SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Reagents

All chemicals were purchased commercially and used without further purification. NODAGA-NHS (2,2'-(7-(1-carboxy-4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutyl)-1,4,7-triazonane-1,4-diyl)diacetic acid), NODAGA(tBu)$_3$ (4-(4,7-bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7-triazacyclononan-1-yl)-5-(tert-butoxy)-5-oxopentanoic acid) and NOTA(^{t}Bu)$_2$ (2-(4,7-bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)acetic acid) were purchased from Chematech. $^{64}CuCl_2$ was obtained from the University of Wisconsin, Medical Physics Department. Sodium [18F]fluoride (in water) was purchased from Cardinal Healthcare (Woburn, MA) or from the cyclotron facility at the A. A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital.

High Performance Liquid Chromatography (HPLC)

Preparative HPLC purification was performed on a Varian Prostar system with two Prostar 210 pumps and a Prostar 325 UV/Vis detector, using a Phenomenex Luna C18 column (250x21.2 mm, 10 μm). Liquid chromatography-electrospray mass spectrometry (LC-MS) was performed using an Agilent 1100 Series apparatus with a Phenomenex Luna C18 column (100×2 mm, 5 μm), equipped with an LC-MSD trap and a Daly conversion dynode detector. Radio-HPLC analyses were obtained on an analytical Agilent 1100 Series system using a Phenomenex Luna C18 column (150x4.6 mm, 5 μm; to check radiochemical yields and purity) or a MetaChem Polaris C18 (150x4.6 mm, 5 μm; for analysis of blood samples). Six different HPLC methods were used depending on whether HPLC was being used for purification (Method 1 and 2), for LC-MS

analysis (Method 3), to check radiochemical yields and purity (Method 4 for Cu Probes and Method 5 for AlF probes) or to analyze blood metabolites (Method 6). For Methods 1, 3, 4, 5 and 6 the mobile phase A was H_2O with 0.1% trifluoroacetic acid (TFA) and mobile phase B was CH_3CN with 0.1% TFA. In Method 2 the mobile phase A was replaced by 10 mM ammonium acetate. Method 1 and 2: flow rate of 15 mL/min, gradient = 0 – 5 min, 15% B; 5 – 35 min, 15 to 55% B; 35 – 40 min, 55 to 95% B; 40 – 41 min, 95 to 15% B; 41 – 45 min, 15% B. Method 3: flow rate of 0.8 mL/min, gradient = 0 – 1 min, 5% B; 1 – 10 min, 5 to 95% B; 10 – 12 min, 95% B; 12 – 12.5 min, 95 to 5% B; 12.5 – 15 min, 5% B. Method 4: flow rate of 0.8 mL/min, gradient = 0 – 12 min, 5 to 80% B; 12 - 12.1 min, 80 to 5% B; 12.1 – 13 min, 5% B. Method 5: flow rate of 0.8 mL/min, gradient = 0 – 1 min, 10% B; 1 - 6 min, 10 to 65% B; 6 – 7 min, 65% B; 7 – 8 min, 65 to 10% B; 8 – 10 min, 10% B. Method 6: flow rate of 1 mL/min, gradient = 0 – 17 min, 0 to 100% B; 17 – 17.1 min, 100 to 0% B; 17.1 – 18 min, 0% B.

Synthesis of FHCHypY(3-Cl)DLCHIL-PXD

The linear peptide FHCHypY(3-Cl)DLCHIL-PXD (Pep, Hyp=*L*-4-hydroxyproline, Y(3-Cl)=*L*-3-chlorotyrosine, PXD=*para*-xylenediamine) was synthesized on a 0.5 mmol scale using microwave assisted solid phase peptide synthesis using 1,4-bis-(aminoethyl)-benzene trityl resin (1.3 mmol/g Novabiochem, CA), Fmoc protected amino acids (EMD Chemicals, NJ), and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) coupling chemistry. The protected amino acids were dissolved in dimethylformamide (DMF; 0.2 M); the activator HBTU solution was prepared also in DMF (0.45 M) and the activator base diethylisopropylamine (DIEA) in N-methyl-2-pyrrolidone (NMP; 2 M). The deprotection solution was prepared as a 20% v/v solution of piperidine in DMF with 0.1 M 1-hydroxybenzotriazole (HOBt) hydrate. The synthesis was accomplished using the CEM Liberty Microwave Peptide synthesis system. 384 mg of trityl resin (0.5 mmol) were subjected to the deprotection solvent (7 mL) and irradiated by

