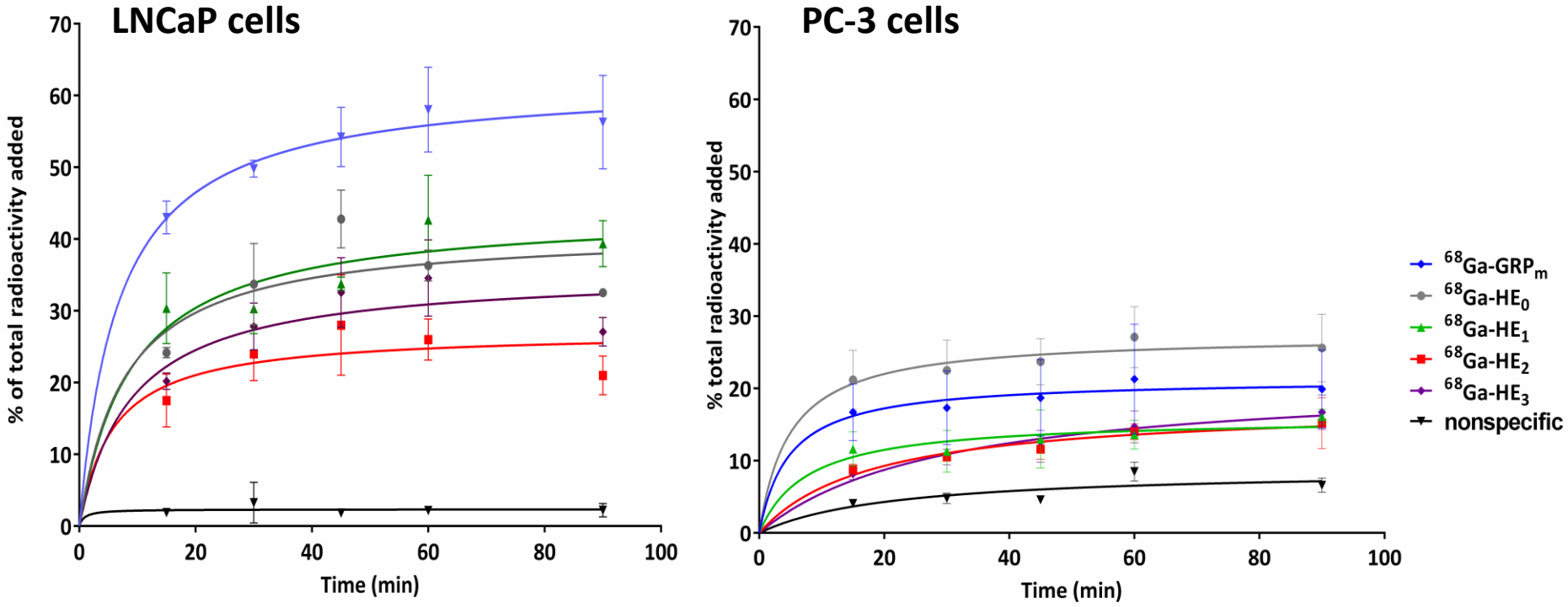
Competition binding assay for GRP on PC-3 cells (10^6), AR42J (10^6) and PSMA on LNCaP cells (10^6).

	compound	IC_{50} (nM) ± Std.Er	ANOVA vs monomer
PC-3	GRPr$_m$	3.65 ± 1.11	-
	HE$_0$	7.72 ± 1.20	NS*
	HE$_1$	7.28 ± 1.17	NS
	HE$_2$	4.40 ± 1.29	NS
	HE$_3$	7.09 ± 1.23	NS
AR42J	GRPr$_m$	1.29 ± 1.23	-
	HE$_0$	3.33 ± 1.17	**
	HE$_1$	2.58 ± 1.15	*
	HE$_2$	5.06 ± 1.20	****
	HE$_3$	3.68 ± 1.17	***
LNCaP	PSMA-11	7.5 ± 1.29	-
	HE$_0$	25.4 ± 1.09	**
	HE$_1$	17.4 ± 1.07	*
	HE$_2$	25.2 ± 1.23	**
	HE$_3$	42.4 ± 1.09	****

[*] NS: not statistically significant difference. Significant differences against the monomers GRPr$_m$ and PSMA-11 in each assay are presented with stars (P<0.05)

