Supporting Information for:

Hybrid peptide dendrimers for imaging of chemokine receptor 4 (CXCR4) expression

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General

All chemicals were obtained from commercial sources and used without further purification. The reactions were monitored by thin layer chromatography (TLC). NMR spectra were taken using a Bruker Ultrashield 300 spectrometer (300 MHz ^{1}H NMR, 75 MHz ^{13}C NMR) and the chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS). Abbreviations used include singlet (s), doublet (d), doublet of doublets (dd) and unresolved multiplet (m). MS (ESI) spectra were measured on a Waters LCT^{TM} Orthogonal Acceleration Time of Flight Mass Spectrometer equipped with a Waters 2795 Seperation Module (Alliance HT) and a Waters 2996 Photodiode Array Detector (190 – 750 nm). HPLC was performed on a Waters HPLC system using a 1525EF pump and a 2489 UV detector. For preparative HPLC a Dr. Maisch GmbH Reprosil-Pur 120 C18-AQ 10 μm (250 × 20 mm) column was used and a gradient of 0.1% TFA in H_2O/CH_3CN 9:1 to 0.1% TFA in H_2O/CH_3CN 1:9 in 40 minutes (12 mL/min) was employed. For analytical HPLC a Dr. Maisch GmbH Reprosil-Pur C18-AQ 5 μm (250 × 4.6 mm) column was used and a gradient of 0.1% TFA in H_2O/CH_3CN 95:5 to 0.1% TFA in H_2O/CH_3CN 5:95 in 20 minutes (1 mL/min) was employed.

Molecular modeling

The structures of MSAP label (**1**) (the succinimidyl group was omitted), dimer Glu(β-Ala-OH)-β-Ala-OH and tetramer Glu(β-Ala-Glu(β-Ala-OH)-β-Ala-OH)-β-Ala-Glu(β-Ala-OH)-β-Ala-OH were minimized in Chem3D Pro 12.0 using the MM2 force field (minimum RMS gradient = 0.100) and PDB files of these minimized structures were made. The PDB file of CXCR4 complexed with the Ac-TZ14011 analog CVX15 (PDB entry code 3OE0) was loaded in YASARA (http://www.yasara.org).[1] CXCR4 was deleted and the CVX15 peptide was modified to Ac-TZ14011 (Gln^6 → Cit^6; Lys^7 → Arg^7; D-Pro^8 → D-Lys^8; Gly^{15} and D-Pro^{16} → deleted). Ac-TZ14011 was minimized in YASARA: a simulation cell, in which each axis was extended 5.0 Å from the molecule, was defined. The Amber99 forcefield was used and the temperature control was step-10 annealing, starting from 298 K and at every 10 simulation steps the velocity of all atoms was reduced to 90%. The simulation was stopped after 10 000 fs, when the atoms almost did not move anymore. A PDB file was made of the minimized Ac-TZ14011 peptide.

For the monomer Ac-TZ14011-MSAP (**3**) the PDB files of the minimized Ac-TZ14011 and MSAP label (**1**) were loaded and the MSAP label (**1**) was properly placed with respect to the amine of D-Lys^{8} and a peptide bond was created, yielding a model of monomer Ac-TZ14011-MSAP (**3**).

For the dimer $(Ac\text{-}TZ14011)_2$-MSAP (**7**) the PDB files of the minimized Ac-TZ14011 (2 ×), dimer Glu(β-Ala-OH)-β-Ala-OH and MSAP label (**1**) were loaded and the molecules were properly placed with respect to each other and peptide bonds were created, yielding a model of dimer $(Ac\text{-}TZ14011)_2$-MSAP (**7**).

For the tetramer $(Ac\text{-}TZ14011)_4$-MSAP (**10**) the PDB files of the minimized Ac-TZ14011 (4 ×), tetramer Glu(β-Ala-Glu(β-Ala-OH)-β-Ala-OH)-β-Ala-Glu(β-Ala-OH)-β-Ala-OH and MSAP label (**1**) were loaded and the molecules were properly placed with respect to each other and peptide bonds were created, yielding a model of tetramer $(Ac\text{-}TZ14011)_4$-MSAP (**10**).

For the models of compounds **3**, **7** and **10** binding to CXCR4 (see Figure S1), the PDB files of CXCR4 with the CVX15 peptide (PDB entry code 3OE0) and of compound **3**, **7** or **10** were loaded. An Ac-TZ14011 peptide in compound **3**, **7** or **10** was overlaid onto CVX15 and CVX15 was deleted, yielding models of compounds **3**, **7** and **10** binding to CXCR4.

To estimate the size of compounds **3**, **7** and **10**, the largest distances between two atoms in the models were established. The sizes were 27.5 Å, 48.5 Å and 71.2 Å for compounds **3**, **7** and **10**, respectively.

