Electronic Supplementary Material (ESI) for Dalton Transactions.

A comprehensively revised strategy that improves the specific activity and long-term stability of clinically relevant ^{89}Zr-immuno-PET agents

Nikunj B. Bhatt[1†], Darpan N. Pandya[1†], Stephanie Rideout-Danner[2], Howard D. Gage[2], Frank C. Marini[1,3] and Thaddeus J. Wadas*[1,]

[1]Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, NC 27157 USA.

[2]Department of Radiology, Wake Forest School of Medicine, Winston-Salem, NC 27157 USA.

[3]Wake Forest Institute of Regenerative Medicine, Winston-Salem, NC 27157 USA.

[†]These authors contributed equally to this work.

[*]Corresponding author:

Thaddeus J. Wadas, Ph.D.

Assistant Professor of Cancer Biology and Radiology

Wake Forest School of Medicine

Medical Center Blvd.

Winston-Salem, NC 27157

phone: (336) 716-5696

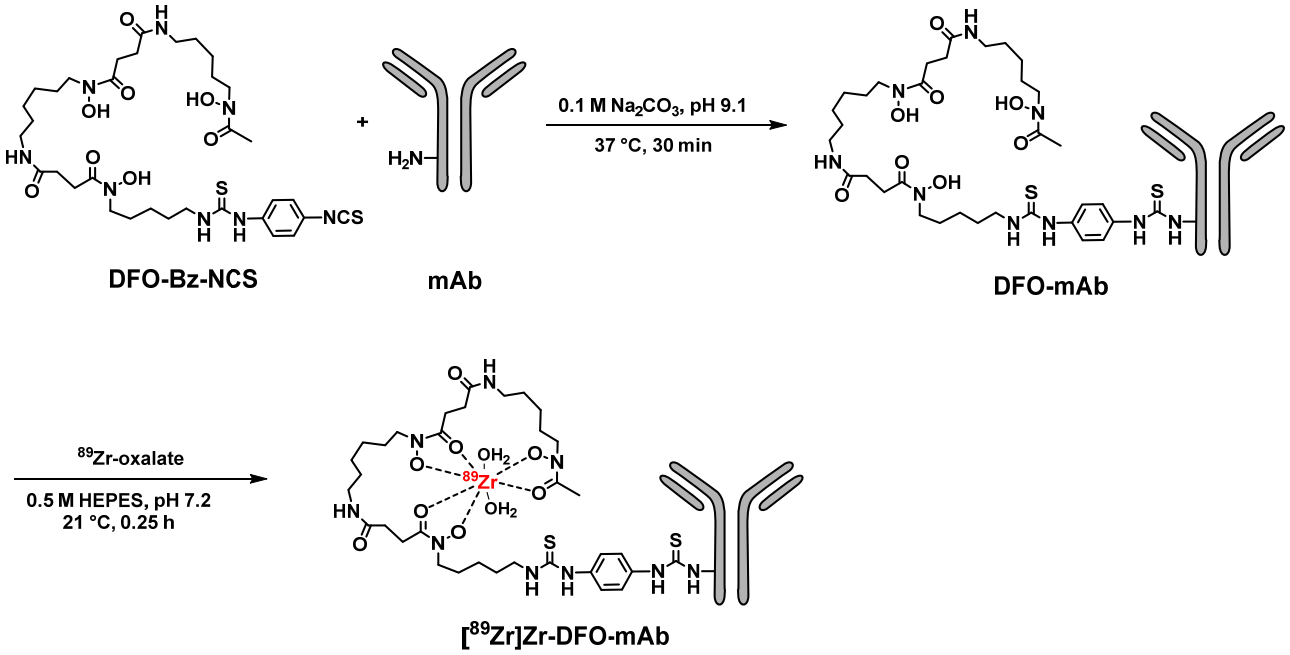
fax: (336) 716-0255

e-mail: twadas@wakehealth.edu

Table of Contents

Reagents setup

- 2 M sodium carbonate: Dissolve 10.599 g of sodium carbonate in 35 mL of Milli-Q water and adjust volume to 50 mL with Milli-Q water.
- 20 mM histidine/240 mM sucrose: Dissolve 0.310 g of histidine and 8.215 g of sucrose in 50 mL of Milli-Q water and adjust volume to 100 mL with Milli-Q water, mix well and check the pH. Adjust to 5.5 to 5.7.
- 0.25 M sodium acetate buffer containing 5 $mg \cdot mL^{-1}$ gentisic acid: Dissolve 3.4 g sodium acetate trihydrate and 0.5 g gentisic acid in 80 mL of Milli-Q water and adjust volume to 100 mL with Milli-Q water, mix well and check the pH. Adjust to 5.5 to 5.7.
- 0.25 M sodium acetate buffer containing 5 $mg \cdot mL^{-1}$ *n*-acetyl-L-cysteine: Dissolve 3.4 g sodium acetate trihydrate and 0.5 g *n*-acetyl-L-cysteine in 80 mL of Milli-Q water and adjust volume to 100 mL with Milli-Q water.
- 0.25 M sodium acetate buffer containing 0.5 $mg \cdot mL^{-1}$ *n*-acetyl-L-cysteine: Dissolve 3.4 g sodium acetate trihydrate and 0.05 g *n*-acetyl-L-cysteine in 80 mL of Milli-Q water and adjust volume to 100 mL with Milli-Q water.
- 0.5 M HEPES buffer (pH 7.1 - 7.3): Mix 20 mL of Milli-Q water with 20 mL of 1 M HEPES solution and check the pH. Adjust to 7.0 to 7.5.
- 20 mM Citric acid (pH 5.0): Dissolve 0.42 g citric acid monohydrate and 1.0 mL of 2 M Na_2CO_3 in 80 mL of Milli-Q water, and adjust volume to 100 mL with Milli-Q water.
- DFO-Bz-NCS solution: Dissolve 1.53 mg of DFO-Bz-NCS in 60 µL of DMSO.
- $ZrCl_4$: Dissolve 7.87 mg of $ZrCl_4$ in 80 mL of Milli-Q water, and adjust volume to 100 mL with Milli-Q water.



Supplementary Scheme S1. Modified preparative route used to prepare [^{89}Zr]Zr-DFO-mAbs.

Conjugation of DFO with mAbs

DFO-mAbs were prepared using a reported procedure with modifications.[1] Briefly, mAb (6 mg) was dissolved in saline (600 µl), and the pH adjusted to pH 8.9-9.1 with 0.1 M Na_2CO_3 (60 µL). A five-fold molar excess of DFO-Bz-NCS (153 µg in 6 µL DMSO) was added and the resulting solution was incubated for 30 min. at 37 °C using a thermomixer at 550 r.p.m. To remove non-conjugated DFO-Bz-NCS, DFO-mAb was purified by PD-10 column using saline (0.9% NaCl). The purified DFO-mAb conjugate was stored at 4 °C and used for ^{89}Zr-radiochemistry.

Determination of chelator to antibody ratio

Chelator to antibody ratio was determined by the following reported process.[2] Briefly, [^{89}Zr]Zr-oxalate (~50 µCi in 2-3 µL1.0 M oxalic acid) was added to the freshly prepared $ZrCl_4$ (10-fold molar excess to the DFO-mAbs used for this experiment). pH of the reaction mixture was adjusted to 6.8-7.2 by using 2 M Na_2CO_3 and mixture was incubated at room temperature for 3 min. followed by pH adjustment to 6.8-7.2 using 0.5 M HEPES buffer (300 µL, pH 7.2). 50 µg of DFO-mAbs conjugate in saline (18.4 µL) was then added; the resulting mixture was incubated at 21 °C for 20 min. The reaction was quenched with ethylenediaminetetraacetic acid solution (50 µL, 50 mM EDTA) and incubated for 10 min. The reaction mixture was then spotted on ITLC and developed in 50 mM EDTA (pH 5.0). Finally, the ITLC strip was cut in two parts and the radioactivity (as counts per minute) associated with each part was measured by gamma counting. The chelator to antibody ratio was calculated using the equation below.

$$\text{Moles of chelator} = \text{Moles of } ZrCl_4 \times \left(\frac{\text{cpm } (R_f < 0.5)}{\text{cpm (total)}} \right)$$

Each experiment was also carried out by using 15- and 20-fold molar excess of $ZrCl_4$ and was done in triplicate.

