

Supplementary Materials

Bispecific GRPR-antagonistic anti-PSMA/GRPR heterodimer for PET and SPECT diagnostic imaging of prostate cancer

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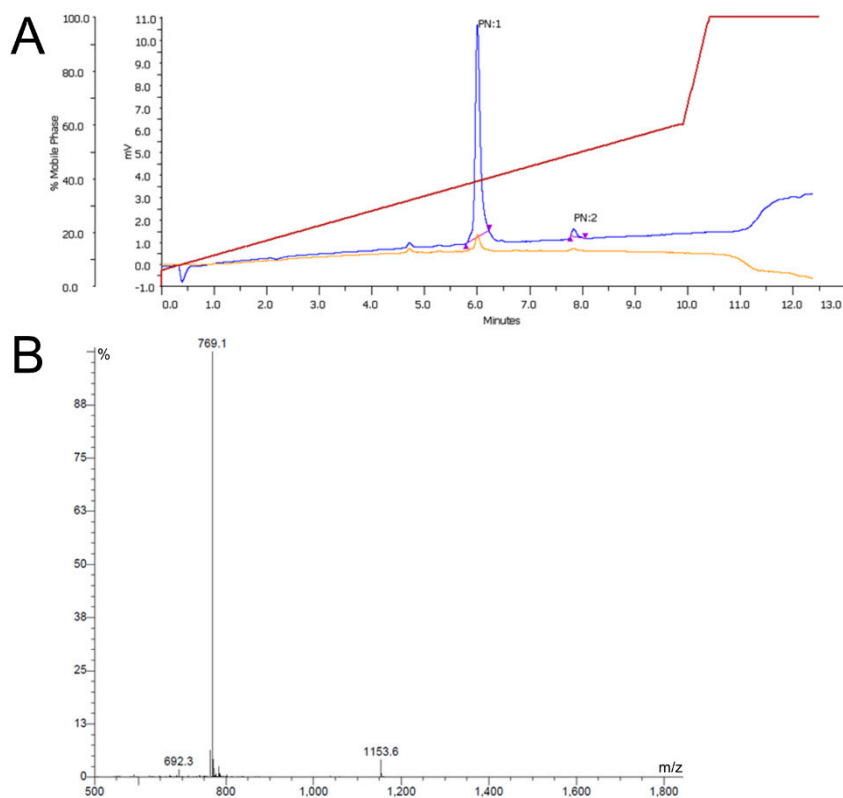


Figure S1. Characterization of heterodimer **6**, Glu-Urea-Glu-Aoc-Lys(NOTA)-(PEG)₆-RM26. Analytical RP-HPLC chromatogram (a) and MS spectrum (b) of purified bispecific heterodimeric molecule **6**.

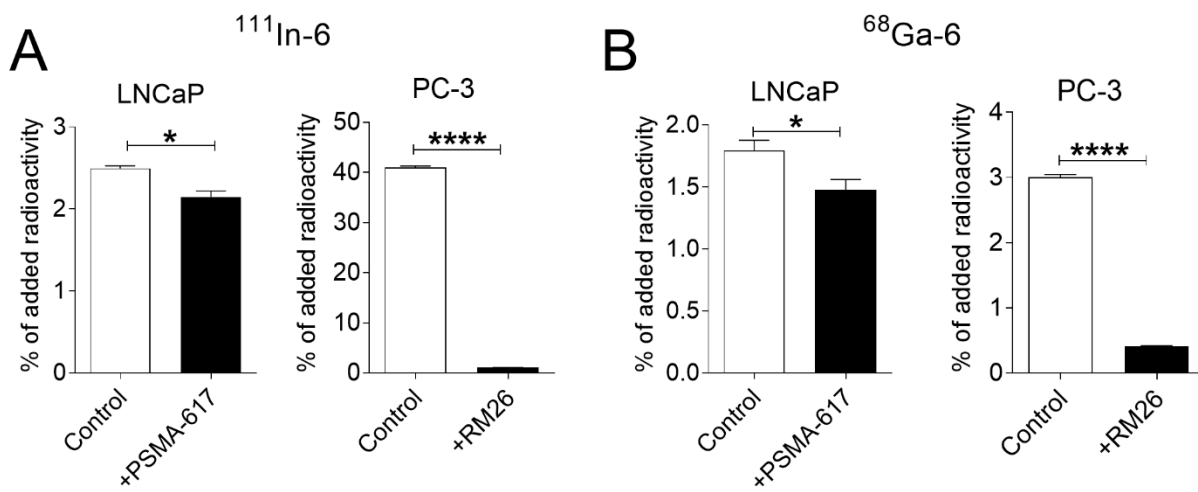


Figure S2. Binding specificity of $^{111}\text{In-6}$ (a) and $^{68}\text{Ga-6}$ (b) to LNCaP cells (PSMA positive) and PC-3 (GRPR positive). Cell containing dishes (n=3) were incubated with radiolabeled conjugates (1 nM). One set of dishes was pre-saturated with excess amount (300 nM) of non-labeled PSMA-617 (+ PSMA-617), or RM26 (+ RM26). Data are presented as average \pm standard deviation.