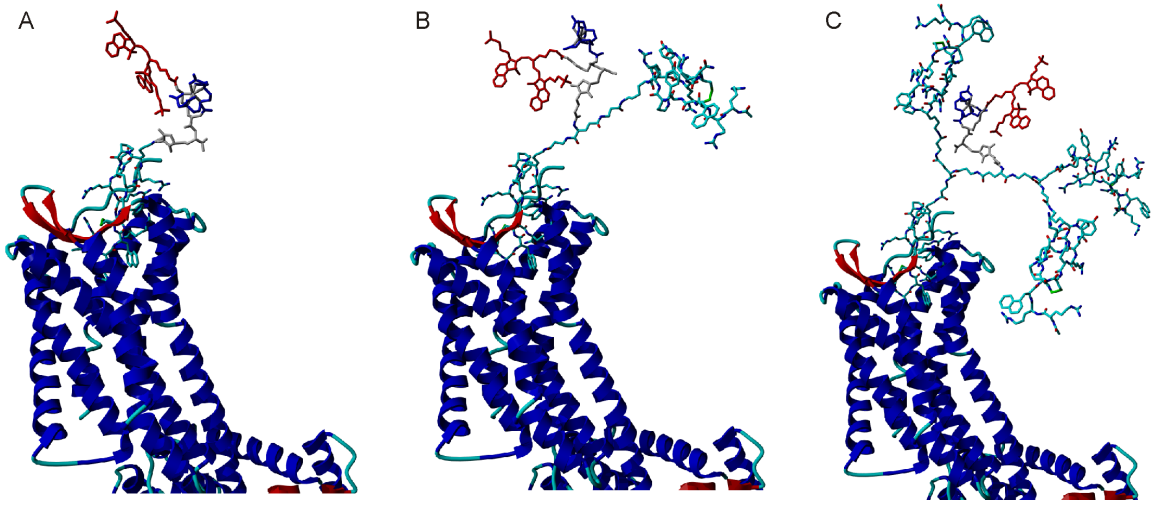

Figure S1. Molecular models of the Ac-TZ14011-MSAP derivatives binding to CXCR4. CXCR4 is shown in ribbon representation and monomer Ac-TZ14011-MSAP (**3**) (A), dimer (Ac-TZ14011)$_2$-MSAP (**6**) (B) and tetramer (Ac-TZ14011)$_4$-MSAP (**10**) (C) as stick models, which is colored by element. For clarity, the MSAP label is not colored by element: the CyAL-5.5$_b$ fluorophore is displayed in red, the indium-bound DTPA chelate in blue and the spacer in grey.

MSAP reagent (1)

The multifunctional single attachment point (MSAP) reagent (**1**) was synthesized based on previously described procedures.[2]

Ac-TZ14011 (2)

Ac-TZ14011 was synthesized according to previously described procedures.[3]

Ac-TZ14011-MSAP (3)

Ac-TZ14011-MSAP was synthesized according to previously described procedures.[2]

Boc-Glu(β-Ala-OH)-β-Ala-OH (4)

Boc-Glu-OH (9.10 g, 36.8 mmol), β-alanine methyl ester hydrochloride (14.0 g, 100 mmol) and N-hydroxysuccinimide (8.60 g, 75 mmol) were dissolved in 500 mL of DMF. DiPEA (17.4 mL, 100 mmol) was added and the solution was cooled to 0 °C. DCC (15.5 g, 75 mmol) was added and the mixture was stirred overnight at 20 °C. DMF was evaporated and H_2O was added. The aqueous phase was extracted with EtOAc (3 ×). The

combined organic phases were washed with 0.1 M Na_2CO_3 (3 ×), 5% citric acid (3 ×) and brine, dried with $MgSO_4$, filtered and concentrated affording 9.8 g (23.5 mmol, 64%) of Boc-Glu(β-Ala-OMe)-β-Ala-OMe. The product was used without further purification.