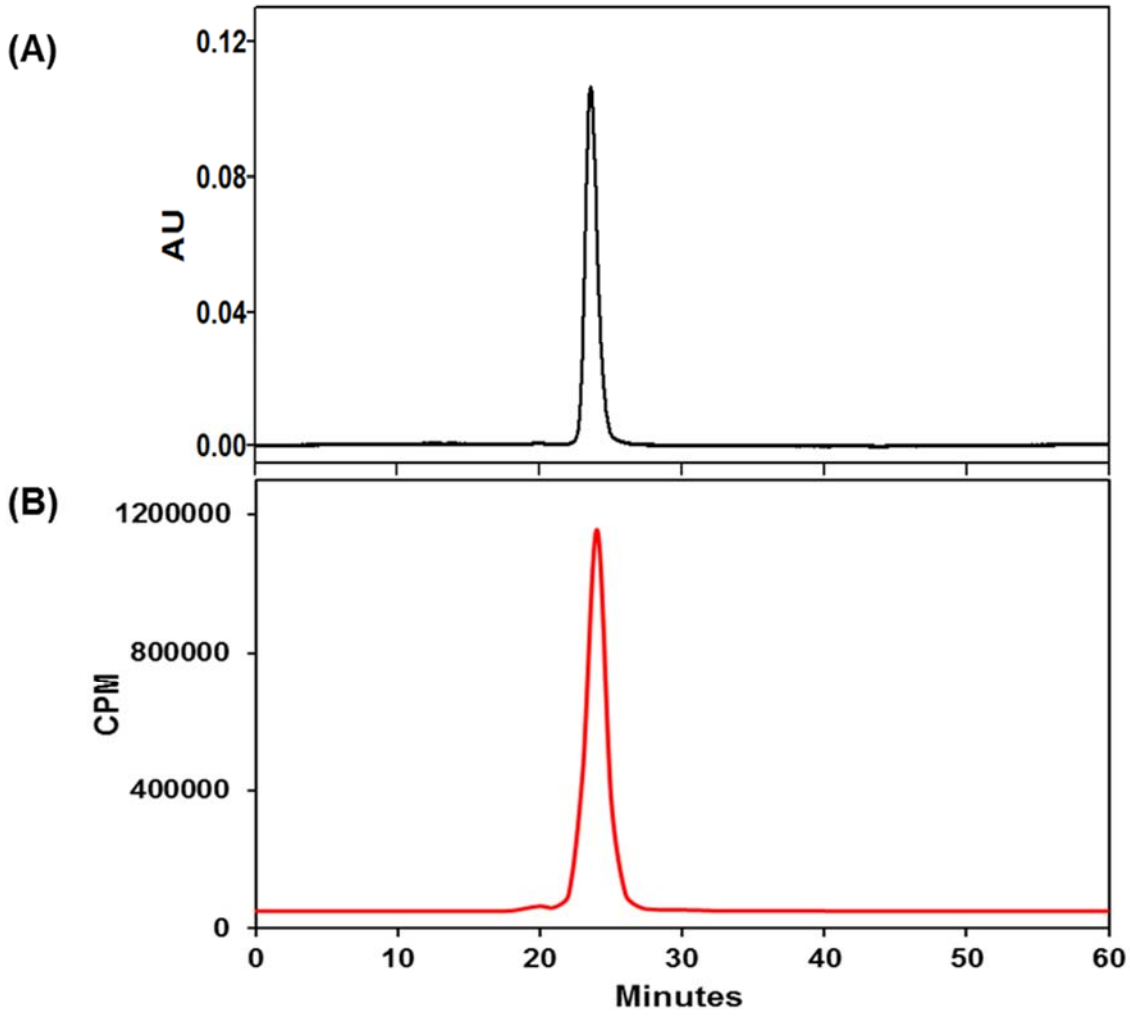
Supplementary Table S1. Chelator to antibody ratio obtained using the isotopic dilution method

Antibody	Chelator per antibody
Cetuximab	3.48 ± 0.2
Trastuzumab	2.91 ± 0.1

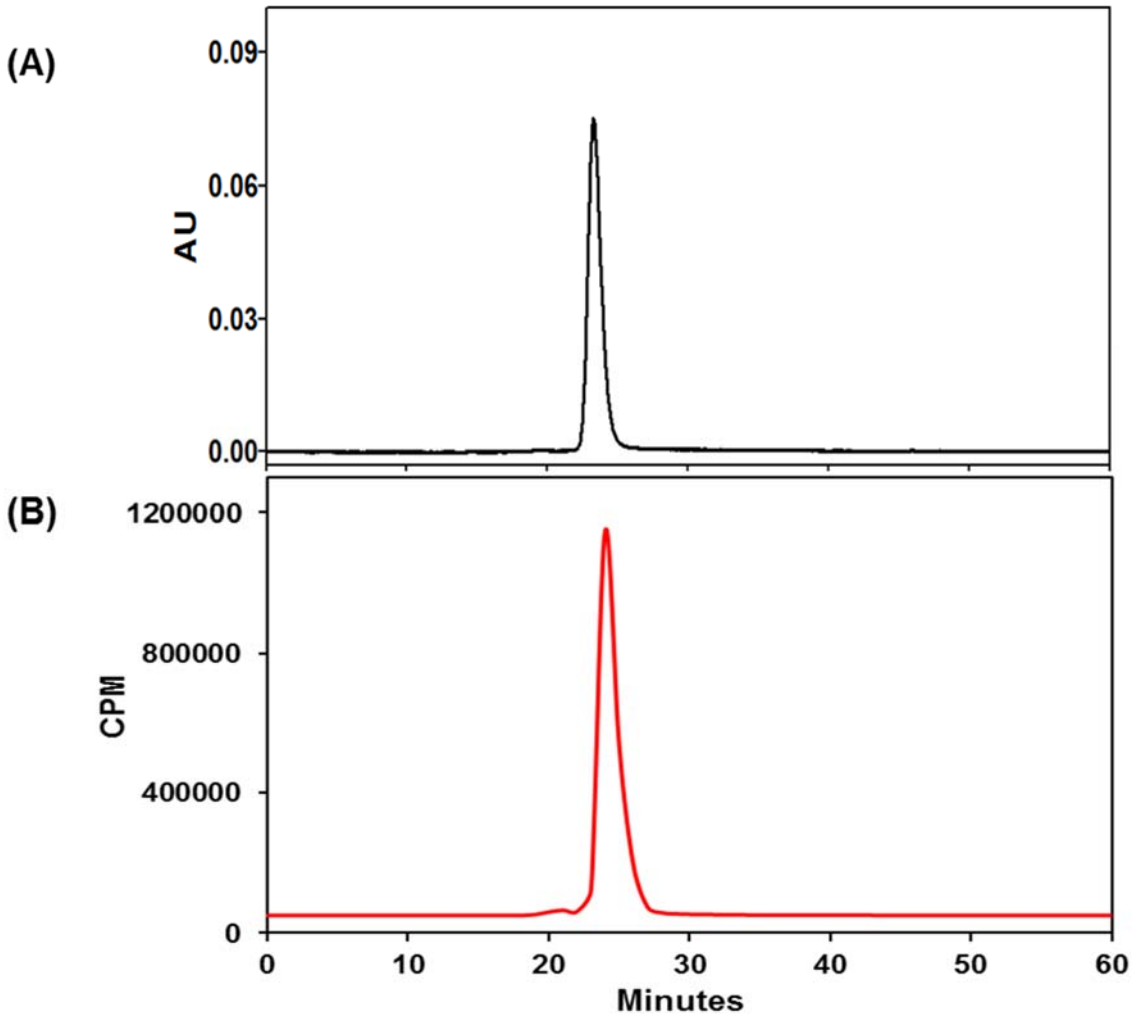
Supplementary Table S2. Summary of optimized mAb mass used to prepare [^{89}Zr]Zr-DFO-mAbs[a]