Results and discussion - *in vitro*

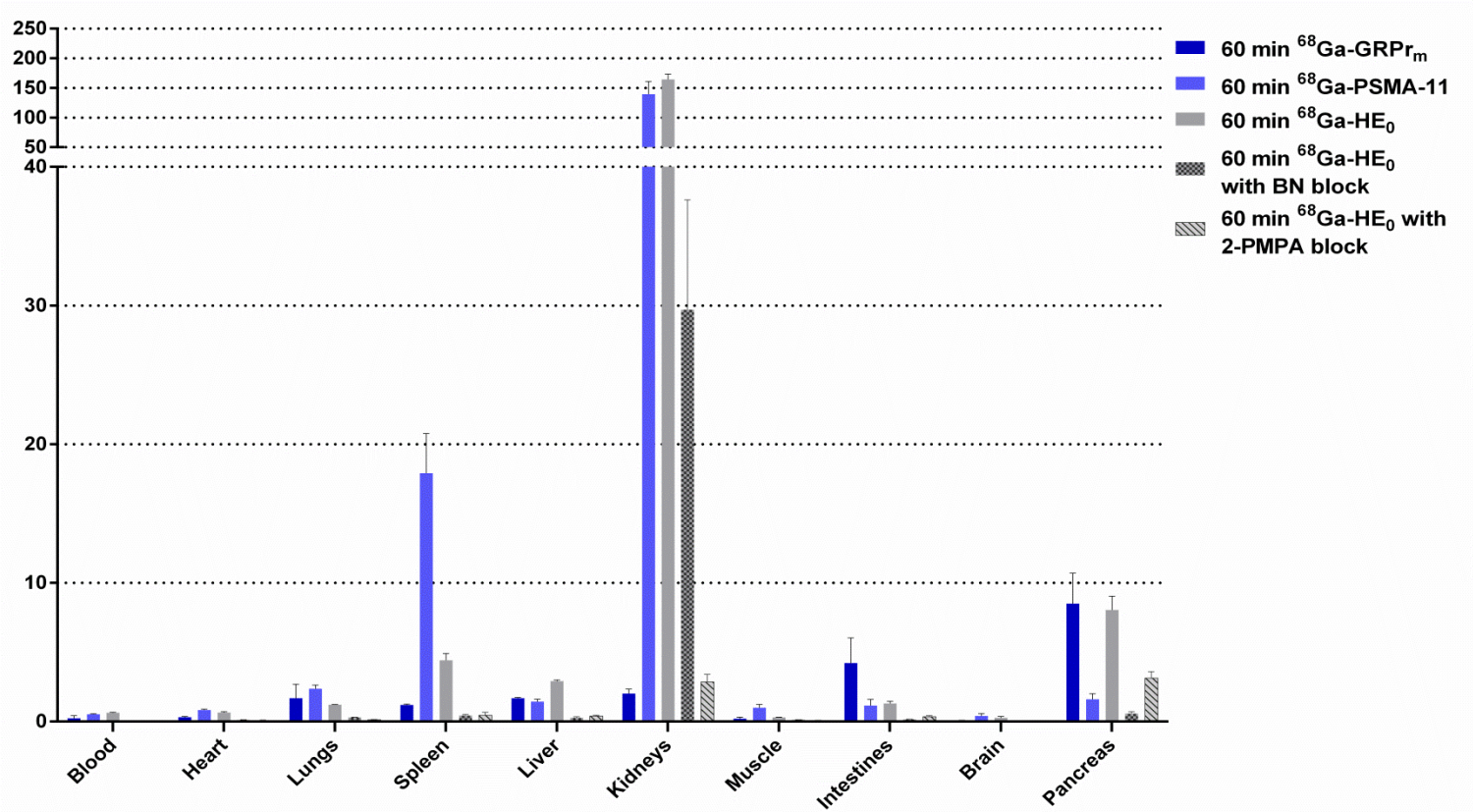
Total cell related radioactivity over time for ^{68}Ga-labelled versions of monomers PSMA-11 and $GRPr_m$ and heterodimers **HE_n**, n=0-3 (30 nM) on LNCaP and PC-3 cells.



(Non-specific binding was determined by adding a blocking solution of 2-PMPA or native BN, x 1000-fold concentration as compared with the respective radioligand, 30 µM).