Boc-Glu(β-Ala-OMe)-β-Ala-OMe (1.47 g, 10 mmol) was dissolved in 105 mL of dioxane and 37.5 mL of MeOH. 7.5 mL of 4 M NaOH (30 mmol) was added and the solution was stirred for 1 hour at 20 °C. The volatiles were evaporated and H_2O was added. The aqueous phase was washed with EtOAc (2 ×), acidified with citric acid and extracted with EtOAc (5 ×). The combined organic phases were washed with 5% citric acid and brine, dried with $MgSO_4$, filtered and concentrated. The product was purified by silica gel column chromatography (1% AcOH in CH_2Cl_2/MeOH 93:7) yielding 2.79 g (72%) of a Boc-Glu(β-Ala-OH)-β-Ala-OH as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ = 1.37 (s, 9H, 3 CH_3), 2.03-2.07 (m, 2H, Glu β CH_2), 2.33-2.39 (m, 4H, Glu δ CH_2 and C*H*$_2$COOH), 2.62 (d, 2H, C*H*$_2$COOH), 3.18-3.28 (m, 4H, 2 C*H*$_2$CH$_2$COOH), 3.82 (dd, 1H, Glu α CH), 6.79 (d, 1H, NH Boc), 7.79-7.86 (m, 2H, 2 β-Ala NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 28.0 (Glu β CH_2), 28.1 (3 CH_3), 31.2 (Glu δ CH_2), 33.8, 33.9, 34.7, 34.7 (4 β-Ala CH_2), 71.8 (Glu α CH), 78.0 (Boc C), 155.1 (Boc CO), 171.2, 171.6 (2 amide CONH), 172.8 (2 COOH).

Boc-protected dimer (Ac-TZ14011)$_2$ (5)

Boc-Glu(β-Ala-OH)-β-Ala-OH (**4**) (1.95 mg, 5 μmol), Ac-TZ14011 (**2**) (35 mg, 12.5 μmol) and BOP (5.53 mg, 12.5 μmol) were dissolved in 2 mL of DMSO. DiPEA (17 μL, 100 μmol) was added and the mixture was stirred overnight at 20 °C, after which the mixture was purified by preparative HPLC. The product was obtained as a white fluffy solid (16.5 mg, 58%) after pooling of the appropriate fractions and lyophilization. MS (ESI): $[M+3H]^{3+}$ calculated 1522.78, found 1522.45; $[M+4H]^{4+}$ calculated 1142.34, found 1142.06; $[M+5H]^{5+}$ calculated 914.07, found 914.08; $[M+6H]^{6+}$ calculated 761.89, found 761.71.

Dimer (Ac-TZ14011)$_2$ (6)

Boc-protected dimer (Ac-TZ14011)$_2$ (**5**) (4.68 mg, 0.844 μmol) was dissolved in H_2O (100 μL) and TFA (2 mL) and the solution was stirred for 3 hours at 20 °C. TFA was

evaporated and the product was lyophilized from CH_3CN/H_2O yielding the product in quantitative yield as a white fluffy solid. MS (ESI): $[M+4H]^{4+}$ calculated 1117.32, found 1117.56; $[M+5H]^{5+}$ calculated 894.06, found 894.25; $[M+6H]^{6+}$ calculated 745.22, found 745.39; $[M+7H]^{7+}$ calculated 638.90, found 639.06.

Dimer (Ac-TZ14011)$_2$-MSAP (7)

Dimer (Ac-TZ14011)$_2$ (**6**) (2.27 mg, 0.397 μmol) and MSAP (**1**) (1.34 mg, 0.793 μmol) were dissolved in 800 μL of DMSO. DiPEA (2.6 μL, 15 μmol) was added and the solution was stirred overnight at 20 °C, after which the mixture was purified by preparative HPLC. The product was obtained as a blue fluffy solid (1.97 mg, 69%) after pooling of the appropriate fractions and lyophilization. MS (ESI): $[M+6H]^{6+}$ calculated 1006.82, found 1006.67; $[M+7H]^{7+}$ calculated 863.13, found 863.19; $[M+8H]^{8+}$ calculated 755.36, found 755.30.

Boc-protected tetramer (Ac-TZ14011)$_4$ (8)

Boc-protected dimer (Ac-TZ14011)$_2$ (**5**) (11.42 mg, 1.46 μmol) was dissolved in H_2O (200 μL) and TFA (2.8 mL) and the solution was stirred for 3 hours at 20 °C. TFA was evaporated and the residue was dissolved in 1 mL of DMSO. DiPEA (5 μL, 29 μmol), Boc-Glu(β-Ala-OH)-β-Ala-OH (**4**) (0.19 mg, 0.50 μmol) and BOP (0.66 mg, 1.5 μmol) were added and the solution was stirred overnight at 20 °C, after which the mixture was purified by preparative HPLC. The product was obtained as a white fluffy solid (2.61 mg, 45%) after pooling of the appropriate fractions and lyophilization. MS (ESI): $[M+8H]^{8+}$ calculated 1161.47, found 1161.76; $[M+9H]^{9+}$ calculated 1032.53, found 1032.73; $[M+10H]^{10+}$ calculated 929.37, found 929.87; $[M+11H]^{11+}$ calculated 844.98, found 845.05; $[M+12H]^{12+}$ calculated 774.65, found 774.64.

