Supplemental material

Experimental details

Synthesis of the PSMA binding motif

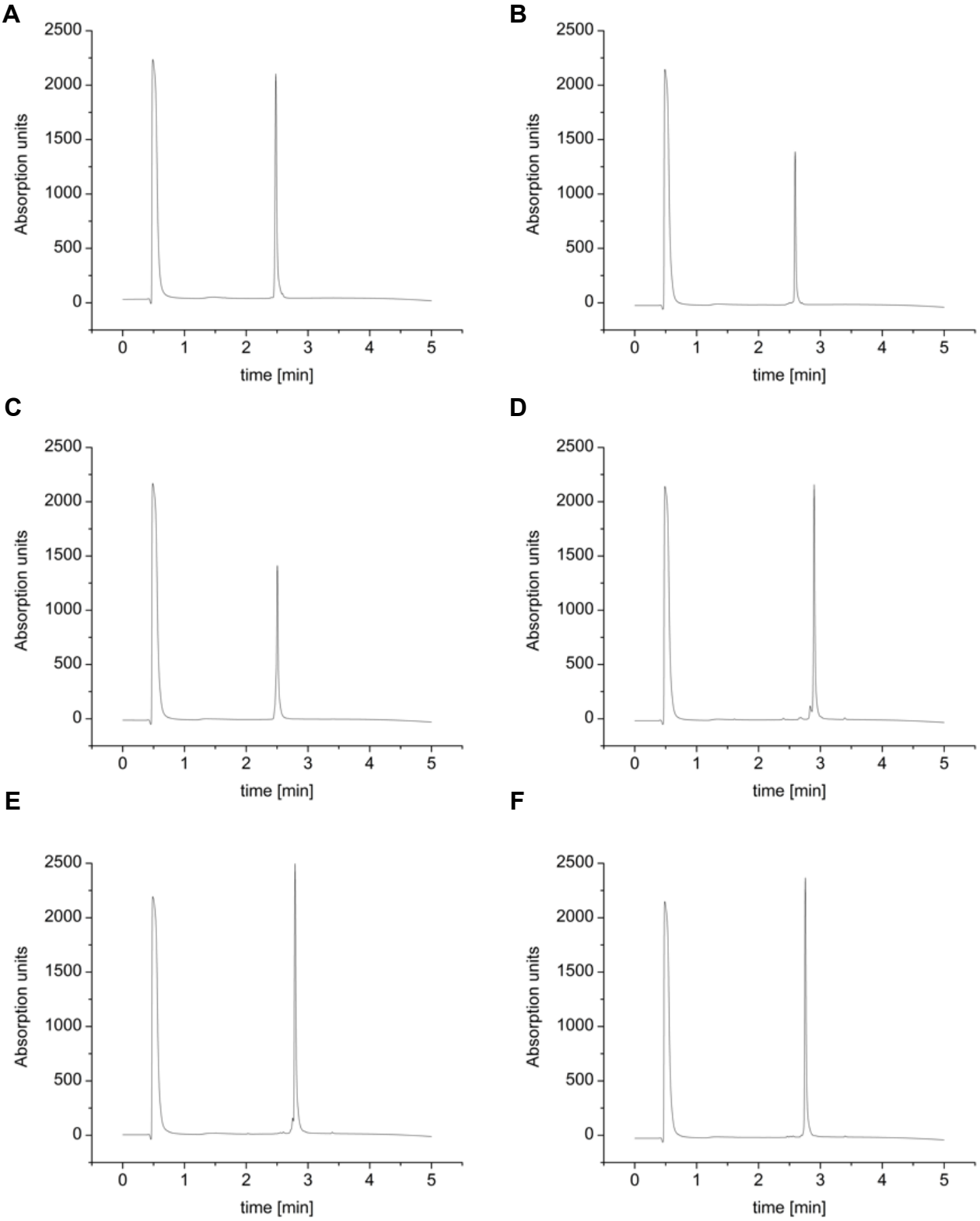
Fmoc-Lys(Alloc)-OH (Orpegen Peptide Chemicals GmbH, Heidelberg, Germany) was immobilized on an equimolar amount of 2-chlorotritylresin. 0.3 mmol of Fmoc-Lys(Alloc)-OH and 4 equivalents of *N*,*N*-diisopropylethylamine (DIPEA) were dissolved in dry dichloromethane (DCM) and agitated with the 2-CT resin for two hours. 3 mmol of H-Glu-(O*t*Bu)-O*t*Bu × HCl were dissolved in 150 ml of DCM and 2 ml DIPEA were added. A solution of 1 mmol triphosgene in 10 ml of DCM was cooled in an ice bath and the solution of H-Glu(O*t*Bu)-O*t*Bu × HCl and DIPEA was added to the stirred solution drop wise over 3-4 hours. Subsequently, the solution was stirred another hour at room temperature. The remaining 2-chlorotritylgroups on the resin were capped under application of a solution of DCM, methanol, DIPEA (17 : 2 : 1). Afterwards, the Fmoc-group of the immobilized lysine was cleaved by using a mixture of dimethylformamide (DMF, Carl Roth, Karlsruhe, Germany) and piperidine (Biosolve BV, Valkenswaard, the Netherlands) (1:1) for 2 and 5 min.