Results and discussion – *in vivo*

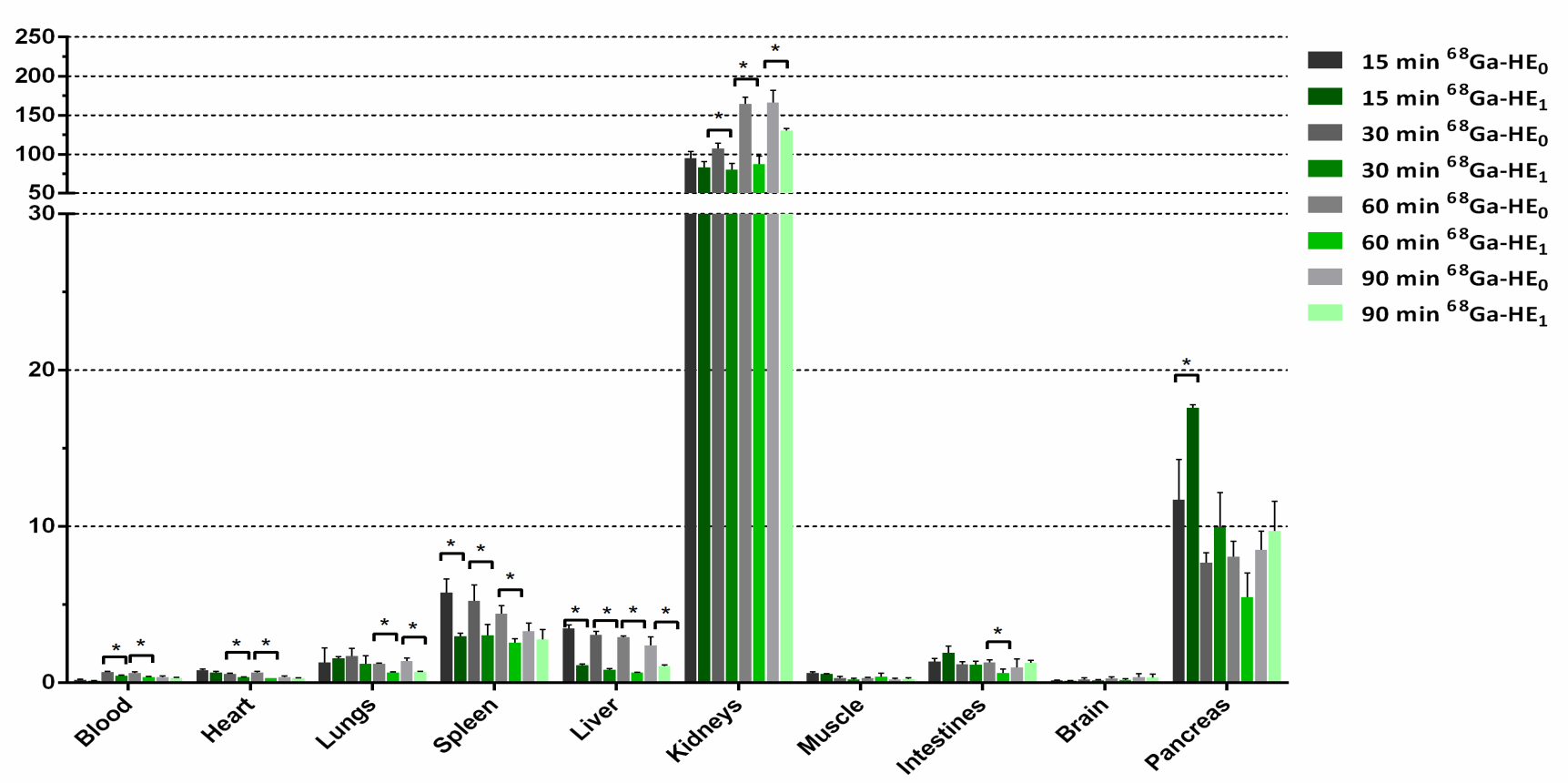
Biodistribution studies (1 h p.i.) between ^{68}Ga-HE$_0$ and ^{68}Ga-PSMA-11 and ^{68}Ga-GRPr$_m$ in mice.



Results are expressed as percentage of the injected dose per g (% ID/g) for each organ or tissue. Blocking experiments: co-injecting native BN (1 µL of a 100 mM solution) or 2-PMPA (15 µL of a 100 mM solution) along with the radiolabelled ligand.

Results and discussion – *in vivo*

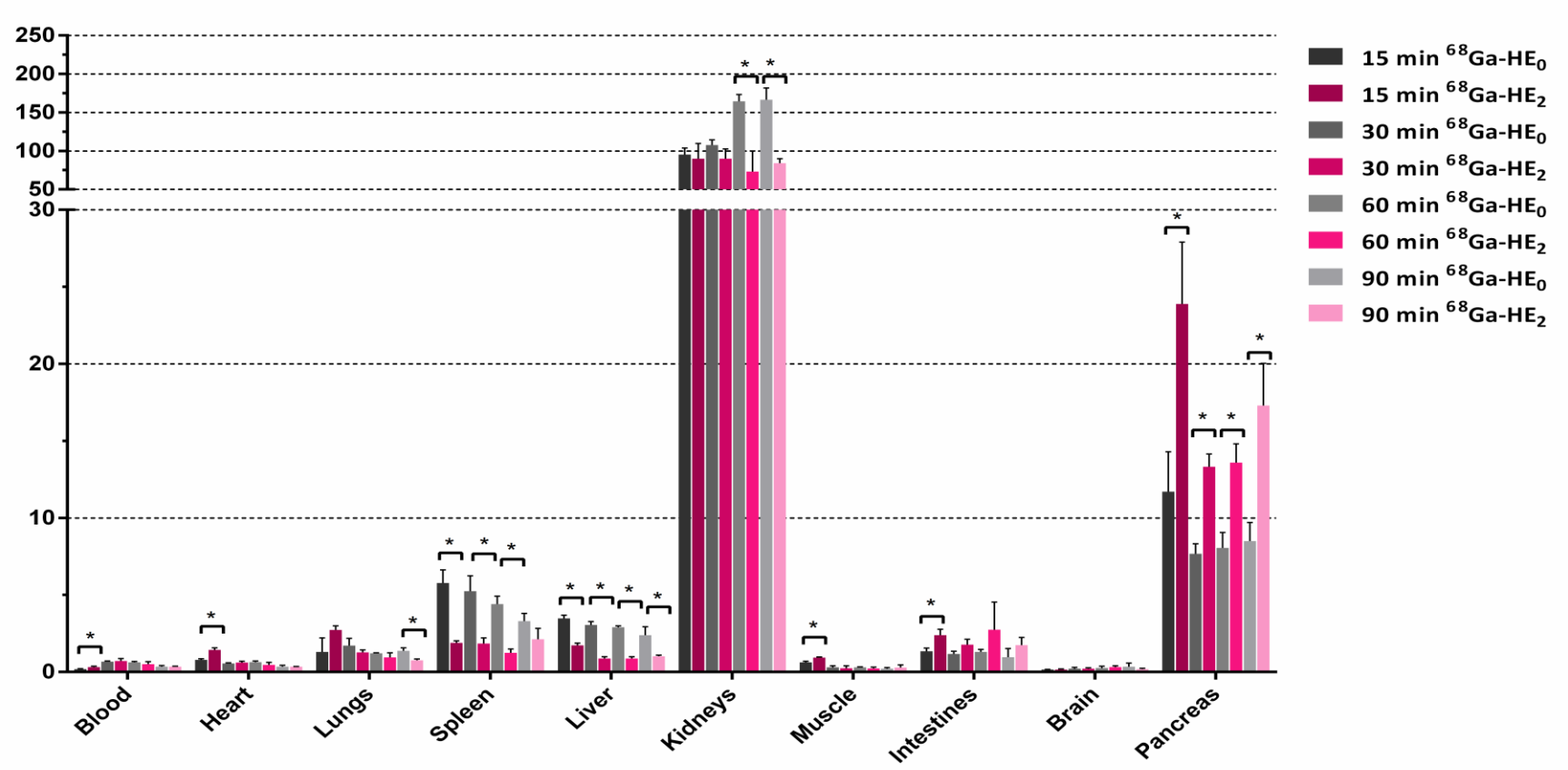
Biodistribution studies in mice between the ^{68}Ga-HE$_0$ and ^{68}Ga-HE$_1$