Conjugates (DFO-mAbs)	Mass of conjugate (µg)	$^{89}Zr(Ox)_2$ added (MBq)	Radiochemical purity[b] by Radio-ITLC (%)	Radiochemical yield[c] (%)	Radiochemical purity[c] by SE-HPLC (%)	Specific activity (A_s; MBq µg^{-1})
DFO-cetuximab	500	55.3	99.9 ± 0.1	98.8 ± 0.2	98.7 ± 0.3	0.109 ± 0.001
	400	55.1	99.8 ± 0.2	98.2 ± 0.3	98.3 ± 0.3	0.134 ± 0.002
	350	50.2	99.9 ± 0.1	97.8 ± 0.4	97.9 ± 0.5	0.139 ± 0.004
	330	**49.8**	**99.9 ± 0.2**	**97.3 ± 0.4**	**97.5 ± 0.4**	**0.144 ± 0.003**
	300	48.1	99.8 ± 0.3	95.2 ± 0.5	95.5 ± 0.7	0.151 ± 0.002
DFO-trastuzumab	500	55.2	99.8 ± 0.1	98.5 ± 0.3	98.6 ± 0.2	0.108 ± 0.001
	400	54.6	99.9 ± 0.2	98.0 ± 0.2	98.1 ± 0.5	0.131 ± 0.004
	350	50.4	99.7 ± 0.4	97.6 ± 0.6	97.8 ± 0.4	0.139 ± 0.002
	330	**49.9**	**99.9 ± 0.1**	**97.5 ± 0.5**	**97.7 ± 0.5**	**0.145 ± 0.002**
	300	47.9	99.8 ± 0.2	94.9 ± 0.4	95.1 ± 0.6	0.150 ± 0.002

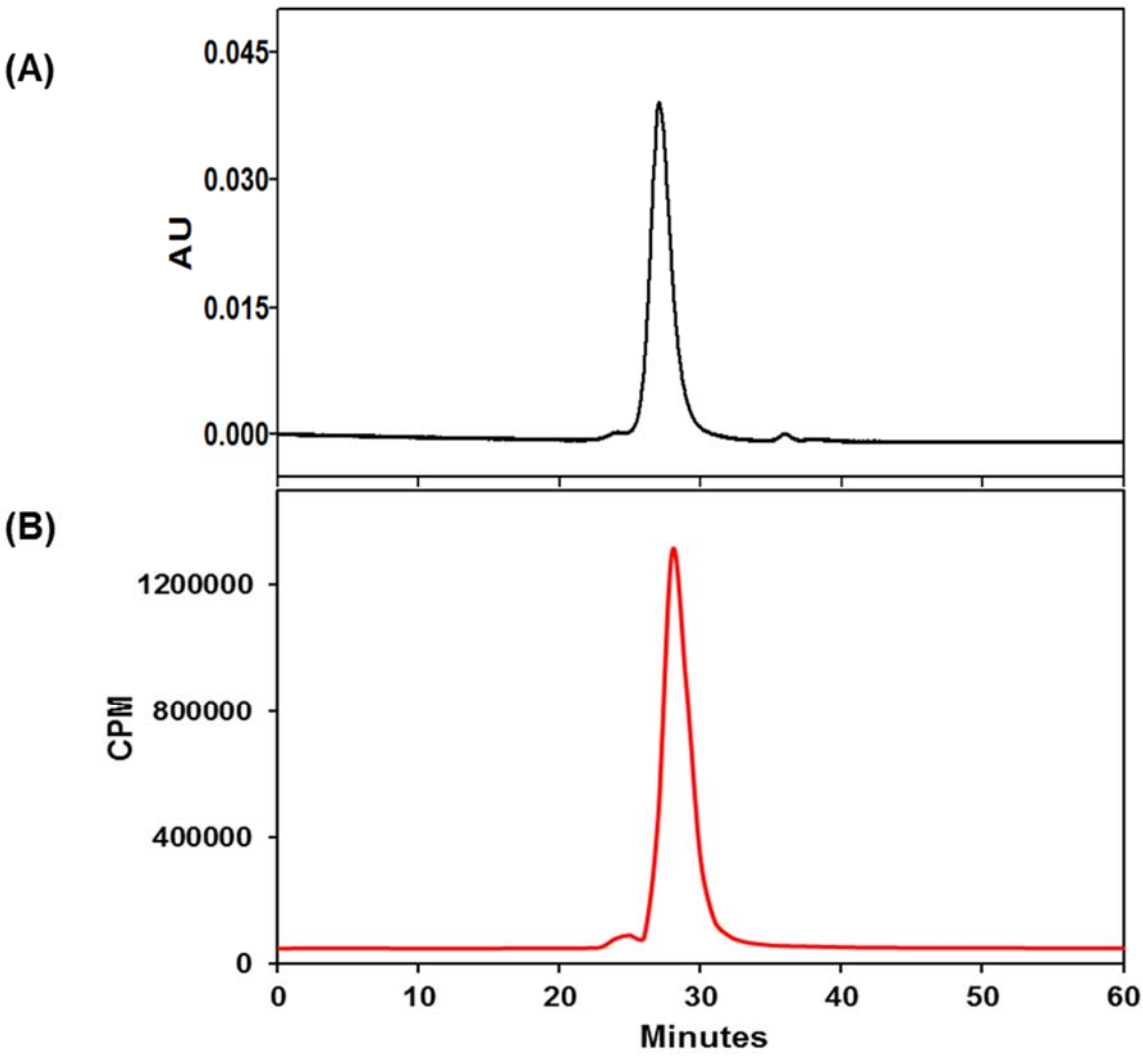
[a]DFO-mAbs were labeled with [^{89}Zr]Zr-oxalate using 0.5 M HEPES buffer (500 µL, pH 7.2) and *n*-acetyl-L-cysteine (100 uL, 5 mg·mL^{-1} in 0.5 M sodium acetate, pH 6.8-7.0) at 21 °C for 15 min. [b]Unchelated ^{89}Zr was not present in the original reaction mixture as determined by Radio-ITLC. [c]Final purity and yield reflect the presence of high and low molecular weight species, which were additionally determined by SE-HPLC.



Supplementary Figure S1: Quality control of [^{89}Zr]Zr-DFO-cetuximab using radio-HPLC. UV-HPLC chromatogram (220 nm) of DFO-cetuximab (A) compared with radio-HPLC chromatogram of [^{89}Zr]Zr-DFO-cetuximab (B).



Supplementary Figure S2: Quality control of [^{89}Zr]Zr-DFO-trastuzumab using radio-HPLC. UV-HPLC chromatogram (220 nm) of DFO-trastuzumab (A) compared with radio-HPLC chromatogram of [^{89}Zr]Zr-DFO-trastuzumab (B).



Supplementary Figure S3: Quality control of [^{89}Zr]Zr-DFO-IgG using radio-HPLC. UV-HPLC chromatogram (220 nm) of DFO-IgG (A) compared with radio-HPLC chromatogram of [^{89}Zr]Zr-DFO-IgG (B).

Supplementary Table S3: Chronological stability study of [^{89}Zr]Zr-DFO-cetuximab formulated in different buffer-excipient combinations and analyzed by centrifugal filtration analysis