microwave at 75°C for 300 seconds. The resin was filtered and washed with DMF (15 mL) and again subjected to another cycle of deprotection (7 mL of 20% piperidine/HOBt; irradiation at 75°C and 20 watts for 300 seconds). The solution was filtered, washed with DMF (4x15 ml) and filtered again. The first Fmoc-protected amino acid was added (10 eq., 25 mL), followed by HBTU (10 eq., 11.1 mL) and finally the base DIEA (10 eq., 2.5 mL). The reaction vessel was heated to 75°C (only 50°C for histidine/cysteine) using microwave irradiation at 20 watts for 300 seconds. The resin was filtered and washed with DMF (3x15 mL). Each amino acid was added sequentially from the C-terminus to the N-terminus using the same procedure. Upon completion of the sequence, the resin was filtered and deprotected with 5 mL of a cocktail of TFA:methanesulfonic acid (MSA, 85% solution):triisopropylsilane (TIS):dithiothreitol (DDT) (86.5:4.5:4.5:4.5) for 2 to 4 hours. The solution was isolated by filtration, triturated with diethylether and centrifuged. The filtrate was removed by decanting, and the pellet dried under high vacuum to remove residual solvents. Analysis of the crude by LC-MS revealed the presence of the desired compound (usually represented as $[M+2H]^{2+}$ or $[M+3H]^{3+}$ ion). The peptide was cyclized using 15% DMSO, 10% acetonitrile, and 75% water (10 mL/mmol crude) over 24 - 72 h. The crude mixture was purified using Method 1. General purities obtained were 90-95%.

Synthesis of $(NODAGA)_2$Pep

The cyclic fibrin-binding peptide FHCHypY(3-Cl)DLCHIL-PXD (Pep; 25 mg, 16.4 μmol) was dissolved in 10 mM ammonium acetate buffer, pH 6, followed by 2.2 eq. of NODAGA-NHS preactivated ester (26 mg, 36.0 μmol) and stirred at room temperature. The reaction was monitored by LC-MS for conversion to the desired product, which was isolated following HPLC purification using Method 1. Lyophilization of the desired peak provided 6 mg of white powder (theoretical MW for $[M+2H]^{2+}$=1121.9, observed 1121.5). Alternatively, $NODAGA(tBu)_3$ (100

mg, 0.184 mmol) was dissolved in DMF (10 mL) and pentafluorophenol (PFP, 1.5 eq., 51 mg) and N-ethyl-N-(3-dimethylaminopropyl)carbodiimide polystyrene (PS-EDC, 1.5 eq., 1.26 mmol/g loading capacity, 219 mg) were added to the reaction. The reaction was monitored by LC-MS and filtered to remove the resin. The activated PFP-ester was added in portions without further purification to the cyclic peptide FHCHypY(3-Cl)DLCHIL-PXD (25 mg, 16.4 μmol) dissolved in DMF (5mL). The pH of the reaction was maintained at ca. 6 with DIEA. After overnight reaction the mixture was triturated with water to give a solid precipitate which was dried under vacuum. The wet solid was deprotected using 10 mL of deprotection cocktail TFA:MSA(85% solution):TIS:DDT (86.5:4.5:4.5:4.5) at room temperature for 3 h. Diethyl ether (20 mL) was added to the mixture forming a white precipitate. Centrifugation formed a pellet, which was dissolved in water and purified by HPLC using Method 1. Lyophilization of the desired peak provided 1 mg of white powder (theoretical MW for $[M+2H]^{2+}$=1121.9, observed 1121.6).

Synthesis of (NOTA–monoamide)$_2$Pep

NOTA(^{t}Bu)$_2$ (100 mg, 0.24 mmol) was dissolved in DMF (10 mL) and pentafluorophenol (PFP, 1.5 eq., 66 mg) and N-ethyl-N-(3-dimethylaminopropyl)carbodiimide polystyrene (PS-EDC, 1.5 eq., 286 mg) were added to the reaction. The reaction was monitored by LC-MS and filtered to remove the resin. The activated PFP-ester was added in portions without further purification to the cyclic peptide (25 mg, 16.4 μmol) dissolved in DMF (5mL). The pH of the reaction was maintained at ca. 6 with DIEA. The reaction was monitored by LC-MS. After 30 h of reaction the mixture was triturated with water to give a solid precipitate. The precipitate was dried under vacuum overnight. The wet solid was deprotected with 10 mL of deprotection cocktail TFA:MSA(85% solution):TIS:DDT (86.5:4.5:4.5:4.5) at room temperature for 3 h. Diethyl ether (20 mL) was added to the mixture forming a white precipitate. Centrifugation formed a pellet that was dissolved in water and purified by HPLC using Method 1. Lyophilization of the desired peak