Results are expressed as % ID/g (mean ± SD, n=3-4). Significant differences are presented with stars above the bars that were compared (P<0.05).

Results and discussion – *in vivo*

Biodistribution studies mice between the ^{68}Ga-HE$_0$ and ^{68}Ga-HE$_2$



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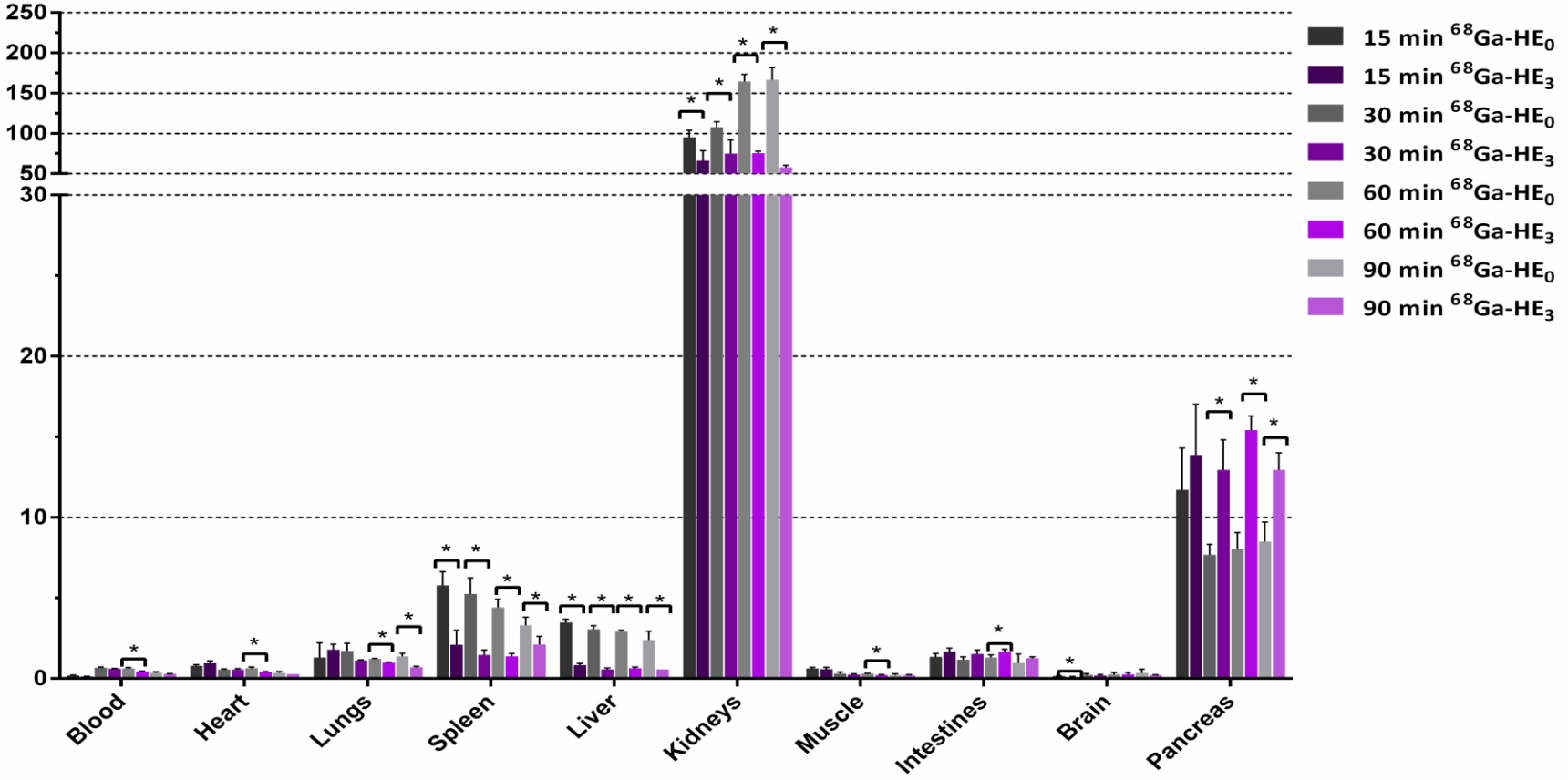
Results and discussion – *in vivo*