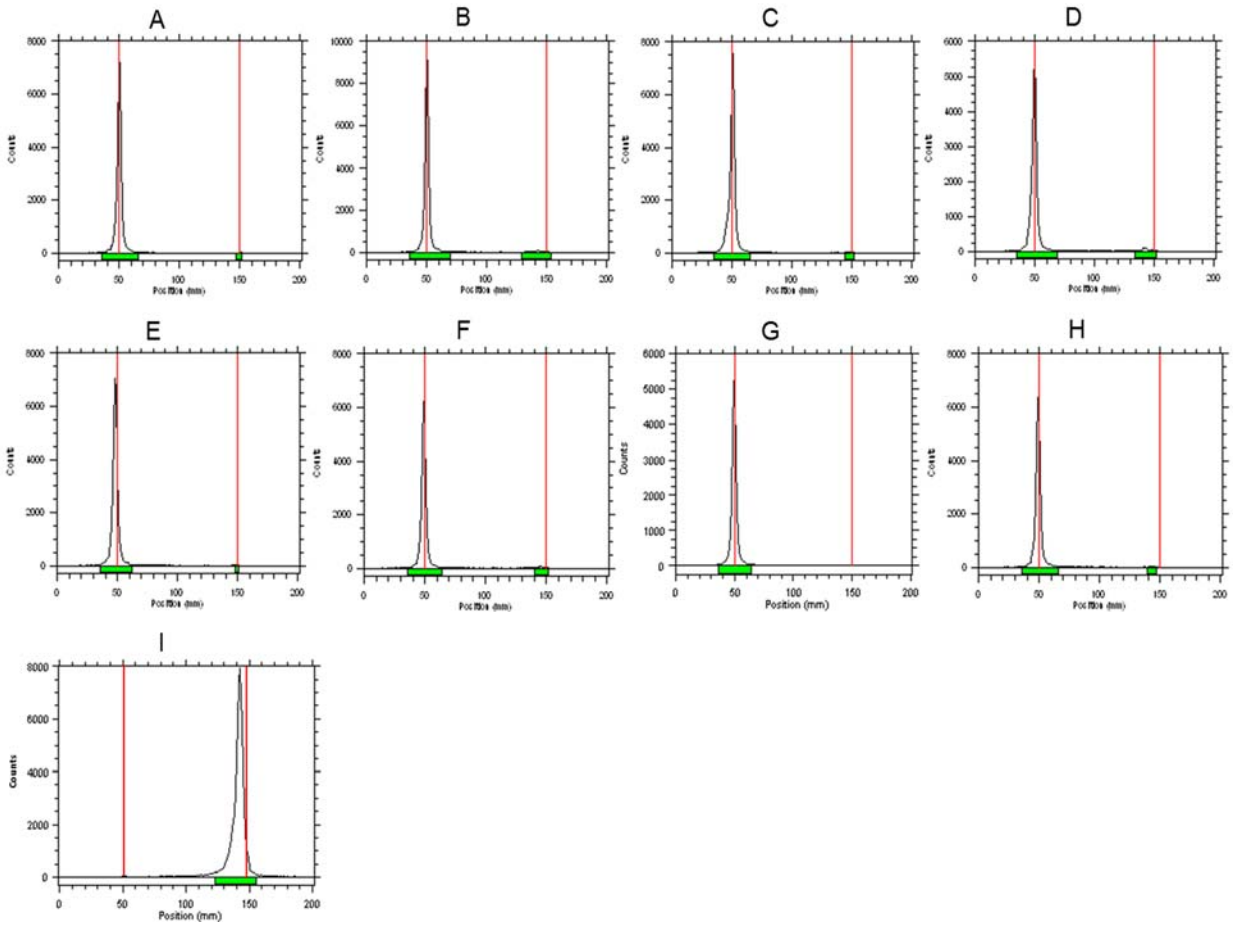
Time point	Temp. (°C)	Intact radiotracer %			
		0.9 % Saline	20 mM Histidine/ 240 mM Sucrose	0.25 M NaOAc + 5 mg·mL^{-1} GA[a]	0.25 M NaOAc + 0.5 mg·mL^{-1} NAC[b]
0 h	21	99.9 ± 0.0	99.9 ± 0.2	99.9 ± 0.0	99.9 ± 0.1
	4	99.9 ± 0.0	99.5 ± 0.1	98.9 ± 0.1	99.8 ± 0.0
1 d	21	99.5 ± 0.1	99.1 ± 0.1	99.2 ± 0.1	99.8 ± 0.1
	4	99.2 ± 0.1	98.6 ± 0.1	98.9 ± 0.1	99.8 ± 0.0
3 d	21	99.2 ± 0.1	98.2 ± 0.0	99.0 ± 0.1	99.7 ± 0.0
	4	99.0 ± 0.2	97.8 ± 0.2	98.9 ± 0.0	99.7 ± 0.0
5 d	21	98.7 ± 0.1	96.6 ± 0.1	98.1 ± 0.0	99.8 ± 0.0
	4	98.8 ± 0.2	97.5 ± 0.1	98.8 ± 0.0	99.8 ± 0.0
7 d	21	97.8 ± 0.2	94.9 ± 1.1	97.4 ± 0.1	99.6 ± 0.1

[a]Gentisic acid. [b]*n*-acetyl-L-cysteine.

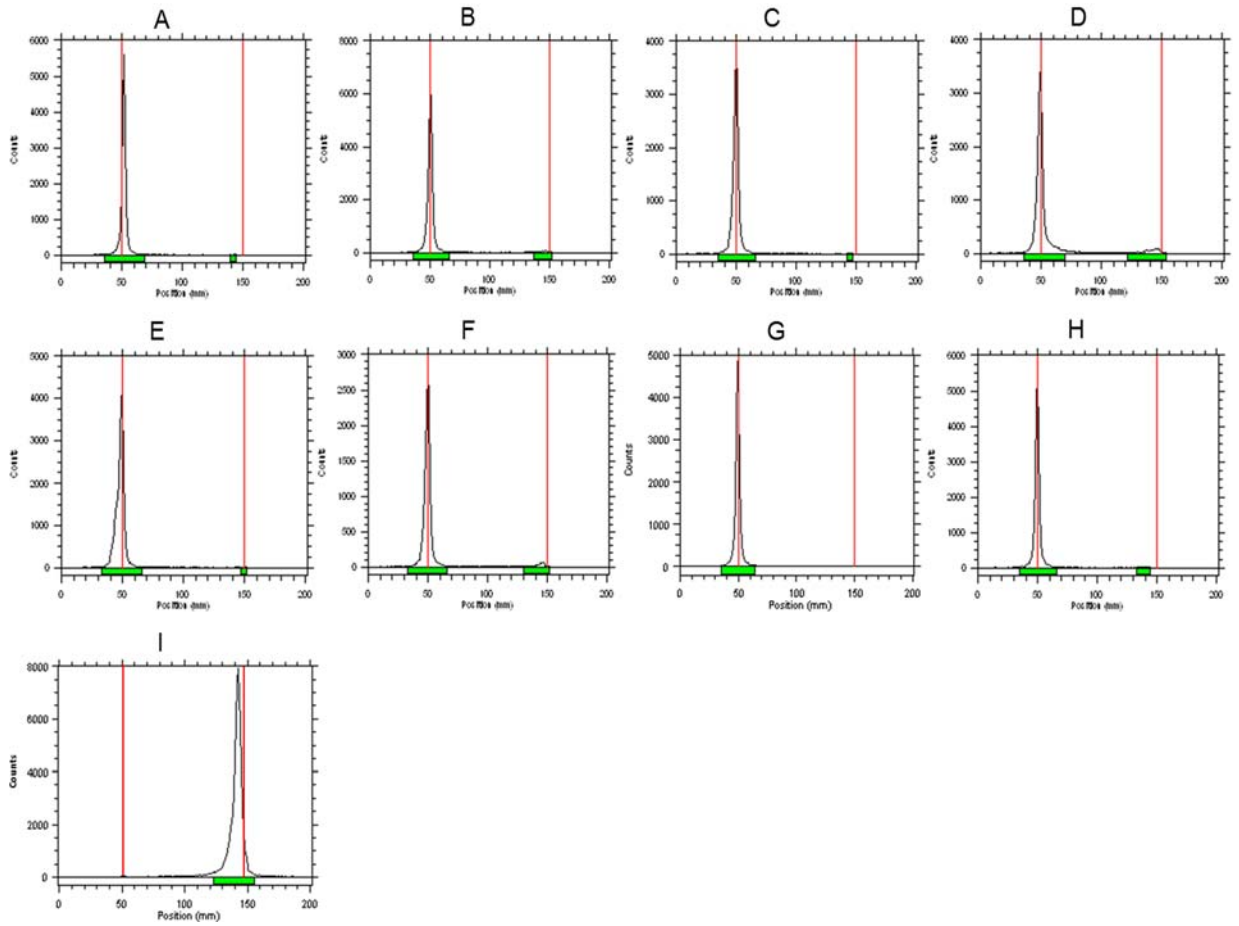
Supplementary Table S4: Chronological stability study of [^{89}Zr]Zr-DFO-trastuzumab formulated in different buffer-excipient combinations and analyzed by centrifugal filtration analysis

Time point	Temp. (°C)	Intact radiotracer %			
		0.9 % Saline	20 mM Histidine/ 240 mM Sucrose	0.25 M NaOAc + 5 mg·mL^{-1} GA[a]	0.25 M NaOAc + 0.5 mg·mL^{-1} NAC[b]
0 h	21	99.9 ± 0.1	99.9 ± 0.0	99.9 ± 0.2	99.9 ± 0.1
1 d	4	99.5 ± 0.1	99.9 ± 0.1	98.5 ± 0.0	99.7 ± 0.1
	21	99.5 ± 0.0	99.4 ± 0.0	99.7 ± 0.0	99.7 ± 0.0
3 d	4	99.2 ± 0.1	98.5 ± 0.4	98.3 ± 0.0	99.6 ± 0.1
	21	99.4 ± 0.03	97.3 ± 0.2	98.2 ± 0.1	99.7 ± 0.0
5 d	4	99.2 ± 0.1	97.2 ± 0.3	98.3 ± 0.3	99.6 ± 0.1
	21	99.1 ± 0.1	94.2 ± 0.2	97.4 ± 0.2	99.3 ± 0.2
7 d	4	98.9 ± 0.1	96.1 ± 0.1	98.2 ± 0.0	99.4 ± 0.3
	21	98.5 ± 0.0	92.5 ± 0.5	96.7 ± 0.4	98.6 ± 0.6

[a]Gentisic acid. [b]*n*-acetyl-L-cysteine.



