Supporting Information

A novel tumor pretargeting system based on complementary L-configured oligonucleotides

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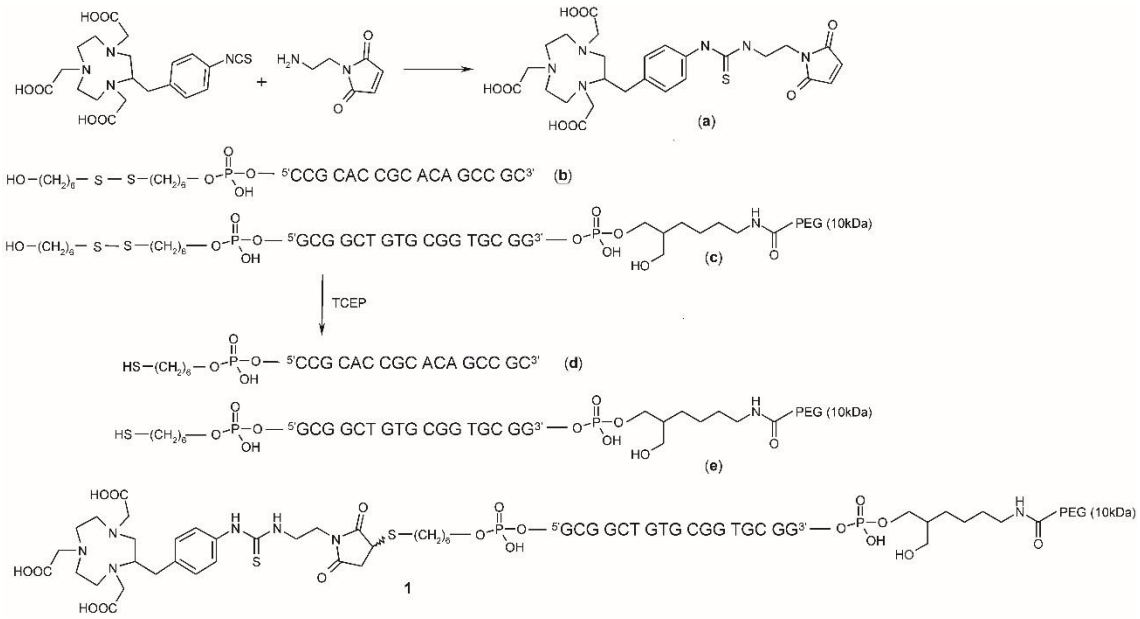
Synthesis of the radionuclide transporting oligonucleotide strand 1 (NOTA'-L-DNA-10kDa-PEG)



Scheme S1. Synthesis of NOTA'-L-DNA-10kDa-PEG

(S)-2-[2-(4-{3-[2-(2,5-dioxo-2,5-dihydro-(1H)-pyrrole-1-yl)ethyl]thiourea} benzyl)-1,4,7-triazonane-1,4,7-triyl]triacetic acid (**a**)

p-SCN-Bn-NOTA × 3HCl (NOTA') (7.77 mg, 13.8 µmol) was dissolved in 105 µL of anhydrous DMF. Afterwards triethylamine (60.75 µmol, 6.15 mg, 8.5 µL) was added, N-(2-aminoethyl)maleimide × TFA (15.75 µmol, 4.0 mg) dissolved in 105 µL of anhydrous DMF was added droppwise to the solution. The reaction mixture was shaken at 26°C for 2 h. In the next step acetic acid (369 µmol, 22.16 mg, 21.1 µL) was added and the reaction mixture was purified by semipreparative HPLC (conditions at Experimental sections). The product fraction was immediately frozen in liquid nitrogen and the content of methanol was reduced in an ice bath under a stream of nitrogen. The residual solvent was then removed by freeze drying to give a white solid (3.33 mg, 41% yield). HPLC A(I): t_R = 11.4 min. HPLC B(I): t_R = 14.5 min. ^{1}H-NMR (400 MHz, D_2O) δ (ppm): 2.92-4.03 (m, 23 H), 7.00 (s, 2 H, CH=CH); 7.32 (d, J = 7.6 Hz, 2 H, aromatic CH), 7.51 (d, J = 7.6 Hz, 2 H, aromatic CH). MS (ESI): m/z = 591 $[M+H]^+$, 613 $[M+Na]^+$.