Biodistribution studies in mice between the ^{68}Ga-HE$_0$ and ^{68}Ga-HE$_2$



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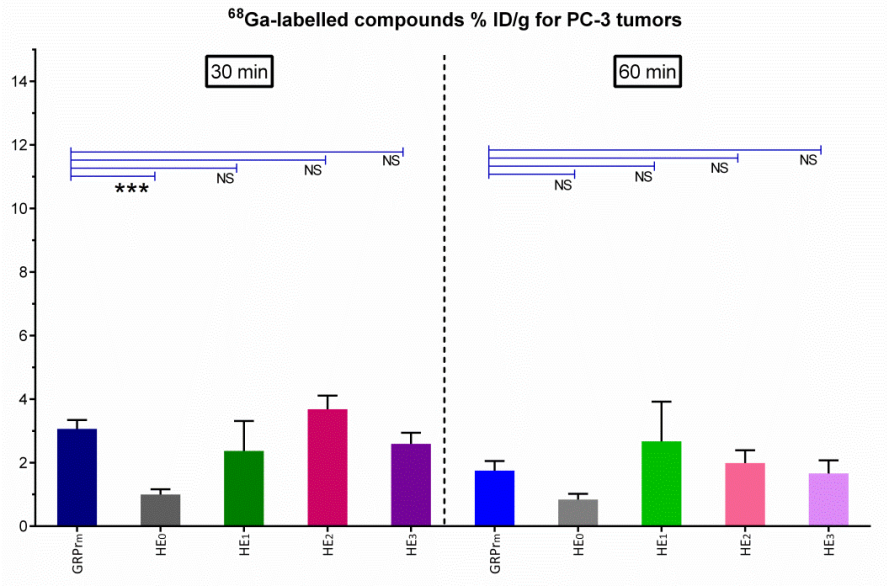
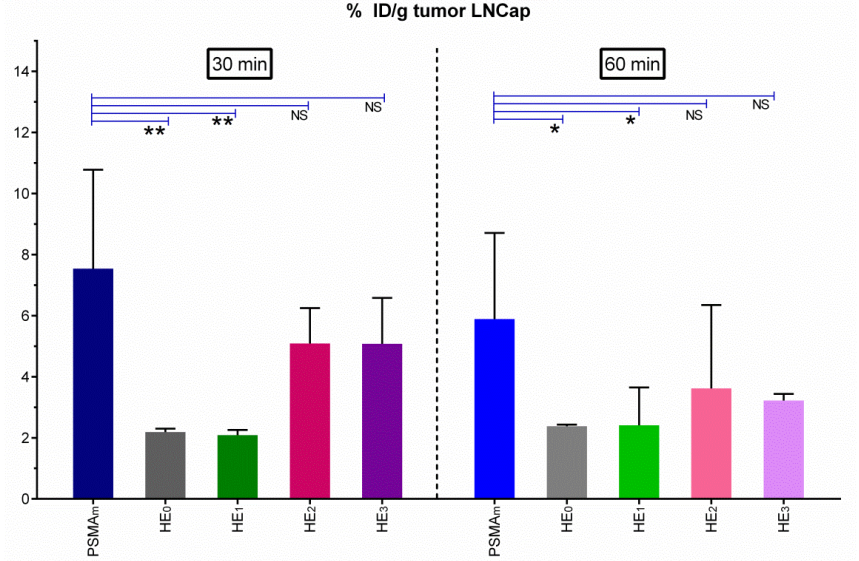
Results and discussion – *in vivo*