Tetramer (Ac-TZ14011)$_2$ (9)

Boc-protected tetramer (Ac-TZ14011)$_4$ (**8**) (2.61 mg, 0.23 μmol) was dissolved in H_2O (100 μL) and TFA (2 mL) and the solution was stirred for 3 hours at 20 °C. TFA was evaporated and the product was lyophilized from CH_3CN/H_2O yielding the product in quantitative yield as a white fluffy solid. MS (ESI): $[M+9H]^{9+}$ calculated 1021.41, found

1021.63; $[M+10H]^{10+}$ calculated 919.37, found 919.50; $[M+11H]^{11+}$ calculated 835.88, found 836.00.

Tetramer (Ac-TZ14011)$_4$-MSAP (10)

Tetramer (Ac-TZ14011)$_4$ (**9**) (2.08 mg, 0.18 μmol) and MSAP (**1**) (0.51 mg, 0.30 μmol) were dissolved in 1 mL of DMSO. DiPEA (10 μL, 57 μmol) was added and the solution was stirred overnight at 20 °C, after which the mixture was purified by preparative HPLC. The product was obtained as a blue fluffy solid (0.97 mg, 50%) after pooling of the appropriate fractions and lyophilization. MS (ESI): $[M+19H]^{19+}$ calculated 567.40, found 568.95; $[M+33H]^{33+}$ calculated 327.08, found 327.86; $[M+34H]^{34+}$ calculated 317.49, found 316.91.

Ac-TZ14011-DTPA (11)

Ac-TZ14011-DTPA was synthesized according to previously described procedures.[3]

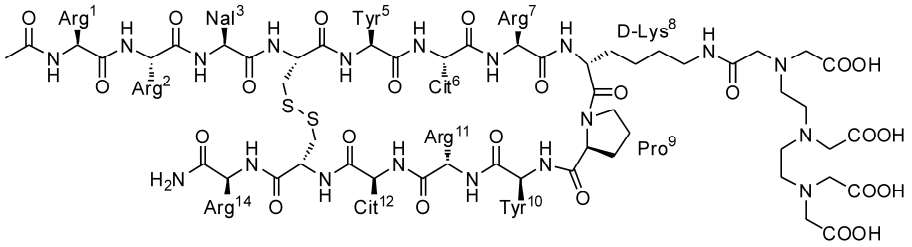

Table S1. Biodistribution of ^{111}In-labeled Ac-TZ14011-DTPA (**11**), MSAP label (**1**), monomer Ac-TZ14011-MSAP (**3**), dimer (Ac-TZ14011)$_2$-MSAP (**7**) and tetramer (Ac-TZ14011)$_4$-MSAP (**10**) in MIN-O tumor bearing mice at 24 h post injecting.

	uptake (%ID/g)				
tissue	**Ac-TZ14011-DTPA (11)**	**MSAP (1)**	**monomer Ac-TZ14011-MSAP (3)**	**dimer (Ac-TZ14011)$_2$-MSAP (7)**	**tetramer (Ac-TZ14011)$_4$-MSAP (10)**
Blood	0.01 ± 0.00***	0.32 ± 0.09*	0.14 ± 0.02	0.14 ± 0.03	0.11 ± 0.01
Brain	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
Lungs	0.13 ± 0.02***	0.65 ± 0.18*	1.02 ± 0.13	2.13 ± 0.60	1.68 ± 0.31
Heart	0.05 ± 0.00**	0.64 ± 0.12	0.89 ± 0.22	0.60 ± 0.12	0.43 ± 0.06*
Liver	5.08 ± 0.52**	5.76 ± 1.11**	22.46 ± 5.46	30.59 ± 5.45	22.43 ± 0.67
Kidneys	27.07 ± 0.74***	6.49 ± 2.99	7.50 ± 1.39	6.46 ± 1.36	4.37 ± 0.52*
Spleen	1.14 ± 0.32**	0.93 ± 0.13**	4.76 ±1.23	7.01 ± 1.45	4.66 ± 0.46
Stomach	0.06 ± 0.01**	0.81 ± 0.45	0.81 ± 0.16	0.97 ± 0.52	1.09 ± 0.52
Intestines	0.10 ± 0.01***	1.02 ± 0.31*	1.85 ± 0.08	2.06 ± 0.26	1.56 ± 0.46
Tumor	0.19 ± 0.03	n.d.	1.10 ± 0.60	0.57 ± 0.19	0.42 ± 0.10
Muscle (paw)	0.03 ± 0.00***	0.22 ± 0.06	0.31 ± 0.01	0.07 ± 0.03***	0.08 ± 0.02***

The significance with respect to ^{111}In-labeled monomer Ac-TZ14011-MSAP (**3**) is indicated by the asterisks (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

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