*General procedure for deprotection of thiol-modified L-oligonucleotides (**b**, **c**)*

Deprotection of thiol-modified L-oligonucleotides was performed as previously reported[1] with slight modifications. To a stock solution of 17mer-c-L-DNA (**b**) (3.2 mM) or L-DNA-10kDa-PEG (**c**) (3.2 mM) a TCEP solution was added (40.7 mM) in 25-fold molar excess compared to **b** and **c**. All compounds were dissolved in phosphate buffered saline (pH = 8.0). The pH-value of the reaction mixture was adjusted to 4-5 before shaking in a thermomixer at 26°C. After 2 h azido pentanoic acid (APA) was dissolved in phosphate buffer (pH = 8.0) to prepare a 0.5 M stock solution and added in a 4-fold molar excess compared to TCEP to the reaction mixture. The adjustment of the pH-value with 1 M NaOH to 8-9 will start the reaction of TCEP with APA as indicated by the formation of gas bubbles. The reaction mixture was shaken in a thermomixer for 4 h at 26°C until no more gas bubbles ascended. Prior to addition of any

maleimide functionalized chelator or antibody conjugate the pH-value was adjusted to 6 with 1 M HCl.

*NOTA'-L-DNA-10kDa-PEG (**1**).* Conjugation of L-DNA **e** with modified chelator **a** was accomplished as previously reported with slight modifications.[1] 300 nmol of **c** were deprotected according to the protocol described before. Subsequently 1.2 µmol of **a** (0.71 mg) dissolved in 100 µL of phosphate buffer (pH = 6.0) was added to the reaction mixture. After shaking over night at 26°C the product **1** was separated by HPLC purification (conditions at Experimental sections). The residual solvent was removed by freeze drying to give a white solid (169 nmol, 56% yield). HPLC C(III): t_R = 22.8 min.