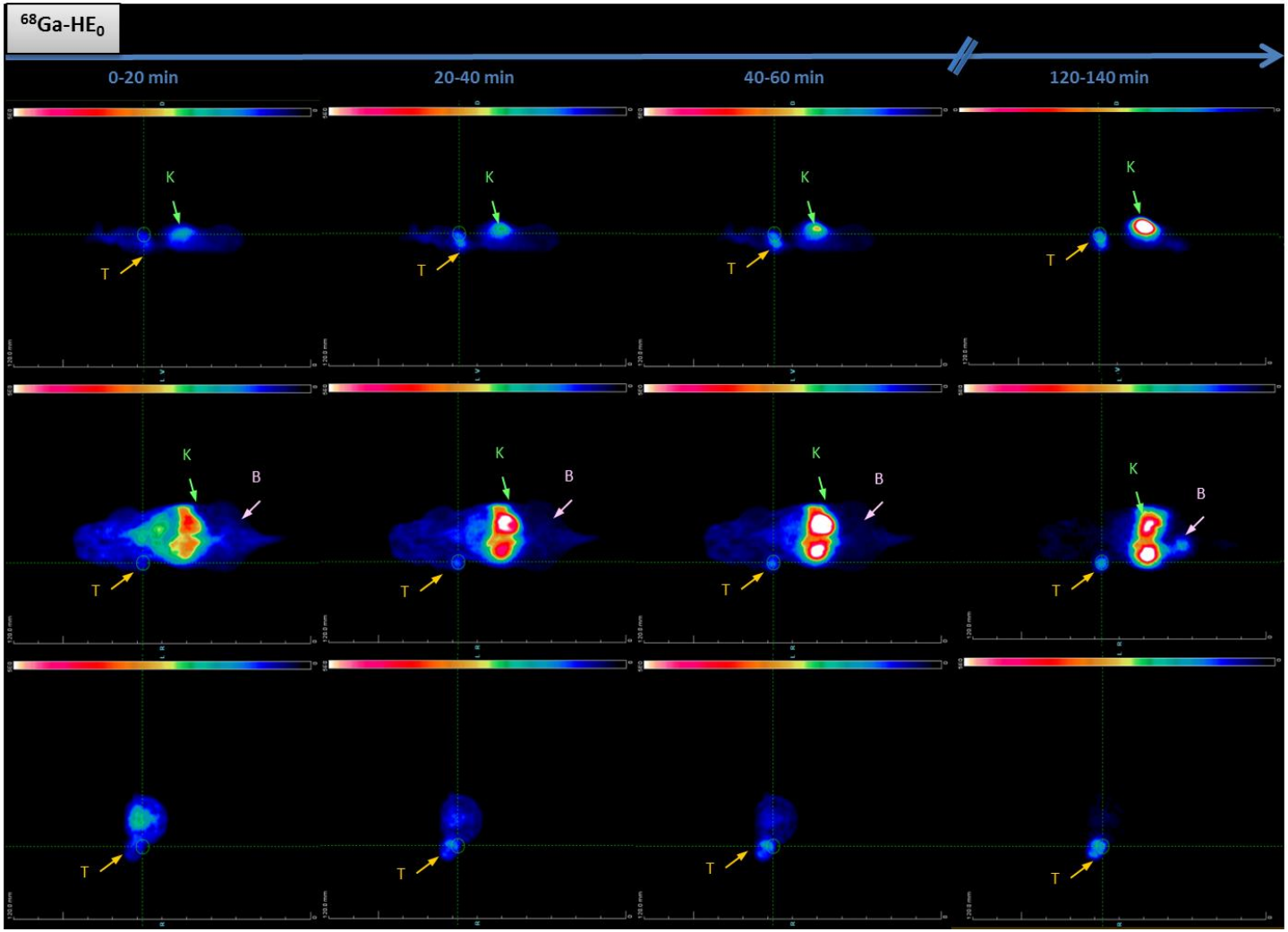




Tumor uptake determined from biodistribution studies (30, 60 min p.i.) in balb/c nu/nu mice bearing: (a) LNCaP and (b) PC-3 tumors, after i.v. administration of the ^{68}Ga-PSMA-11, ^{68}Ga-GRPr$_m$ and heterodimers **HE$_n$** (n=0-3).

Significant differences are presented with stars above the bars that were compared (P<0.05). The values are expressed as % ID/g (mean ± SD, n=3-4)