Supplementary Figure S4: Chronological stability analysis of [^{89}Zr]Zr-DFO-cetuximab formulated in different buffer-excipient combinations at 21 °C by radio-ITLC. 0.9% saline (A) 0 h, (B) 7 d; 20 mM histidine/240 mM sucrose (C) 0 h, (D) 7 d; 0.25 M sodium acetate buffer containing gentisic acid (5 mg·mL^{-1}) (E) 0 h, (F) 7 d; 0.25 M sodium acetate buffer containing *n*-acetyl-L-cysteine (0.5 mg·mL^{-1}) (G) 0 h, (H) 7 d; (I) [^{89}Zr]Zr-Oxalate. In this ITLC-SG system, free ^{89}Zr eluted with the solvent front ($R_f \sim 1$), while [^{89}Zr]Zr-DFO-cetuximab remained at the origin ($R_f \sim 0$).



Supplementary Figure S5: Chronological stability analysis of [^{89}Zr]Zr-DFO-trastuzumab formulated in different buffer-excipient combinations at 21 °C by radio-ITLC. 0.9% saline (A) 0 h, (B) 7 d; 20 mM histidine/240 mM sucrose (C) 0 h, (D) 7 d; 0.25 M sodium acetate buffer containing gentisic acid (5 mg·mL^{-1}) (E) 0 h, (F) 7 d; 0.25 M sodium acetate buffer containing *n*-acetyl-L-cysteine (0.5 mg·mL^{-1}) (G) 0 h, (H) 7 d; (I) [^{89}Zr]Zr-Oxalate. In this ITLC-SG system, free ^{89}Zr eluted with the solvent front ($R_f \sim 1$), while [^{89}Zr]Zr-DFO-trastuzumab remained at the origin ($R_f \sim 0$).

Supplementary Table S5: Chronological stability study of [^{89}Zr]Zr-DFO-cetuximab at 21 °C formulated in different buffer-excipient combinations and analyzed by Radio-ITLC

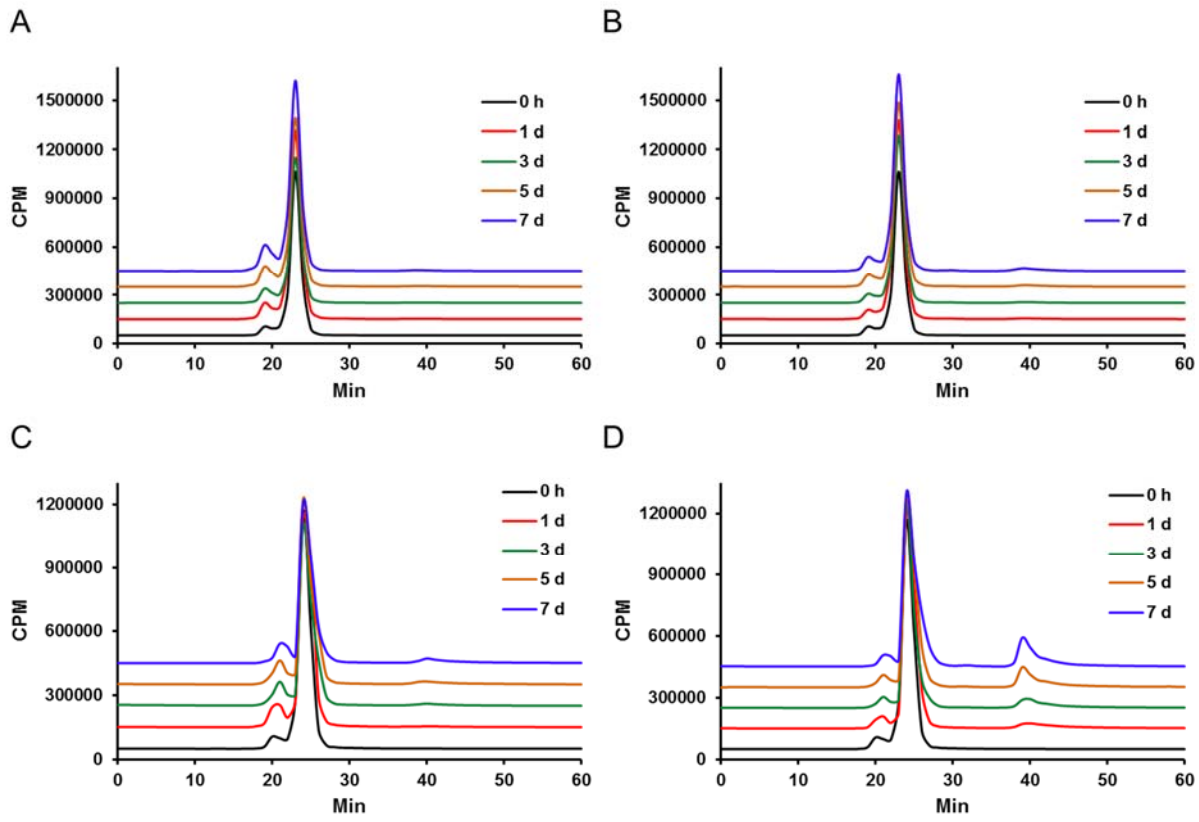
Time point	Intact radiotracer %			
	0.9 % Saline	20 mM Histidine/ 240 mM Sucrose	0.25 M NaOAc + 5 mg·mL^{-1} GA[a]	0.25 M NaOAc + 0.5 mg·mL^{-1} NAC[b]
0 h	99.7 ± 0.1	99.6 ± 0.2	99.9 ± 0.0	100
1 d	99.1 ± 0.4	98.9 ± 0.3	99.2 ± 0.1	99.8 ± 0.2
3 d	98.3 ± 0.2	97.4 ± 0.1	98.4 ± 0.3	99.6 ± 0.3
7 d	97.2 ± 0.3	96.5 ± 0.5	97.5 ± 0.7	99.4 ± 0.1

[a]Gentisic acid. [b]*n*-acetyl-L-cysteine.

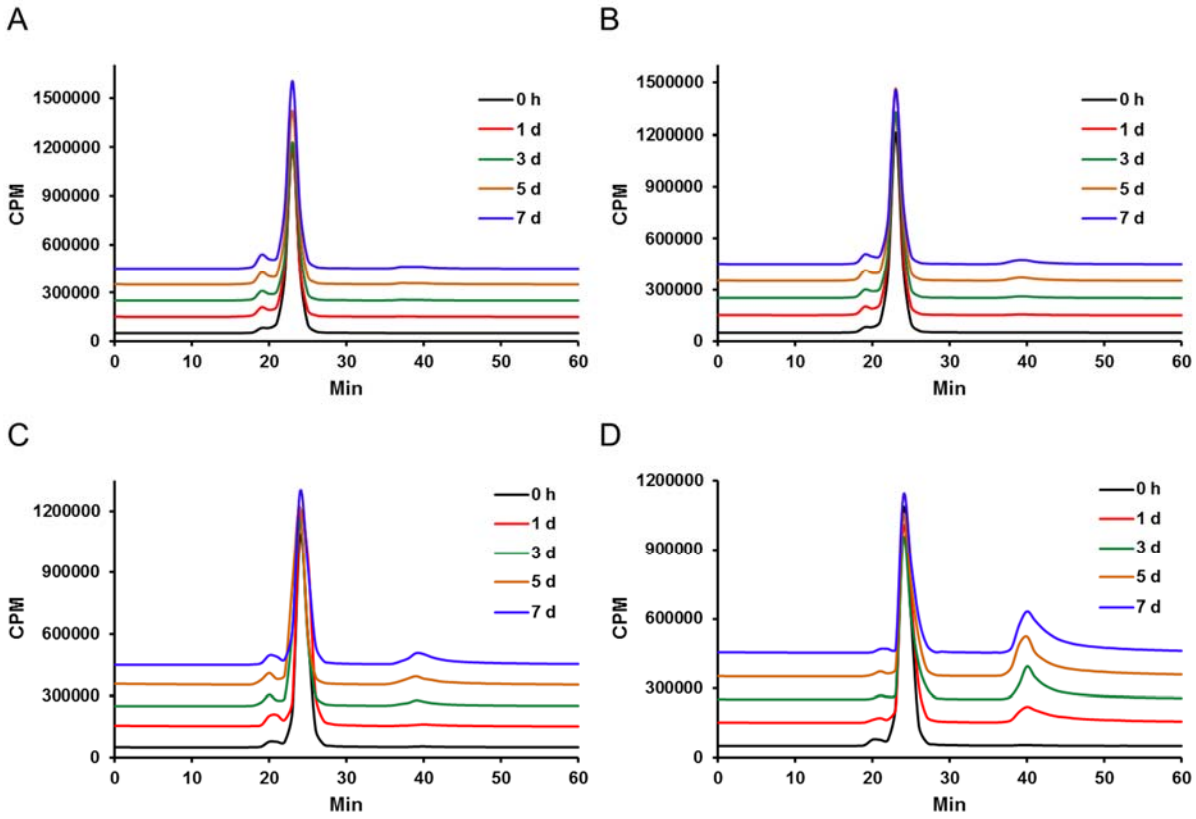
Supplementary Table S6: Chronological stability study of [^{89}Zr]Zr-DFO-trastuzumab at 21 °C formulated in different buffer-excipient combinations and analyzed by Radio-ITLC