provided 3 mg of white powder (theoretical MW for $[M+H]^+$=2098.9, observed 2098.3). Alternatively, NOTA(tBu)2 (50 mg, 0.120 mmol) was dissolved in 2-(N-morpholino)ethanesulfonic acid (MES) buffer pH 4.5 (10 mL) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC, 1.5 eq., 35 mg) and N-hydroxysulfosuccinimide (NHS-sulfo, 1.5 eq., 39 mg) were added to the reaction and stirred for 16 h. Mercaptoethanol (1 eq. to EDC, 14 mg) was added to reaction to quench excess EDC. The cyclic peptide (25 mg, 16.4 μmol) was also dissolved in MES buffer (5 mL). The activated ester was added directly to the solution, and the reaction monitored by LC-MS for conversion to the desired product. Conversion was complete with the total addition of ~4 eq. of activated ester. The reaction was frozen and lyophilized to provide the protected peptide. The solid was deprotected using 10 mL of deprotection cocktail TFA:MSA(85% solution):TIS:DDT (86.5:4.5:4.5:4.5) at room temperature for 3 h. Diethyl ether (20 mL) was added to the mixture forming a white precipitate. Centrifugation formed a pellet that was dissolved in water and purified by HPLC using Method 1. Lyophilization of the desired peak provided 3 mg of white powder (theoretical MW for $[M+H]^+$=2098.9, observed 2098.5).

Preparation of the Non-Radioactive FBP Complexes

FBP8. $(NODAGA)_2$Pep ligand (15 mg, 6.7 μmol) was dissolved in 1.47 mL of a 10 mM solution of $CuSO_4$ (2.2 eq., 14.7 μmol). The mixture was heated at 50°C for 30 min. EDTA (1 mL of 10 mM solution) was added and the mixture stirred an additional 20 min to scavenge any excess Cu^{2+}. The compound was purified by HPLC using Method 2 to isolate the bis-copper complex FBP8 as a white powder (13 mg, 5.5 μmol; theoretical MW for $[M+2H]^{2+}$=1182.5, observed 1182.9; theoretical MW for $[M+3H]^{3+}$=788.6, observed 789.0).

FBP9. This compound was prepared as described for FBP8. Starting with 1.5 mg of $(NOTA–monoamide)_2$Pep (0.715 μmol) and 157.4 μL of a 10 mM solution of $CuSO_4$ (2.2 eq., 1.57 μmol) a

white powder was obtained after purification by HPLC using Method 1 (0.6 mg, 0.27 μmol; theoretical MW for $[M+2H]^{2+}$=1111.4, observed 1111.7).

FBP10. $(NODAGA)_2Pep$ (1.6 mg, 0.71 μmol) was mixed with 785 μL of a 2 mM solution of $AlCl_3$ (2.2 eq., 1.57 μmol) and 157 μL of a 50 mM solution of NaF (11 eq., 7.85 μmol) in the same acetate buffer. The reaction mixture was heated for 90 min at 104°C until complete consumption of the ligand was observed by HPLC analysis. The mono-aluminum-fluoride complex was purified by HPLC (Method 1) and isolated as a 30:70 mixture of two isomers (1.5 mg, 0.657 μmol), presumably due to aluminum complexation at either the C-terminal or N-terminal NODAGA. These isomers differed by 0.4 min retention time using Method 3, and resulted in the same mass spectral analysis (theoretical MW for $[M+3H]^{3+}$=762.6, observed 762.7). Efforts to prepare the bis(AlF) complex were unsuccessful.

FBP11. 250 μL of a 2.5 mM solution of $(NOTA–monoamide)_2Pep$ (0.625 μmol) in 0.1 mM NaOAc buffer pH 4.1 were mixed with 687 μL of a 2 mM solution of $AlCl_3$ (2.2 eq., 1.37 μmol) and 62.5 μL of a 50 mM solution of NaF (5 eq., 3.13 μmol) in the same acetate buffer. The reaction mixture was heated for 50 min at 104°C until complete consumption of the ligand was observed by HPLC analysis. The desired bis-complex was purified by HPLC (Method 1) as a 50:50 mixture of two isomers which could not be isolated in a pure form (0.6 mg, 0.274 μmol; MS: theoretical MW for $[M+2H]^{2+}$=1093.8, observed 1093.6). The formation of coordination isomers for other aluminum-fluoride complexes has been reported in the literature and it may arise from position of the fluoride ligand with respect to the N and O donors of the NOTA ligand (*(1, 2)*).