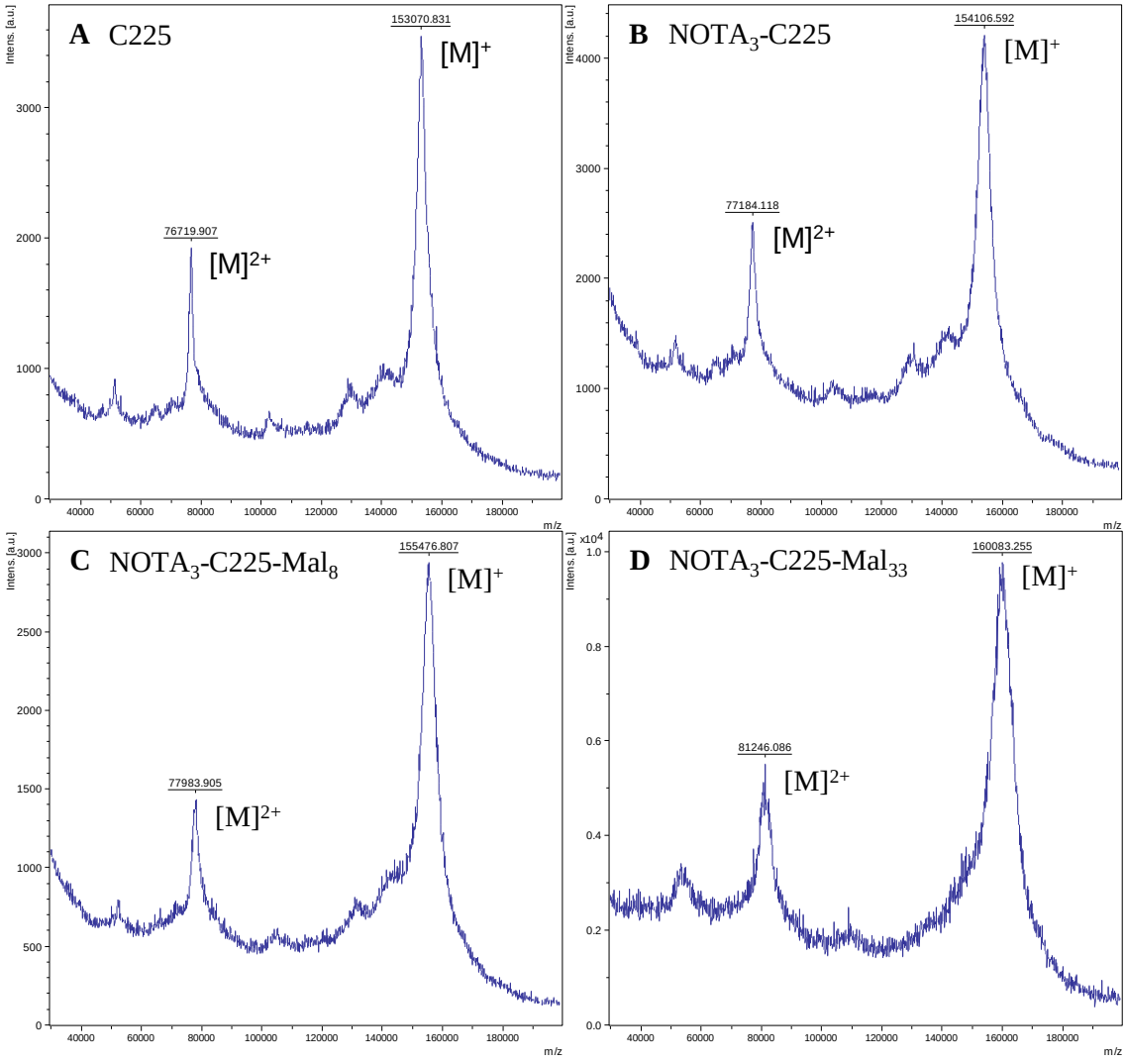


Figure S1. Comparison of MALDI-ToF mass spectra of (**A**) C225, (**B**) NOTA'$_3$-C225, (**C**) NOTA'$_3$-C225-Mal$_8$ and (**D**) NOTA'$_3$-C225-Mal$_{33}$.

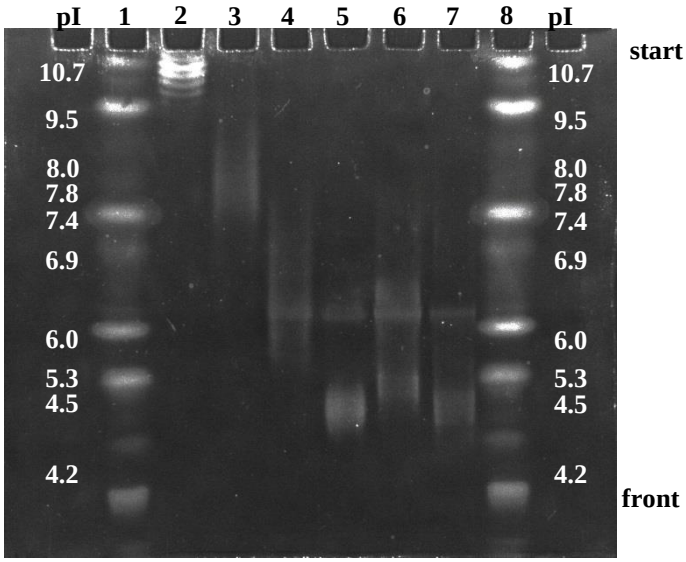


Figure S2. Isoelectronic focusing analysis of modified C225 conjugates on polyacrylamide gel. Lane 1 and 8: isoelectronic focusing marker; lane 2: C225; lane 3: NOTA'$_3$-C225; lane 4: NOTA'$_3$-C225-Mal$_8$; lane 5: NOTA'$_3$-C225-Mal$_{33}$, lane 6: NOTA'$_3$-C225-(c-L-DNA)$_{1.5}$, lane 7: NOTA'$_3$-C225-(c-L-DNA)$_5$.