Time point	Intact radiotracer %			
	0.9 % Saline	20 mM Histidine/ 240 mM Sucrose	0.25 M NaOAc + 5 mg·mL^{-1} GA[a]	0.25 M NaOAc + 0.5 mg·mL^{-1} NAC[b]
0 h	99.6 ± 0.2	99.7 ± 0.1	99.8 ± 0.1	100
1 d	99.3 ± 0.3	98.1 ± 0.2	98.9 ± 0.3	99.6 ± 0.1
3 d	98.4 ± 0.4	95.4 ± 0.4	97.3 ± 0.5	98.9 ± 0.3
7 d	97.1 ± 0.2	92.1 ± 1.1	95.9 ± 0.3	98.4 ± 0.1

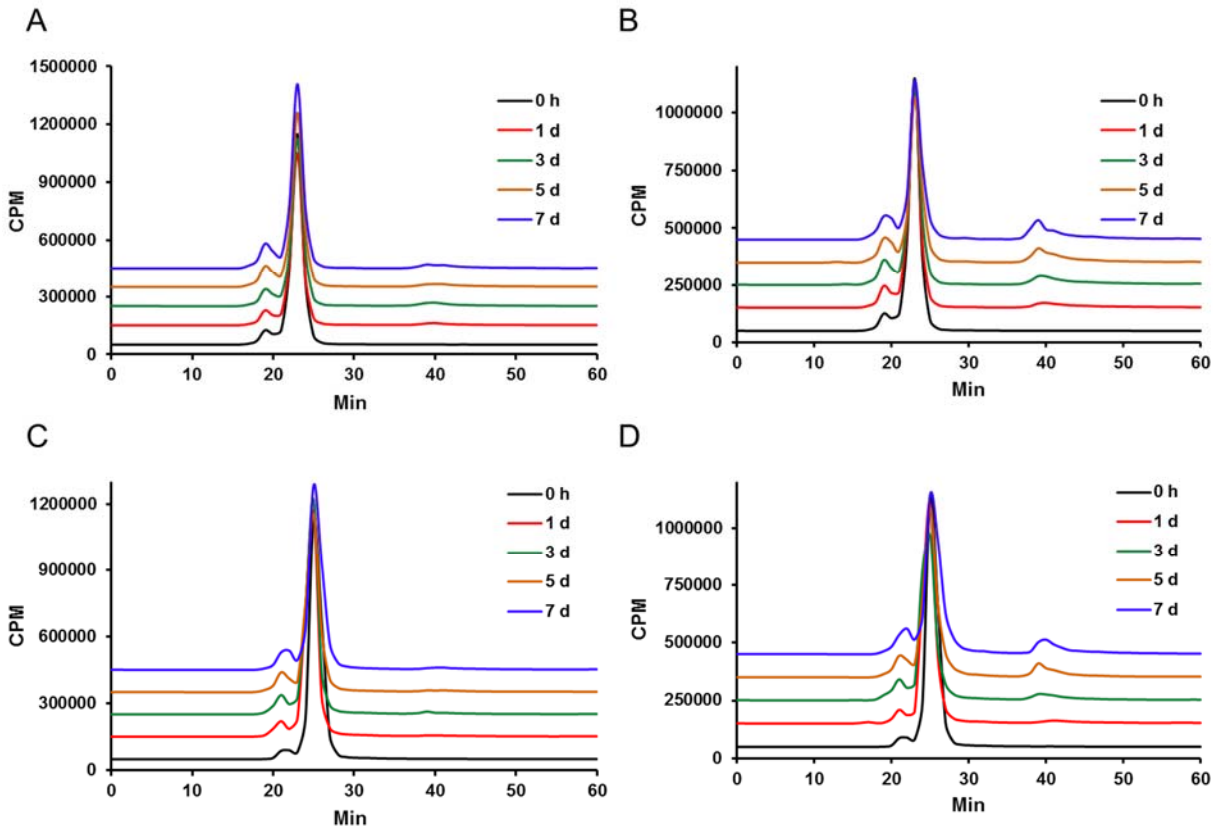
[a]Gentisic acid. [b]*n*-acetyl-L-cysteine.



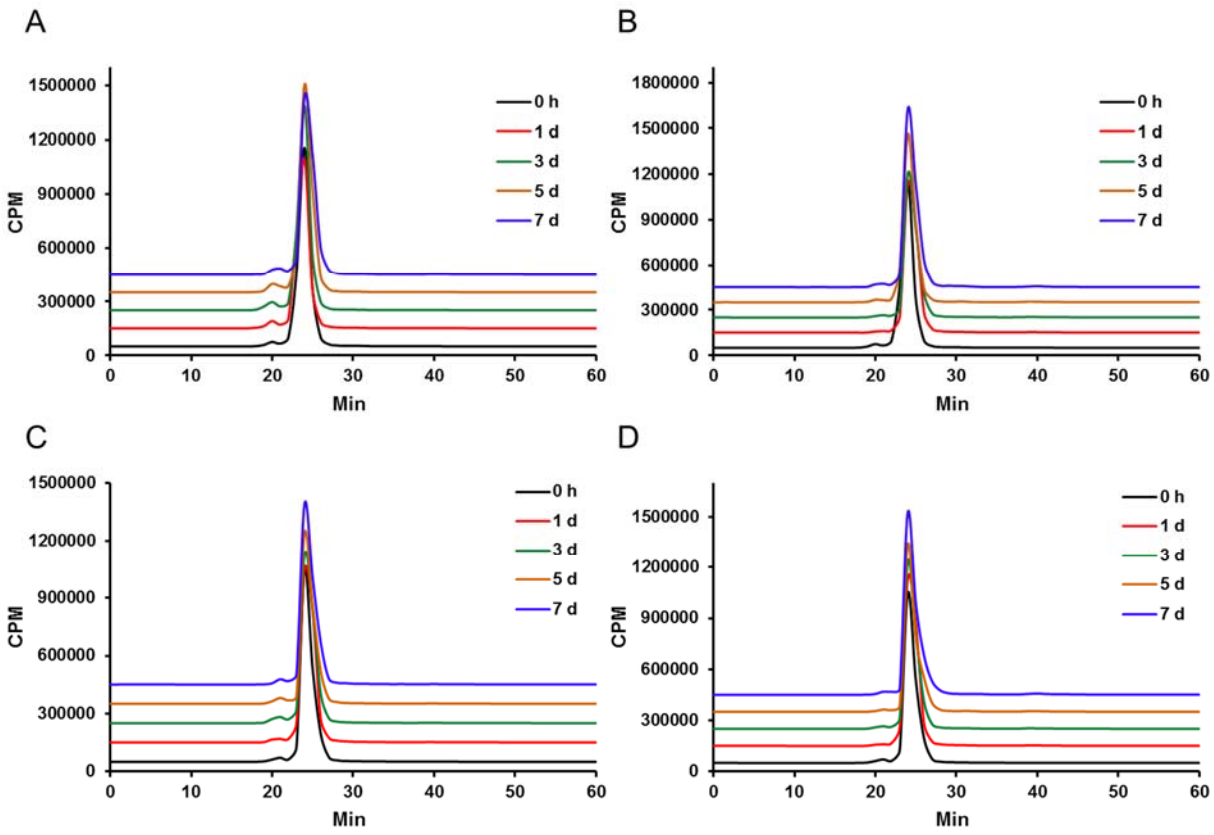
Supplementary Figure S6: Chronological stability analysis of [^{89}Zr]Zr-DFO-mAbs preserved in 0.9% saline. (A) [^{89}Zr]Zr-DFO-cetuximab at 4°C, (B) [^{89}Zr]Zr-DFO-cetuximab at 21°C, (C) [^{89}Zr]Zr-DFO-trastuzumab at 4°C and (D) [^{89}Zr]Zr-DFO-trastuzumab at 21°C. Chromatograms were generated using the SEC methods described in the Supplementary Information. In the size exclusion chromatogram the radiopharmaceutical product was observed to elute at 23-30 min. while high-molecular weight and low-molecular weight species were observed to elute at 18-22 min. and 38-46 min., respectively. An ethylenediaminetetraacetic acid (EDTA) blank injection was run after each SEC injection of [^{89}Zr]Zr-DFO-mAbs to ensure that any free ^{89}Zr was observed (^{89}Zr-EDTA, t_R: 38-40 min.).