Radiochemistry

^{64}Cu-labeling. A 50 μL aliquot of a 1 mg/mL solution of $(NODAGA)_2Pep$ or $(NOTA–monoamide)_2Pep$ (23 nmol) was combined with a solution of $^{64}CuCl_2$ (10 mCi) in NaOAc (10 mM, pH 5.5, total volume 450 μL). After reaction at 50°C for 30 min the reaction was monitored

by analytical HPLC. Under these conditions FBP8 and FBP9 were obtained in quantitative yields, at a ligand concentration of 50 μM and with specific activities of 0.21-0.43 mCi/nmol.

^{18}F-labeling. A Sep-Pak Light Accell Plus QMA cartridge (130 mg, Waters) was washed with 5 mL of 0.4 M $KHCO_3$ followed by 10 mL of deionized water. ^{18}F fluoride, received in 2 mL of water, was loaded onto the cartridge. The cartridge was washed with 4 mL of deionized water and the ^{18}F eluted from the cartridge in 100 μL fractions with 0.4 M $KHCO_3$. Most of the activity was in the second and third fractions. The pH of the solution was adjusted to 4.1 with 5 μL of glacial acetic acid/100 μL of solution. The (NOTA–monoamide)$_2$-Pep ligand (15 μL of 2.5 mM solution, 37.5 nmol), dissolved in 0.1 M acetate buffer pH 4.1, was mixed with $AlCl_3$ (3 μL of a 2 mM solution of $AlCl_3 \cdot 6H_2O$ prepared in 0.1 M acetate buffer pH 4.1), 75 μL of Na^{18}F (25 mCi), and 25 μL of DMF. The pH of the reaction solution was measured using a microelectrode (Microelectrodes, Inc.) and carefully adjusted to 4.1 with acetic acid. After heating at 104°C for 15 min in a sealed vial the radiochemical yield was ascertained by radio-HPLC. A labeling yield of 81±5% was obtained using a peptide concentration of 317 μM. In the case of (NODAGA)$_2$Pep the highest labeling yield (57±6%) was obtained using the same conditions but replacing DMF for ethanol. The crude products were applied on a Sep-Pak C18 Plus Light Cartridge (130 mg, Waters) to remove unincorporated ^{18}F and Al^{18}F. The cartridges were washed with 4 mL of water and the labeled peptides eluted with 2 mL of ethanol. Before *in vivo* studies the peptides were diluted with 0.9% NaCl and the ethanol solution removed by rotary evaporation. FBP10 and FBP11 were obtained with high radiochemical purity determined by radio-HPLC (>97%) and with specific activities of 0.30-0.36 mCi/nmol. The effect of the pH, concentration of the peptide and $AlCl_3$, reaction time, total volume, and organic solvent on the labeling yield were investigated and the results are summarized in Supplemental Table 1 and 2.

In vitro stability of probes in bovine serum and to a NOTA challenge

A 100 μL aliquot of each probe (100 μCi) was incubated in 100 μL of bovine serum at 37°C for 1, 2, 4 and 18 h for copper probes and 1 and 3 h for aluminum-fluoride probes. After incubation the mixture was filtered through a 0.22-μm Millipore filter and analyzed by radio-HPLC. All probes were highly stable under these conditions (≥97% intact).

For the NOTA challenge, 100 μL aliquots of FBP10 and FBP11 (100 μCi, 37.5 nmol) were added to 20 μL of a 10 mM solution of free NOTA chelator. The resulting solutions were incubated at 37°C for 30 min and at 104°C for 5, 10, 15, 20 and 30 min. After incubation the percentage of intact probe was determined by radio-HPLC analysis (Supplemental Table 3). There was no evidence of transchelation observed for FBP10 and FBP11 after 30 min at 37°C. However, when incubated at 104°C, complete transchelation was observed within 30 min for both probes (Supplemental Table 3). At short incubation times the NOTA–monoamide-containing probe (FBP11) showed greater inertness than the NODAGA containing probe (FBP10). For example, 31% and 60% of the intact FBP10 and FBP11 were found at 5 min of incubation at 104°C, respectively (Supplemental Table 3). The higher degree of exchange found for FBP10 is in accordance with the low labeling efficiencies found for this probe and its lower *in vivo* stability.