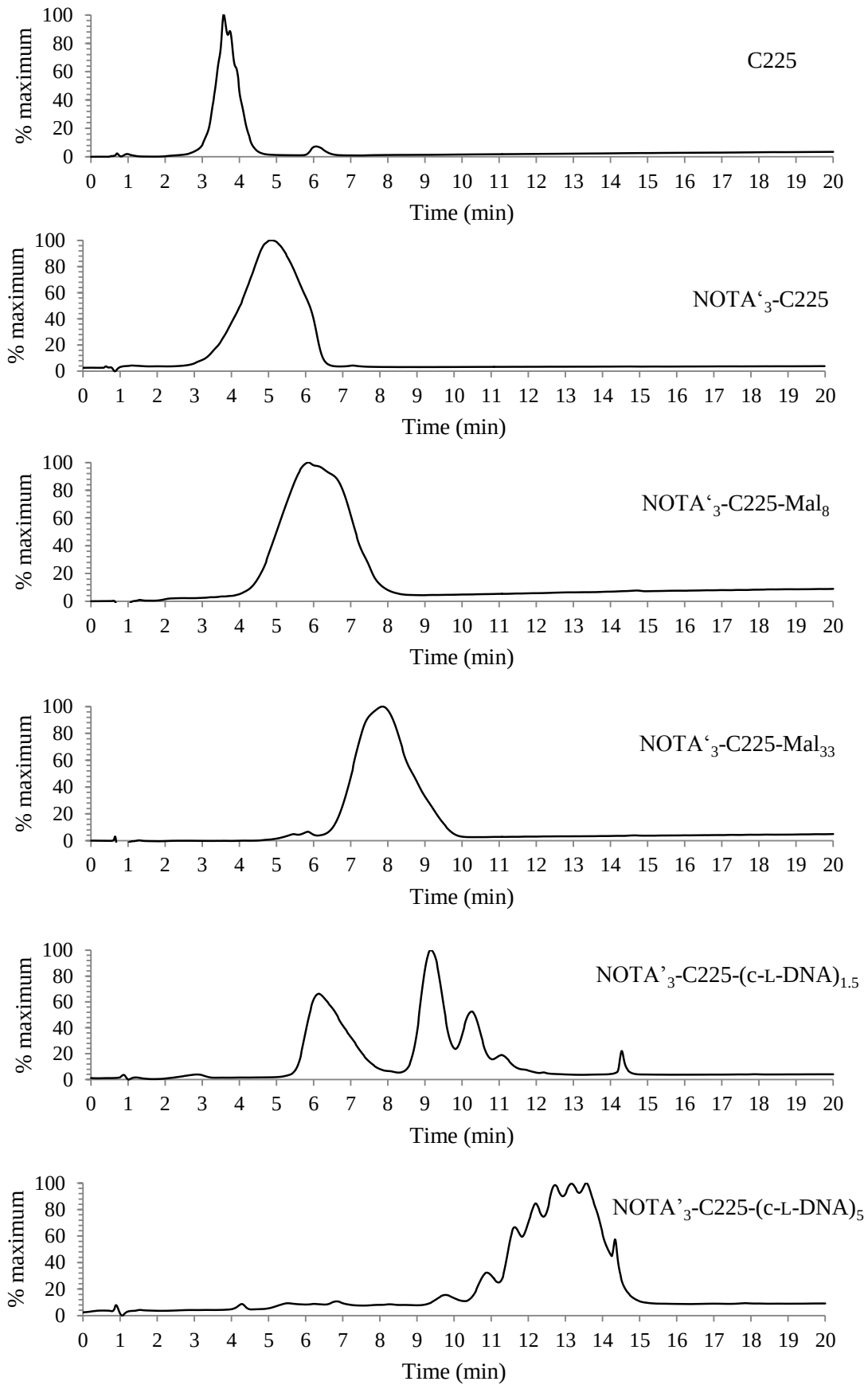


Figure S3. Comparison of HPLC chromatograms (220 nm) of modified C225 conjugates.