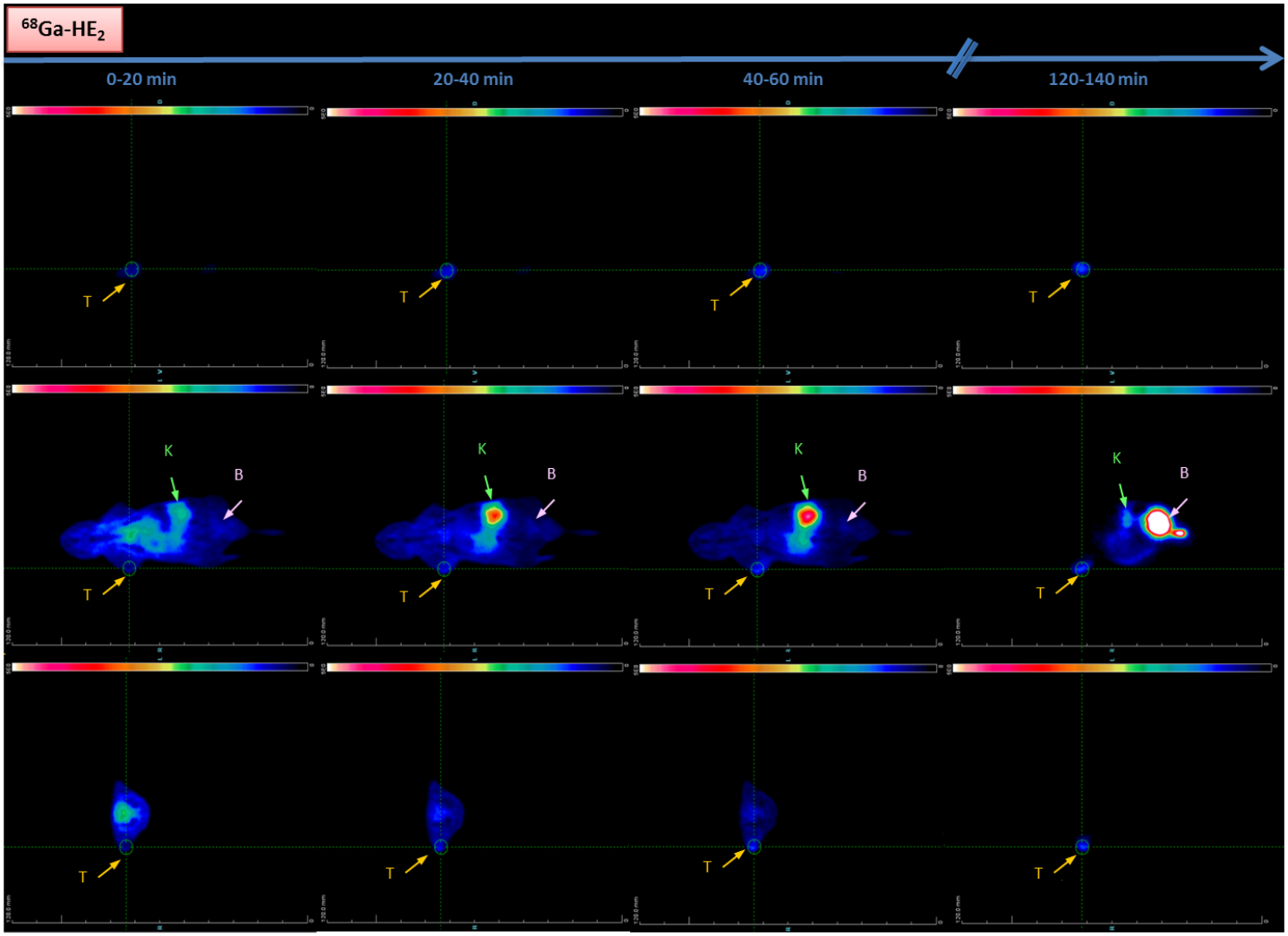
Results and discussion – *in vivo*



Whole-body µPET (axial, coronal, saggital, from top to bottom) images of male nu/nu mice bearing LNCaP tumor xenografts, for **^{68}Ga-HE$_0$**

Where: T = Tumor; K= kidneys, B = bladder as indicated with arrows.

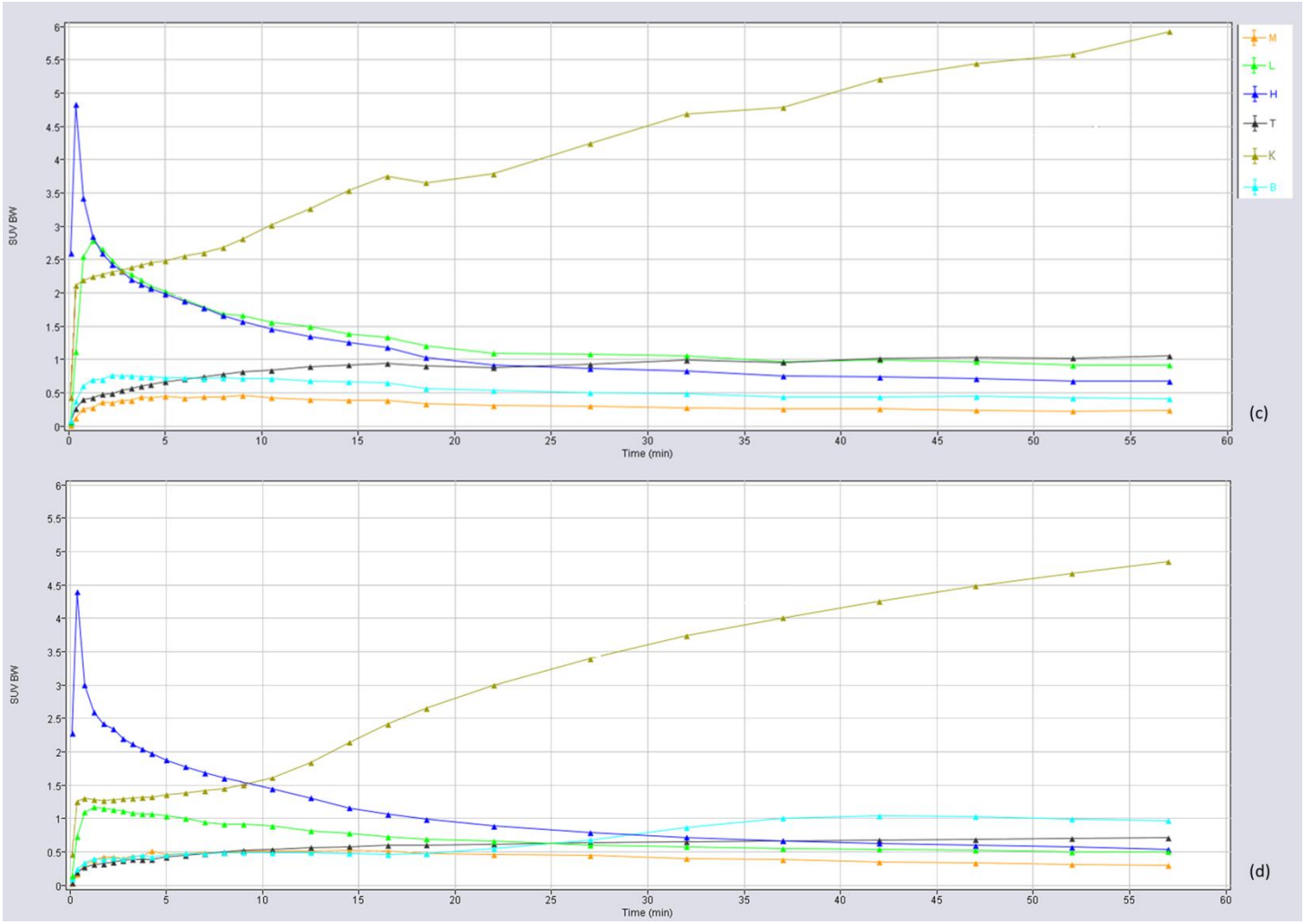
Results and discussion – *in vivo*



Whole-body µPET (axial, coronal, saggital, from top to bottom) images of male nu/nu mice bearing LNCaP tumor xenografts, for **^{68}Ga-HE$_2$**

Where: T = Tumor; K= kidneys, B = bladder as indicated with arrows.