Supplementary Figure S7: Chronological stability analysis of [^{89}Zr]Zr-DFO-mAbs preserved in 20 mM histidine/240 mM sucrose. (A) [^{89}Zr]Zr-DFO-cetuximab at 4°C, (B) [^{89}Zr]Zr-DFO-cetuximab at 21°C, (C) [^{89}Zr]Zr-DFO-trastuzumab at 4°C and (D) [^{89}Zr]Zr-DFO-trastuzumab at 21°C. Chromatograms were generated using the SEC methods described in the Supplementary Information. In the size exclusion chromatogram the radiopharmaceutical product was observed to elute at 23-30 min. while high-molecular weight and low-molecular weight species were observed to elute at 18-22 min. and 38-46 min., respectively. An ethylenediaminetetraacetic acid (EDTA) blank injection was run after each SEC injection of [^{89}Zr]Zr-DFO-mAbs to ensure that any free ^{89}Zr was observed (^{89}Zr-EDTA, t_R: 38-40 min.).



Supplementary Figure S8: Chronological stability analysis of [^{89}Zr]Zr-DFO-mAbs preserved in 0.25 M sodium acetate buffer containing gentisic acid. (A) [^{89}Zr]Zr-DFO-cetuximab at 4°C, (B) [^{89}Zr]Zr-DFO-cetuximab at 21°C, (C) [^{89}Zr]Zr-DFO-trastuzumab at 4°C and (D) [^{89}Zr]Zr-DFO-trastuzumab at 21°C. Chromatograms were generated using the SEC methods described in the Supplementary Information. In the size exclusion chromatogram the radiopharmaceutical product was observed to elute at 23-30 min. while high-molecular weight and low-molecular weight species were observed to elute at 18-22 min. and 38-46 min., respectively. An ethylenediaminetetraacetic acid (EDTA) blank injection was run after each SEC injection of [^{89}Zr]Zr-DFO-mAbs to ensure that any free ^{89}Zr was observed (^{89}Zr-EDTA, t_R: 38-40 min.).



Supplementary Figure S9: Chronological stability analysis of [^{89}Zr]Zr-DFO-mAbs preserved in 0.25 M sodium acetate buffer containing *n*-acetyl-L-cysteine. These radiopharmaceuticals were prepared without using Na_2CO_3. The presence of high-and low-molecular weight species is significantly reduced, and initial chromatograms (t = 0 h) demonstrate that elimination of this procedural step does not diminish radiopharmaceutical integrity. (A) [^{89}Zr]Zr-DFO-cetuximab at 4°C, (B) [^{89}Zr]Zr-DFO-cetuximab at 21°C, (C) [^{89}Zr]Zr-DFO-trastuzumab at 4°C and (D) [^{89}Zr]Zr-DFO-trastuzumab at 21°C. Chromatograms were generated using the SEC methods described in the Supplementary Information. In the size exclusion chromatogram the radiopharmaceutical product was observed to elute at 23-30 min. while high-molecular weight and low-molecular weight species were observed to elute at 18-22 min. and 38-46 min., respectively. An ethylenediaminetetraacetic acid (EDTA) blank injection was run after each SEC injection of [^{89}Zr]Zr-DFO-mAbs to ensure that any free ^{89}Zr was observed (^{89}Zr-EDTA, t_R: 38-40 min.).

Supplementary Table S7: Summary of SEC data describing the chronological stability of [^{89}Zr]Zr-DFO-cetuximab formulated in different buffer-excipient combinations

Time point	Temp. (°C)	Species %											
		0.9 % Saline			20 mM Histidine/240 mM Sucrose			0.25 M NaOAc + 5 mg·mL^{-1} GA[a]			0.25 M NaOAc + 0.5 mg·mL^{-1} NAC[b]		
		HMW[c]	Intact radiotracer	LMW[d]	HMW[c]	Intact radiotracer	LMW[d]	HMW[c]	Intact radiotracer	LMW[d]	HMW[c]	Intact radiotracer	LMW[d]
0 h	21	6.0	94.0	0.0	3.5	96.5	0.0	7.5	92.5	0.0	2.6	97.4	0.0
1 d	4	9.9 ± 1.7	89.5 ± 1.6	0.7 ± 0.1	6.5 ± 0.1	93.0 ± 0.1	0.7 ± 0.1	9.5 ± 0.8	87.2 ± 0.8	3.4 ± 0.1	4.2 ± 0.2	95.6 ± 0.1	0.3 ± 0.1
	21	5.3 ± 0.3	93.7 ± 0.2	1.1 ± 0.1	4.8 ± 0.1	94.1 ± 0.2	1.2 ± 0.1	9.2 ± 0.4	84.9 ± 0.3	6.0 ± 0.7	2.1 ± 0.4	97.3 ± 0.2	0.6 ± 0.0
3 d	4	10.9 ± 1.2	88.4 ± 1.3	0.8 ± 0.1	6.7 ± 0.1	92.0 ± 0.1	1.5 ± 0.1	10.1 ± 0.1	84.7 ± 0.1	5.3 ± 0.2	4.3 ± 0.3	95.3 ± 0.3	0.3 ± 0.0
	21	5.8 ± 0.1	92.9 ± 0.1	1.3 ± 0.1	5.3 ± 0.1	92.3 ± 0.3	2.4 ± 0.1	11.6 ± 0.5	77.7 ± 0.6	10.8 ± 1.1	2.1 ± 0.1	97.3 ± 0.0	0.6 ± 0.1
5 d	4	12.0 ± 0.1	87.0 ± 0.2	1.1 ± 0.1	7.4 ± 0.1	90.5 ± 0.1	2.3 ± 0.2	11.8 ± 0.6	82.4 ± 1.0	5.9 ± 1.6	4.7 ± 0.4	95.0 ± 0.4	0.4 ± 0.0
	21	6.8 ± 0.3	91.2 ± 0.2	2.1 ± 0.1	5.9 ± 0.1	89.8 ± 0.5	4.4 ± 0.4	13.1 ± 0.1	73.5 ± 0.8	13.5 ± 0.8	1.9 ± 0.1	97.5 ± 01	0.6 ± 0.0
7 d	4	13.4 ± 0.4	85.3 ± 0.4	1.4 ± 0.1	7.6 ± 0.1	89.5 ± 0.1	3.1 ± 0.1	13.3 ± 0.4	80.8 ± 0.8	6.0 ± 0.5	4.6 ± 0.1	95.1 ± 0.1	0.4 ± 0.0
	21	7.2 ± 0.3	89.6 ± 0.2	3.3 ± 0.1	6.2 ± 0.2	87.5 ± 0.6	6.4 ± 0.4	12.9 ± 0.1	71.5 ± 0.3	15.7 ± 0.1	2.1 ± 0.1	97.2 ± 0.1	0.8 ± 0.1

[a]Gentisic acid. [b]*n*-acetyl-L-cysteine. [c]High-molecular weight, radioactive protein species. [d]Low-molecular weight, radioactive protein species, or unchelated ^{89}Zr.