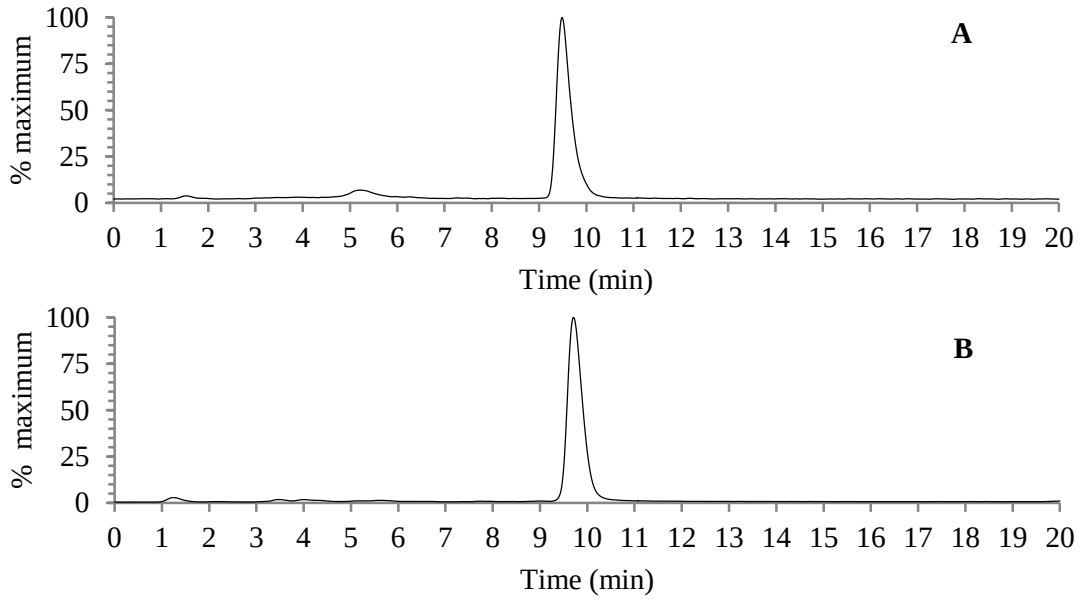


Figure S4. Analytical radio-HPLC chromatograms of (**A**) [^{68}Ga]Ga-**1** (t_R = 9.7 min) and (**B**) [^{64}Cu]Cu-**1** (t_R = 9.5 min).