Results and discussion – *in vivo*

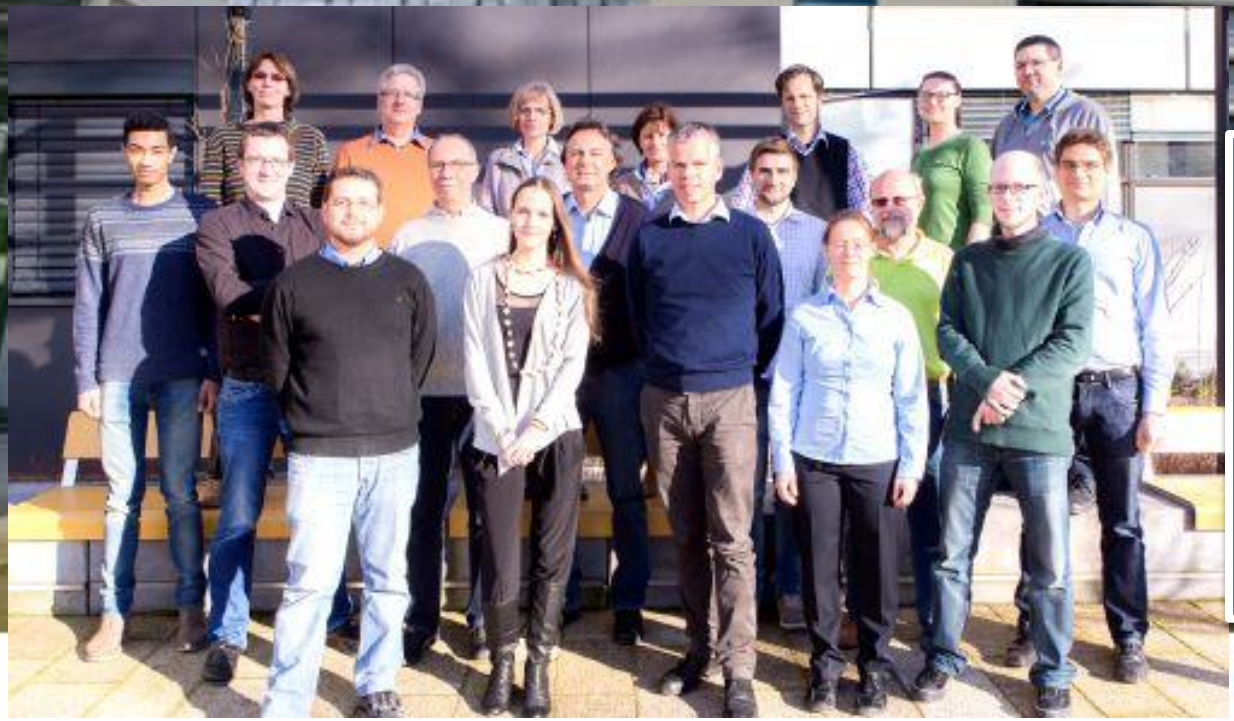


Representative time-activity curves taken from the dynamic PET measurements (0-60 min p.i.) expressed as SUV_{mean} (standardized uptake values) for ^{68}Ga-labelled **HE_0** (top) and **HE_2** (bottom). The SUV time-activity curves for the organs of interest are represented with the following letters, M = muscle, T = tumor, B = bladder, K = kidneys, L = liver.

Conclusion

- A series of novel bispecific radioligands (^{68}**Ga-HE$_n$**, n=0-3) were synthesized for the first time and evaluated for PSMA and GRPr targeting properties *in vitro* and *in vivo*.
- Both *in vitro* and *in vivo* studies showed that all low-molecular weight heterodimers under study (^{68}**Ga-HE$_n$**, n = 0-3) could efficiently target PSMA and GRPr on LNCaP and PC-3 prostate cancer cells and tumor xenografts.
- This dual-targeting heterodimer approach can improve the sensitivity of prostate cancer detection due to the synergistic increase of binding interactions for the chosen biological targets, i.e. PSMA and GRPr.
- In addition, their biodistribution profiles were optimized by incorporation of charged linkers (^{68}**Ga-HE$_n$**, n=1-3), which resulted in a significant reduction of normal organ uptake (i.e. kidneys, spleen), while tumor uptake remained at the same levels or was increased in comparison with the monomers (^{68}Ga-PSMA-11, ^{68}Ga-GRPr$_m$).
- These novel low-molecular weight heterodimers could potentially be applied in clinical practice as bispecific radiotracers for the noninvasive imaging of all stages of prostate cancer by means of PET/CT and PET/MRI

Thank you for your attention!



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- Prof. Dr. rer. nat. Klaus Kopka

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