Table S1. In vivo biodistribution of ¹¹¹In-6 and ⁶⁸Ga-6 (50 pmol/animal) over time in PC3-PIP-xenografted BALB/c nu/nu mice. Activity uptake in tissues was calculated as percent injected dose per tissue weight (%ID/g). Data are presented as average ± standard deviation.

Organ	¹¹¹ In-6			⁶⁸ Ga-6	
	1h	3h	24h	1h	3h
Blood	0.4±0.1 ^{a,b}	0.06±0.02	0.009±0.005 ^a	0.33±0.09 ^a	0.07±0.01
Salivary glands	0.18±0.07 ^{a,b}	0.07±0.02	0.06±0.02	0.19±0.08 ^a	0.07±0.02
Lung	0.39±0.09 ^{a,b}	0.13±0.06	0.046±0.008 ^a	0.6±0.2 ^a	0.12±0.06
Liver	0.6±0.1 ^{a,b,c}	0.34±0.02 ^c	0.31±0.05	1.3±0.2 ^a	0.67±0.06
Spleen	0.28±0.06 ^{a,b,c}	0.12±0.03 ^c	0.06±0.04 ^a	0.49±0.06 ^a	0.28±0.09
Pancreas	3.5±0.8 ^{a,b,c}	0.33±0.06	0.10±0.04 ^a	1.8±0.3 ^a	0.2±0.1
Stomach	1.3±0.3 ^{a,b,c}	0.26±0.09	0.04±0.03 ^a	0.8±0.2 ^a	0.1±0.1
Small intestine	1.2±0.5 ^{a,b}	0.17±0.04	0.055±0.003 ^a	0.8±0.3 ^a	0.13±0.08
Kidneys	10±2 ^{a,b,c}	4.9±0.9 ^c	2.6±0.4 ^a	6.6±0.8 ^a	3.0±0.4
Tumor	12±2 ^{a,b,c}	7±1 ^c	1.5±0.4 ^a	8±2 ^a	2.9±0.9
Muscle	0.13±0.05 ^{a,b}	0.04±0.01	0.03±0.02	0.11±0.05 ^a	0.03±0.01
Bone	0.3±0.2	0.14±0.05 ^c	0.10±0.05	0.3±0.2	0.05±0.03
GI tract	0.8±0.3	0.6±0.4	0.2±0.1	0.5±0.2	0.4±0.2
Carcass	2.5±0.7 ^{a,b}	0.9±0.1 ^c	0.44±0.06 ^a	1.9±0.6 ^a	0.6±0.1

The organ uptake values are expressed as a percentage of injected dose per gram of tissue weight (%ID/g) except for carcass and GI tract, for which the values are expressed as a percentage of the injected dose per sample (%ID). Significant difference ($p<0.5$) at the same time point.

- a. Conjugate significantly differs from the 3h pi time-point
- b. Conjugate significantly differs from the 24h pi time-point
- c. Conjugate significantly differs from ⁶⁸Ga-6 at the same time-point

Table S2. Tumor-to-organ ratios of ¹¹¹In-6 and ⁶⁸Ga-6 tested in BALB/c nu/nu mice bearing PC3-PIP-xenografts, 1, 3, 24 h pi for ¹¹¹In-6 and 1, 3 h pi for ⁶⁸Ga-6. Mice were intravenously injected with 50 pmol of ¹¹¹In-6 or ⁶⁸Ga-6. Data are presented as average ± standard deviation.

Organ	¹¹¹ In-6			⁶⁸ Ga-6	
	1h	3h	24h	1h	3h
Blood	30±4	115±13	192±66	24±3	44±6
Salivary glands	72±13	93±10	26±4	43±8	43±8
Lung	32±3	64±30	34±11	15±3	28±17
Liver	22±4	20±2	4.8±0.8	5.9±0.9	4±1
Spleen	44±6	59±16	30±16	16±4	11±1
Pancreas	3.5±0.8	21±1	16±2	4.4±0.6	18±6
Stomach	10±3	29±12	61±52	10±4	45±39
Small intestine	12±4	42±9	27±6	11±3	33±27
Kidneys	1.2±0.2	1.4±0.2	0.6±0.1	1.2±0.1	0.9±0.2
Muscle	101±25	210±90	72±59	78±28	128±57
Bone	75±50	52±22	19±15	30±11	67±21