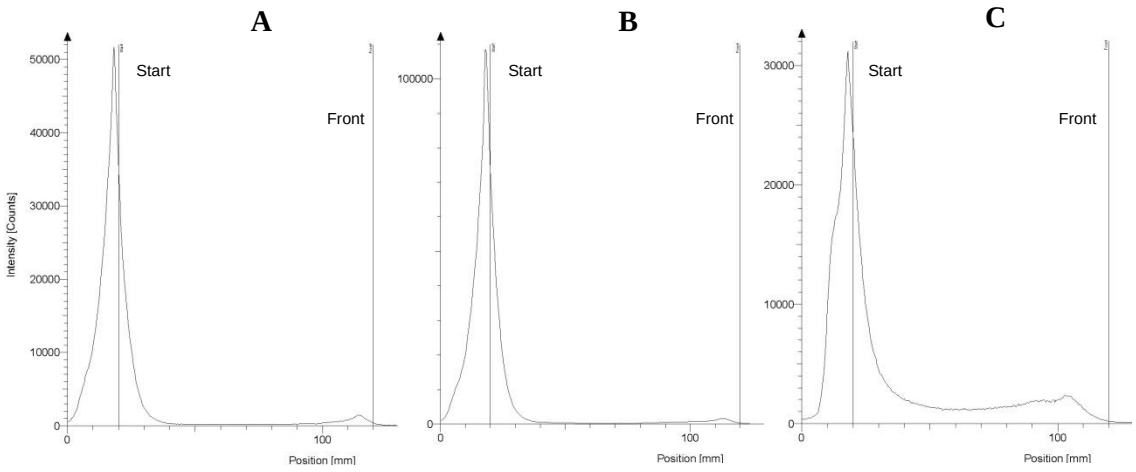


Figure S5. Radio-ITLC chromatograms of (**A**) ([^{64}Cu]Cu-NOTA')$_3$-C225, (**B**) ([^{64}Cu]Cu-NOTA')$_3$-C225-(c-L-DNA)$_{1.5}$ and (**C**) ([^{64}Cu]Cu-NOTA')$_3$-C225-(c-L-DNA)$_5$.

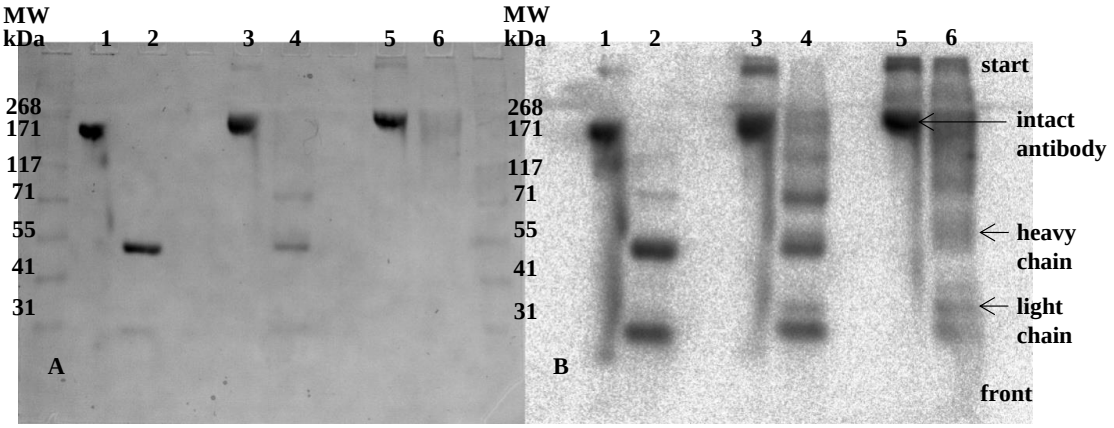


Figure S6. SDS-PAGE analysis of ^{64}Cu-labeled C225-derivatives, (**A**) Coomassie staining, (**B**) Autoradiogram. Lane 1: ([^{64}Cu]Cu-NOTA')$_3$-C225 native; lane 2: ([^{64}Cu]Cu-NOTA')$_3$-C225 reduced; lane 3: ([^{64}Cu]Cu-NOTA')$_3$-C225-(c-L-DNA)$_{1.5}$ native; lane 4: ([^{64}Cu]Cu-NOTA')$_3$-C225-(c-L-DNA)$_{1.5}$ reduced; lane 5: ([^{64}Cu]Cu-NOTA')$_3$-C225-(c-L-DNA)$_5$ native; lane 6: reduced ([^{64}Cu]Cu-NOTA')$_3$-C225-(c-L-DNA)$_5$.