Supplementary Table S8: Summary of SEC data describing the chronological stability of [^{89}Zr]Zr-DFO-trastuzumab formulated in different buffer-excipient combinations

Time point	Temp. (°C)	Species %											
		0.9 % Saline			20 mM Histidine/240 mM Sucrose			0.25 M NaOAc + 5 mg·mL^{-1} GA[a]			0.25 M NaOAc + 0.5 mg·mL^{-1} NAC[b]		
		HMW[c]	Intact radiotracer	LMW[d]	HMW[c]	Intact radiotracer	LMW[d]	HMW[c]	Intact radiotracer	LMW[d]	HMW[c]	Intact radiotracer	LMW[d]
0 h	21	7.7	92.3	0.0	4.6	95.4	0.0	5.6	94.4	0.0	2.7	97.3	0.0
1 d	4	10.5 ± 1.3	87.7 ± 1.0	1.8 ± 0.4	6.1 ± 0.7	91.2 ± 0.6	2.7 ± 0.1	7.6 ± 0.4	90.2 ± 0.3	2.1 ± 0.1	3.5 ± 0.9	96.3 ± 0.9	0.3 ± 0.0
	21	5.9 ± 0.1	87.4 ± 0.1	6.6 ± 0.1	2.7 ± 0.2	82.7 ± 0.7	14.7 ± 1.1	6.1 ± 0.2	89.9 ± 0.1	4.1 ± 0.4	2.0 ± 0.4	97.6 ± 0.2	0.5 ± 0.0
3 d	4	12.5 ± 0.3	84.9 ± 0.1	2.6 ± 0.3	5.9 ± 0.4	86.6 ± 0.6	7.4 ± 0.1	9.5 ± 0.3	87.5 ± 0.2	2.9 ± 0.1	3.3 ± 0.1	96.5 ± 0.1	0.3 ± 0.0
	21	5.6 ± 0.1	84.9 ± 0.1	9.5 ± 0.1	2.3 ± 0.1	71.3 ± 0.2	26.5 ± 0.4	10.3 ± 0.7	82.2 ± 0.2	7.6 ± 0.6	2.0 ± 0.1	97.5 ± 0.0	0.5 ± 0.1
5 d	4	14.6 ± 0.2	81.4 ± 0.2	4.4 ± 0.1	6.1 ± 0.5	81.6 ± 0.6	12.4 ± 0.2	12.9 ± 0.1	83.8 ± 0.1	3.2 ± 0.1	3.4 ± 0.4	96.2 ± 0.4	0.3 ± 0.0
	21	5.6 ± 0.1	79.5 ± 1.1	14.9 ± 1.1	2.6 ± 0.3	62.6 ± 0.2	34.9 ± 0.1	13.3 ± 0.1	76.2 ± 0.1	10.5 ± 0.2	1.7 ± 0.1	97.6 ± 0.1	0.7 ± 0.1
7 d	4	13.1 ± 0.2	80.3 ± 0.9	6.6 ± 1.0	6.4 ± 0.3	78.4 ± 0.2	15.3 ± 0.1	12.4 ± 0.1	84.1 ± 0.6	3.6 ± 0.6	2.9 ± 0.1	96.7 ± 0.1	0.4 ± 0.1
	21	6.7 ± 0.1	73.8 ± 1.2	19.5 ± 1.4	3.2 ± 0.2	57.1 ± 0.2	39.8 ± 0.1	13.2 ± 0.7	73.9 ± 0.6	12.9 ± 1.2	2.1 ± 0.1	97.1 ± 0.2	0.9 ± 0.1

[a]Gentisic acid. [b]*n*-acetyl-L-cysteine. [c]High-molecular weight, radioactive protein species. [d]Low-molecular weight, radioactive protein species, or unchelated ^{89}Zr.

Supplementary Table S9: Chronological *in vitro* serum stability study data of [^{89}Zr]Zr-DFO -mAbs maintained at 37°C (n = 3 each radiopharmaceutical)

Time point	[^{89}Zr]Zr-DFO-cetuximab		[^{89}Zr]Zr-DFO-trastuzumab	
	% Intact	% Unchelated ^{89}Zr	% Intact	% Unchelated ^{89}Zr
0 h	99.9	0.1	99.9	0.1
1 d	99.5 ± 0.1	0.4 ± 0.1	99.5 ± 0.1	0.5 ± 0.1
3 d	99.2 ± 0.2	0.9 ± 0.1	99.4 ± 0.1	0.6 ± 0.2
5 d	99.1 ± 0.5	1.0 ± 0.6	99.3 ± 0.2	0.7 ± 0.1
7 d	98.9 ± 0.5	1.1 ± 0.3	99.3 ± 0.3	0.7 ± 0.1

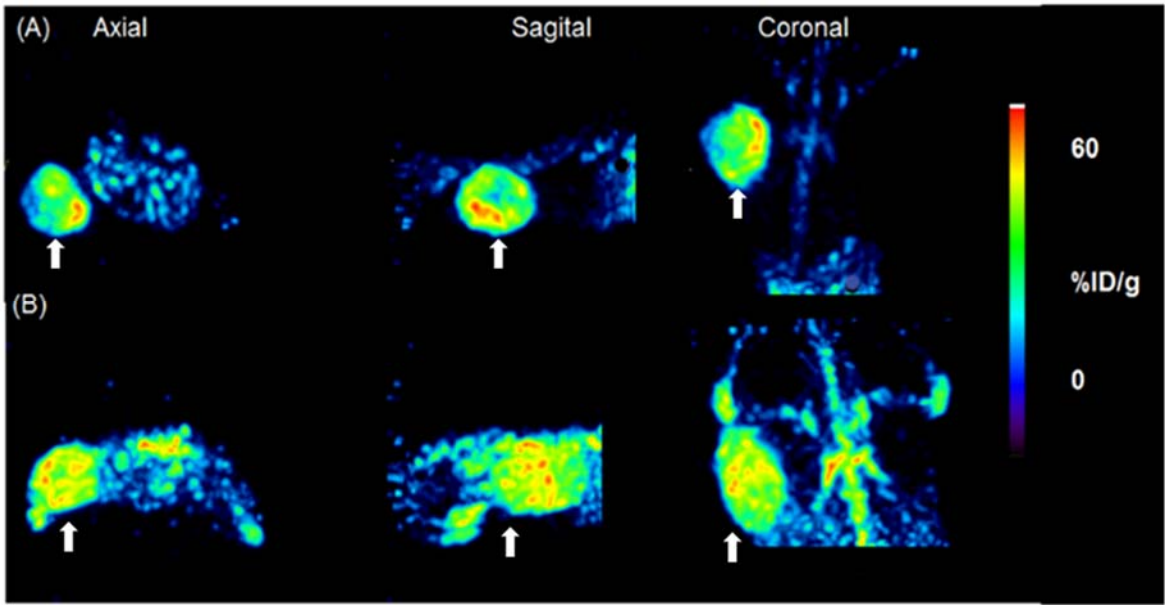


Figure S10. Maximum intensity projection PET images of two mice injected with (A) [^{89}Zr]Zr-DFO-cetuximab or (B) [^{89}Zr]Zr-DFO-IgG. All images were acquired in one bed position and are equally scaled. Tumors are indicated by white arrows.

Supplementary Table S10: Post-PET biodistribution (%ID/g) of [^{89}Zr]Zr-DFO-cetuximab and [^{89}Zr]Zr-DFO-IgG in selected organs at 144 h p.i.

Tissue/Organ	[^{89}Zr]Zr-DFO-Cetuximab	[^{89}Zr]Zr-DFO-IgG
Blood	0.4 ± 0.1	4.5 ± 0.3
Heart	1.2 ± 0.1	2.3 ± 0.1
Lung	2.5 ± 0.2	5.2 ± 0.4
Liver	11.8 ± 0.9	13.7 ± 0.9
Small intestine	3.3 ± 0.5	3.9 ± 0.6
Large intestine	1.4 ± 0.2	1.6 ± 0.2
Kidney	3.2 ± 0.3	4.7 ± 0.3
Spleen	5.4 ± 0.4	6.8 ± 0.4
Pancreas	1.0 ± 0.0	1.5 ± 0.1
Stomach	0.7 ± 0.1	0.8 ± 0.1
Muscle	0.3 ± 0.1	0.5 ± 0.1
Fat	0.7 ± 0.1	0.8 ± 0.2
Bone	12.8 ± 1.7	15.2 ± 1.9
Tumor (+)	35.1 ± 1.8	11.4 ± 1.2

References

1. Vosjan MJWD, Perk LR, Visser GWM, Budde M, Jurek P, Kiefer GE, Van Dongen GAMS. Conjugation and radiolabeling of monoclonal antibodies with zirconium-89 for PET imaging using the bifunctional chelate *p*-isothiocyanatobenzyl-desferrioxamine. Nat. Protoc. 2010; 5: 739-43.
2. Kukis DL, DeNardo GL, DeNardo SJ, Mirick GR, Miers LA, Greiner DP, Meares CF. Effect of the Extent of Chelate Substitution on the Immunoreactivity and Biodistribution of 2IT-BAT-Lym-1 Immunoconjugates. Cancer Res. 1995; 55: 878-84.