In vivo Stability

Blood samples collected at 2, 15, 120 and 240 min were immediately centrifuged for 10 min at 2000 rpm and the plasma passed through a 0.22-μm Millipore filter and injected onto an analytical HPLC column. The eluent was collected every 0.5 min, and the activity of each fraction was measured by the gamma counter. The HPLC analysis was performed in duplicate. Data obtained from the gamma counter were plotted to reconstruct the HPLC chromatograms.

Determination of K_i

The affinity of the probes was assessed using a DD(E) fluorescence polarization displacement assay that was described previously (*(3)*). The displacement of a tetramethylrhodamine labeled peptide (TRITC-Tn6) from DD(E) was detected by observing the corresponding change in fluorescence anisotropy. The K_d of the TRITC-Tn6 probe was determined by titrating it with the DD(E) protein and fitting the resultant fluorescence data as described previously (*(3)*). This experiment was performed at room temperature using a concentration of TRITC-Tn6 of 0.1 μM in the following assay buffer: Tris base (50 mM), NaCl (100 mM), $CaCl_2$ (2 mM), Triton X- 100 (0.01%), pH = 7.8. The anisotropy measurements were made using a TECAN Infinity F200 Pro plate reader equipped with the appropriate filter set for tetramethylrhodamine (excitation 535 nm, emission 590 nm). A series of solutions of the ^{63,65}Cu- and Al^{19}F-probes were prepared through serial dilutions (25 to 0.1 μM). These solutions were then added to a mixture of the DD(E) protein and TRITC-Tn6 peptide. The final concentrations of protein and fluorescent probe used in these experiments were 2 μM and 0.1 μM, respectively. All measurements were performed at room temperature in a 384-well plate from Greiner Bio One. The inhibition constants, K_i of the ^{63,65}Cu- and Al^{19}F-peptides were then calculated using least-squares regression and the known K_d of the fluorescent probe (Supplemental Table 4), as described previously (*3*). Concentrations were determined by analyzing for ^{65}Cu or ^{27}Al by inductively coupled plasma-mass spectrometry.

Functional Fibrin Binding Assay

Human fibrinogen (American Diagnostica) was dialyzed against 50 mM Tris, pH 7.4, 150 mM sodium chloride, 5 mM sodium citrate (TBS·citrate) prior to use. The fibrinogen concentration was adjusted to 5 mg/mL (based on the absorbance at 280 nm and ε_{280} = 1.512 $Lg^{1-}cm^{-1}$), and $CaCl_2$ was added (7 mM). The fibrinogen solution (50 μL) was dispensed into the wells of a 96-well polystyrene microplate (Immulon-II). A solution (50 μL) of human thrombin (2 U/mL) in TBS was added to each well to clot the fibrinogen and to yield a final fibrin concentration close to

2.5 mg/mL (7.3 μM based on fibrinogen MW = 340 kDa). The plates were incubated at 37°C and evaporated to dryness overnight. Blood tubes were centrifuged (2000 rpm for 20 min at 4°C), and then plasma incubated in fibrin immobilized wells as well as in empty wells, and the sealed plate incubated for 2 h on a shaker at 300 rpm and room temperature. The plate was sealed with tape to prevent evaporation and agitated at 300 rpm on a shaker. After incubation, the counts in the supernatant in both the fibrin-containing and empty wells were measured on a gamma counter and divided by the weight of plasma to determine the concentration of unbound probe, [unbound], and total probe, [total], respectively. The amount of ^{64}Cu and Al^{18}F containing species bound to fibrin, [bound], was calculated from [bound] = [total] − [unbound].

As a positive control, an aliquot of the dose was spiked into blood plasma and used to estimate the total possible fibrin binding by each FBPs in the assay (% bound at t = 0). The amount of functional probe in the blood at time t was determined by taking the ratio of the % bound to fibrin at time t compared to the % bound at t = 0, and multiplying this ratio by the measured total %ID/g in the blood.

Histology

Ipsilateral and contralateral common carotid arteries were harvested 120 min after crush injury, carefully rinsed in Phosphate Buffer, embedded in OCT mounting media (Tissue-Tek), and snap-frozen in -45°C isopentane. Arteries were cryosectioned (20 μm thickness) and processed for Hematoxylin and Eosin staining according to the standard protocol. Images were acquired using a Nikon TE-2000 microscope (40X magnification).

Supplemental Table 1. Radiochemical yield for FBP10 under different conditions determined by radio-HPLC analysis of the crude reaction mixtures.