Melting curves. Equimolar amounts of 0.3 - 0.6 mM stock solution of L-DNA-10kDa-PEG (**c**) and complementary 17mer-c-L-DNA (**b**) were mixed and filled into a cuvette. To induce complete de-hybridization the samples were heated to 95°C within 5 min. Thereafter, the cuvette was cooled down to 5°C with a gradient of 5°C/min to achieve complete hybridization of the complementary strands. For melting point determination the cuvette was heated with 2°C/min to a final temperature of 95°C. The melting point T_M corresponds to the maximum peak of the 1st differentiation of the fitted data points (**Figure S7**).

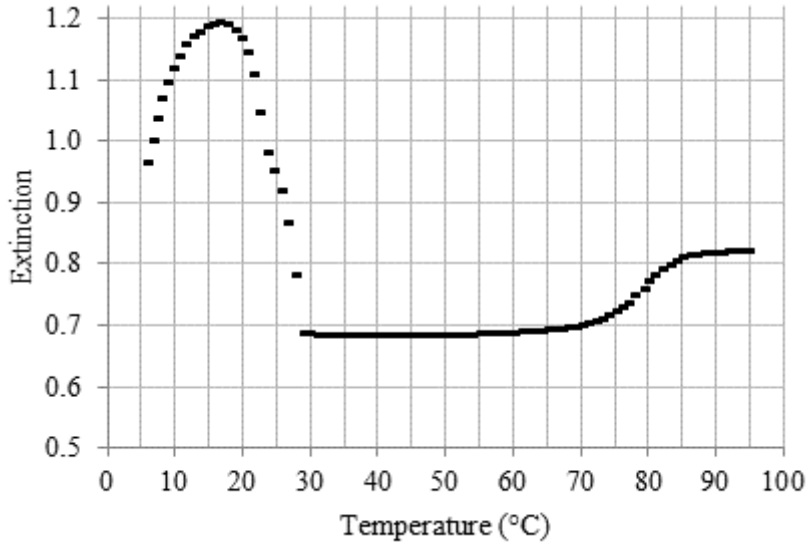


Figure S7. Melting curve of L-DNA-10kDa-PEG (**c**) with 17mer-c-L-DNA (**b**) in 0.9% NaCl solution.

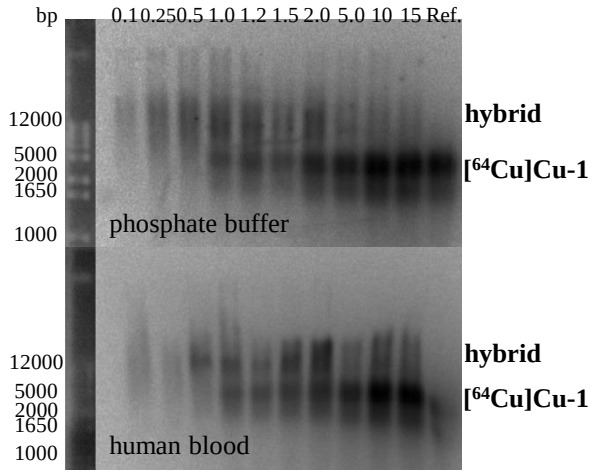


Figure S8. Hybridization of NOTA'$_3$-C225-(c-L-DNA)$_{1.5}$ with [^{64}Cu]Cu-**1**, autoradiographic image of 2% agarose gel.