Table S3. In vivo specificity of ¹¹¹In-6 and ⁶⁸Ga-6 tested in BALB/c nu/nu mice bearing PC3-PIP-xenografts, 1 h pi. Mice were intravenously injected with 50 pmol of ¹¹¹In-6 or ⁶⁸Ga-6 alone or together with 1.5 nmol non-labeled RM26, or 1.5 nmol non-labeled PSMA-617, or both. Activity uptake in tissues was calculated as percent injected dose per tissue weight (%ID/g). Data are presented as average ± standard deviation.

Organ	¹¹¹ In-6				⁶⁸ Ga-6	
	Control	+RM26	+PSMA-617	+Both	Control	+Both
Blood	0.4±0.1	0.3±0.1	0.29±0.02	0.4±0.2	0.33±0.09	0.3±0.2
Salivary glands	0.18±0.07	0.14±0.05	0.12±0.04	0.16±0.06	0.19±0.08	0.11±0.06
Lung	0.39±0.09	0.31±0.09	0.4±0.1	0.6±0.3	0.6±0.2	0.7±0.2
Liver	0.6±0.1	0.55±0.06	0.54±0.07	0.58±0.06	1.3±0.2	0.9±0.1 ^a
Spleen	0.28±0.06	0.17±0.03 ^a	0.20±0.04	0.23±0.07	0.49±0.06	0.28±0.06 ^a
Pancreas	3.5±0.8	0.3±0.1 ^a	2.6±0.2	0.28±0.09 ^a	1.8±0.3	0.3±0.1 ^a
Stomach	1.3±0.3	0.17±0.05 ^a	0.88±0.09 ^a	0.3±0.2 ^a	0.8±0.2	0.3±0.1 ^a
Small intestine	1.2±0.5	0.19±0.04 ^a	0.7±0.2	0.24±0.07 ^a	0.8±0.3	0.3±0.1 ^a
Kidneys	10±2	9±2	16±2 ^a	19±3 ^a	6.6±0.8	14±2 ^a
Tumor	12±2	9±2	8±1 ^a	1.7±0.6 ^a	8±2	0.9±0.4 ^a
Muscle	0.13±0.05	0.06±0.02	0.07±0.03	0.10±0.03	0.11±0.05	0.1±0.1
Bone	0.3±0.2	0.15±0.09	0.12±0.03	0.19±0.08	0.3±0.2	0.11±0.08
GI tract	0.8±0.3	0.4±0.2	0.7±0.1	0.7±0.4	0.5±0.2	0.6±0.5
Carcass	2.5±0.7	1.7±0.7	1.9±0.5	5±4	1.9±0.6	4±4

The organ uptake values are expressed as a percentage of injected dose per gram of tissue weight (%ID/g) except for carcass and GI tract, for which the values are expressed as a percentage of the injected dose per sample (%ID). Significant difference ($p<0.5$) at the same time point.

d. Conjugate significantly differs from control

Table S4. Tumor-to-organ ratios of ¹¹¹In-6 and ⁶⁸Ga-6 tested in BALB/c nu/nu mice bearing PC3-PIP-xenografts, 1 h pi. Mice were intravenously injected with 50 pmol of ¹¹¹In-6 or ⁶⁸Ga-6 alone or together with 1.5 nmol non-labeled RM26, or 1.5 nmol non-labeled PSMA-617, or both. Data are presented as average ± standard deviation.

Organ	¹¹¹ In-6				⁶⁸ Ga-6	
	Control	+RM26	+PSMA-617	+Both	Control	+Both
Blood	30±4	37±14	28±3	4.5±0.7	24±3	3.3±0.5
Salivary glands	72±13	67±9	74±35	11±1	43±8	10±4
Lung	32±3	30±2	24±4	3.1±0.6	15±3	1.4±0.5
Liver	22±4	16±3	15±0.8	2.9±0.8	5.9±0.9	1.1±0.5
Spleen	44±6	54±6	41±11	8±3	16±4	4±1
Pancreas	3.5±0.8	27±3	3.0±0.3	6±3	4.4±0.6	5±3
Stomach	10±3	52±3	9±2	6±4	10±4	4±2
Small intestine	12±4	48±8	12±5	8±4	11±3	4±3
Kidneys	1.2±0.2	0.9±0.1	0.49±0.06	0.09±0.02	1.2±0.1	0.07±0.02
Muscle	101±25	151±27	132±34	17±3	78±28	11±6
Bone	75±50	73±31	69±26	10±6	30±11	11±7