Finally, the resin was washed 6 × with DMF, 6 × with DCM, suspended in DCM and finally drawn out with another syringe to add it to the solution containing the isocyanate of the glutamyl moiety. The reaction mixture was stirred for 16 h.

To cleave the Alloc-group, 30 mg of tetrakis(triphenylphosphine)palladium(0) were dissolved in 3 ml DCM and 300 µl morpholine were added. The resin was washed 6 × with DCM and then incubated 2 × 1 hour in the dark with the Pd/morpholine solution. Next, it was washed 6 × with DMF, 8 × with a solution of 1-2% DIPEA in DMF and then 10 × 5 min each with a solution containing 15-20 mg/ml of sodium diethyldithiocarbamate × 3 H_2O in DMF.

Coupling of the linker region

The linker region comprises a 2-naphthyl-L-alanine and *trans*-4-(aminomethyl)cyclohexanecarboxylic acid. The coupling was achieved using standard peptide synthesis, precisely, 0.98 mmol of *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (≙ 0.98 × 4 equivalents) and 1 mmol of Fmoc-2-naphthylalanine (≙ 4 equivalents) were completely dissolved in DMF. Next, 175 µl of DIPEA (≙ 4 equivalents) were added, the mixture was vortexed and set aside for 2 minutes. This solution was then incubated with the PSMA binding motif coupled to the resin for 45 min. Again, the product was washed with DMF, then incubated with 50% piperidine in DMF for 2 min and 5 min, respectively, and washed again with DMF. Next, 1 mmol of *trans*-4-(Fmoc-aminomethyl)cyclohexanecarboxylic acid was coupled. It was dissolved in 2 ml DMF, 1 mmol of Oxyma Pure and 500 µl of DMF were added and the solution was incubated for 4 min on ice. Next, 160 µl of N,N'-diisopropylcarbodiimide (DIC) were added and the reaction was set on ice another 2 min. The clear solution was incubated with the resin for 1 hour. Finally, the resin was washed 6 × with DMF, DCM and diethyl ether respectively and dried *in vacuo*, yielding the compound comprised of the binding motif and the linker moiety.


A
B
C
D
E
F
Absorption units
time [min]
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500
1000
1500
2000
2500
0
1
2
3
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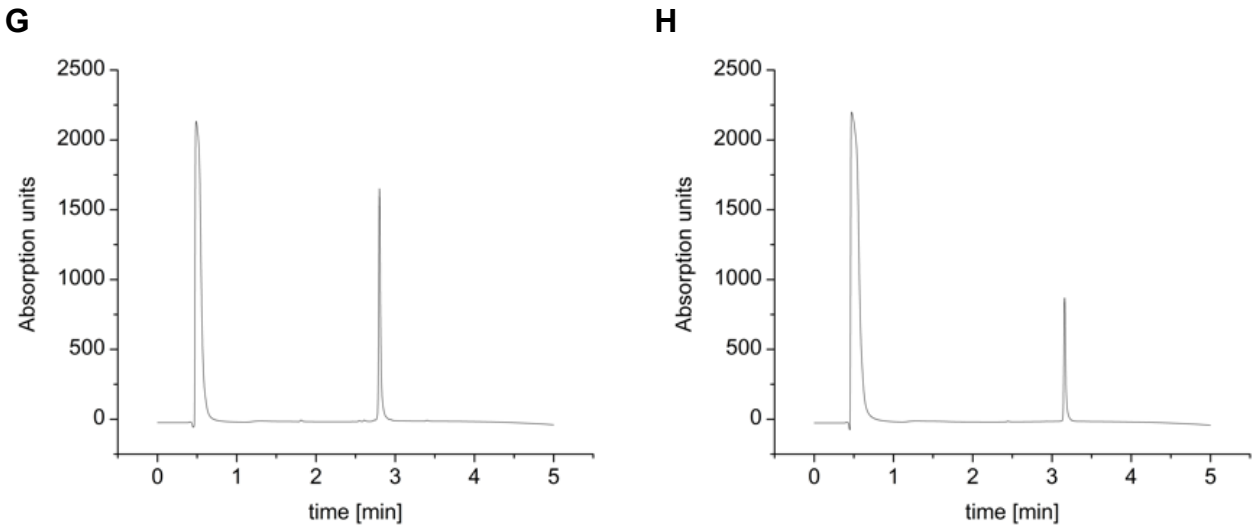

Figure S1: HPLC analysis of the synthesized and purified compounds dissolved in DMSO. **A** DOTA conjugate; **B** NOTA conjugate; **C** DTPA conjugate; **D** CHX-A''-DTPA conjugate; **E** PCTA conjugate; **F** oxo-DO3A conjugate; **G** NODAGA conjugate; **H** CIM conjugate. The first peak corresponds to DMSO, the second to the product.