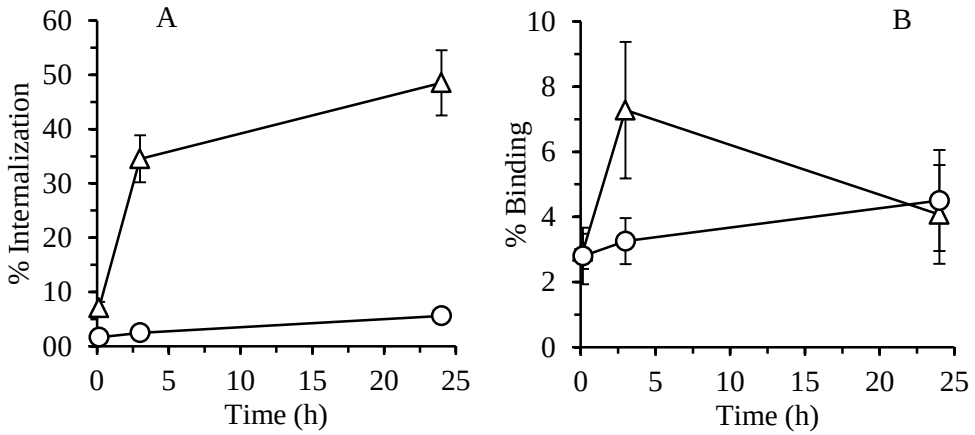


Figure S9. (A) Internalization and (B) binding of ([^{64}Cu]Cu-NOTA')$_3$-C225 after 15 min, 3 h and 24 h of incubation on A431 (triangles) and FaDu cells (circles).

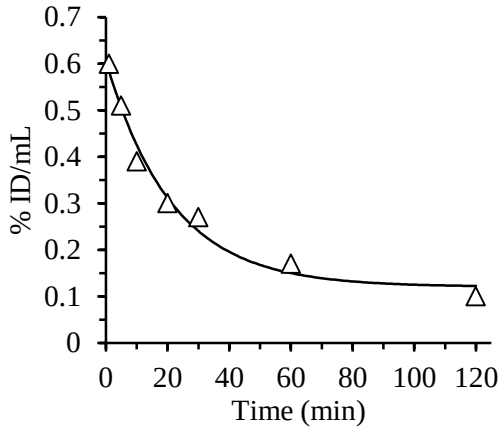


Figure S10. Representative blood clearance curve of [^{64}Cu]Cu-**1** from arterial blood of a rat.

Table S1. Biodistribution of [^{64}Cu]Cu-**1** at 24 h p.i. in FaDu tumor bearing NMRI nu/nu mice without (control experiment; n = 2) and with pretreatment (pretargeting; n = 9) of NOTA'$_3$-C225-(c-L-DNA)$_{1.5}$ 24 h earlier; column p: n.s. = non-significant.

Organ	Control group		Pretargeting group		p
	SUV		SUV		
	Mean	SD	Mean	SD	
Blood	0.06	0.01	0.80	0.19	0.0005
Spleen	0.15	0.01	0.29	0.06	n.s.
Kidney	0.82	0.21	0.99	0.15	n.s
Muscle	0.02	0.00	0.08	0.02	n.s
Liver	0.45	0.01	0.80	0.11	n.s.
Tumor	0.11	0.01	0.88	0.47	0.0498
	%ID		%ID		
	Mean	SD	Mean	SD	
Spleen	0.05	0.00	0.16	0.05	n.s
Kidney	0.96	0.08	1.24	0.22	n.s
Liver	1.99	0.20	3.97	0.40	0.0001
Tumor	0.22	0.04	1.33	0.90	n.s
	Ratio (SUV/SUV)		Ratio (SUV/SUV)		
	Mean	SD	Mean	SD	
Tumor/Blood	1.83	0.27	1.10	0.30	n.s.
Tumor/Spleen	0.73	0.08	3.03	1.11	n.s.
Tumor/Kidney	0.13	0.04	0.89	0.50	n.s.
Tumor/Muscle	5.5	0.29	11.00	5.00	n.s.
Tumor/Liver	0.24	0.01	1.10	0.42	n.s.

(1) Förster, C., Schubert, M., Bergmann, R., Vonhoff, S., Klussmann, S., Walther, M., Pietzsch, J., Pietzsch, H. J., Steinbach, J. (2010) Radiolabeled L-oligonucleotides with tuneable pharmacokinetics - a suitable complementary system for pretargeting approaches. In Mazzi, U., Eckelman, W. C., Volkert, W. A. (Eds.), *Technetium and Other Radiometals in Chemistry and Medicine*, Padova, Italy: S.G.E., 357-362.