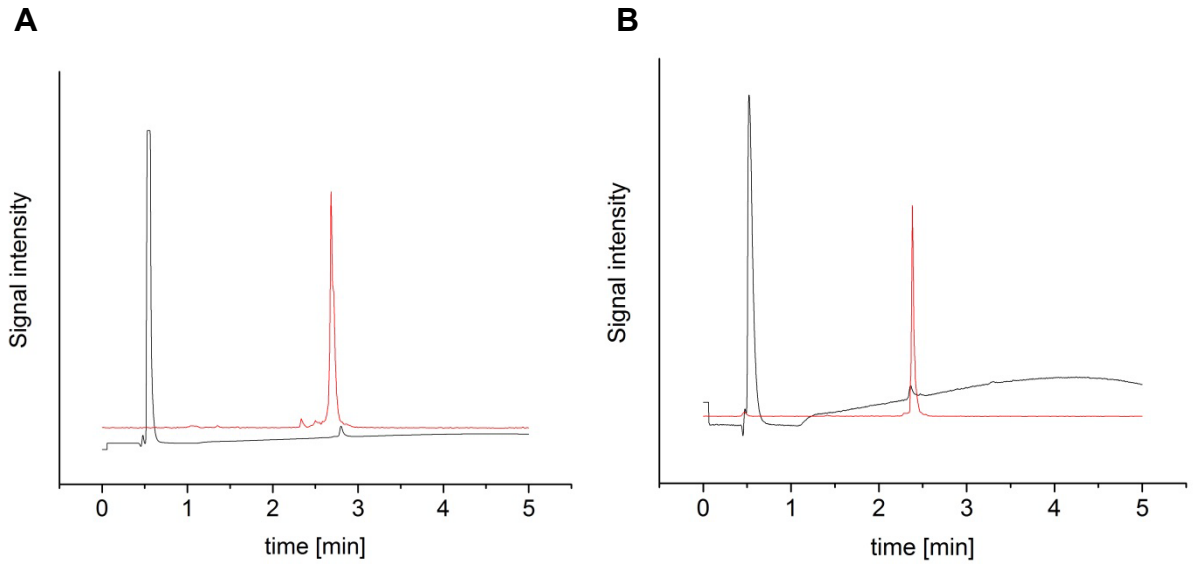

Figure S2: Radio-HPLC of the ^{68}Ga-labeled CHX-A''-DTPA **A** and DOTA **B** conjugates. The red graph represents the NaI counter channel, the black one corresponds to the UV channel (234 nm)

Table S1: Analytical data of all compounds**.**

Substance	Calculated mass [g/mol]	m/z (+ [M^+])	Purity	Retention time [min] (HPLC)
DOTA conjugate	1042.15	1042.43	> 98%	2.4
NOTA conjugate	941.05	994.30 (+[Fe^{3+}])	> 98%	2.39
DTPA conjugate	1031.08	1084.32 (+[Fe^{3+}])	> 98%	2.44
CHX-A''-DTPA conjugate	1250.38	1287.35 (+[K^+]; 70%) 1341.30 (+[K^+]+[Fe^{3+}]; 30%)	> 95%	2.65
PCTA conjugate	1183.34	1252.30 (+3 [Na^+])	> 95%	2.63
oxo-DO3A conjugate	1150.31	1219.39 (+3 [Na^+])	> 98%	2.59
NODAGA conjugate	1013.11	1246.52 (+4 [Fe^{3+}]+[Na^+])	> 98%	2.61
CIM conjugate	1394.64	1282.54 (+2 [Fe^{3+}])	> 98%	2.87

Table S2: Maximal SUV obtained during microPET imaging for the ^{68}Ga-labeled DOTA and CHX-A''-DTPA conjugate and for the ^{68}Ga-labeled CHX-A''-DTPA while blocked with an excess of non-labeled precursor.

DOTA	**heart**	**kidney**	**bladder**	**tumor**
1 h	0.47	1.50	69.0	1.10
2 h	0.15	0.36	8.90	0.94
CHX-A''-DTPA				
1 h	0.91	6.10	75.3	1.60
2 h	0.23	5.00	42.6	1.80
3 h	0.23	4.70	18.0	2.10
CHX-A''-DTPA block				
1 h	0.77	6.80	38.7	0.51
2 h	0.14	0.42	27.6	0.17
3 h	0.11	0.40	25.0	0.16