Entry #	$AlCl_3$ [μM]	$NODAGA_2Pep$		Organic solvent	Final pH	Final volume	Time (m) at 104 °C	Yield %
		nmol	[μM]					
1	51	37.5	317	-	4.1	118	15	2
2	51	62.5	530	-	4.1	118	15	3
3	51	37.5	317	25 μL DMF	4.1	118	45	14
4							30	24
5	51	37.5	317	25 μL DMF	3.7	118	15	28
6					4.1			27
7					4.3			27
8	51	37.5	317	50 μL DMF	4.1	118	15	15
9	25.5			50 μL DMF				11
10	51	37.5	317	50 μL EtOH	4.1	118	15	57 ± 6
11			551	25 μL EtOH		68		50
12	51	62.5	530	50 μL EtOH	4.1	118	15	52

Supplemental Table 2. Radiochemical yield for FBP11 under different conditions determined by radio-HPLC analysis of the crude reaction mixtures.

Entry #	$AlCl_3$ [μM]	(NOTA–monoamide)$_2$Pep		Organic solvent	Final pH	Final volume	Time (m) at 104 °C	Yield %
		nmol	[μM]					
1	51	37.5	317	-	4.1	118	15	5
2					4.1			54
3	51	37.5	317	50 μL EtOH	4.3	118	15	41
4					3.7			39
5					3.7			69
6	51	37.5	317	50 μL DMF	4.1	118	15	75
7					4.3			81
8	51	37.5	317	10 μL DMF	4.1	118	15	41
9				25 μL DMF				81 ± 5
10	51	37.5	107	25 μL DMF	4.1	393	15	37
11			551			68		68
12	51	37.5	317	50 μL DMSO	4.1	118	15	70

Supplemental Table 3. Challenging FBP10 and FBP11 with a 5-fold excess of NOTA. Percentage of intact probe was ascertained by radio-HPLC.

Incubation time (min)	% of intact probe at 37 °C		% of intact probe at 104 °C	
	FBP10	FBP11	FBP10	FBP11
5	-	-	31	60
10	-	-	16	39
15	-	-	12	-
20	-	-	-	3.3
30	100	100	0	2.2

Supplemental Table 4. K_i values (nM) determined for fibrin binding peptides through the competitive displacement of the peptide TRITC-Tn6 from DD(E). EP-2104R was used as positive control (*(4)*).

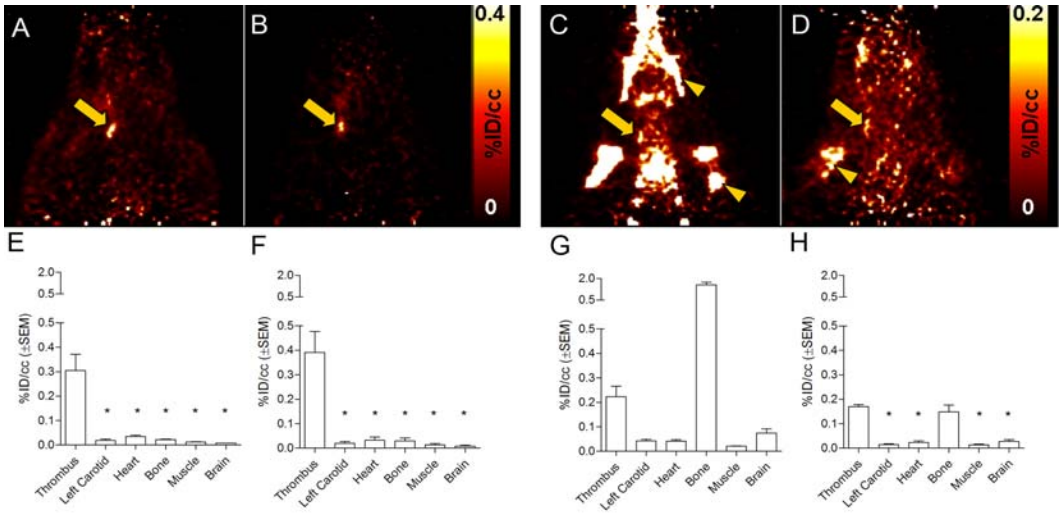
EP-2104R	FBP8	FBP9	FBP10	FBP11
n=4	n=2			
410 ± 69	435 ± 63	480 ± 14	760 ± 42	735 ± 49

Supplemental Table 5. Biodistribution data (%ID/g ± SEM) at 120 min post injection.

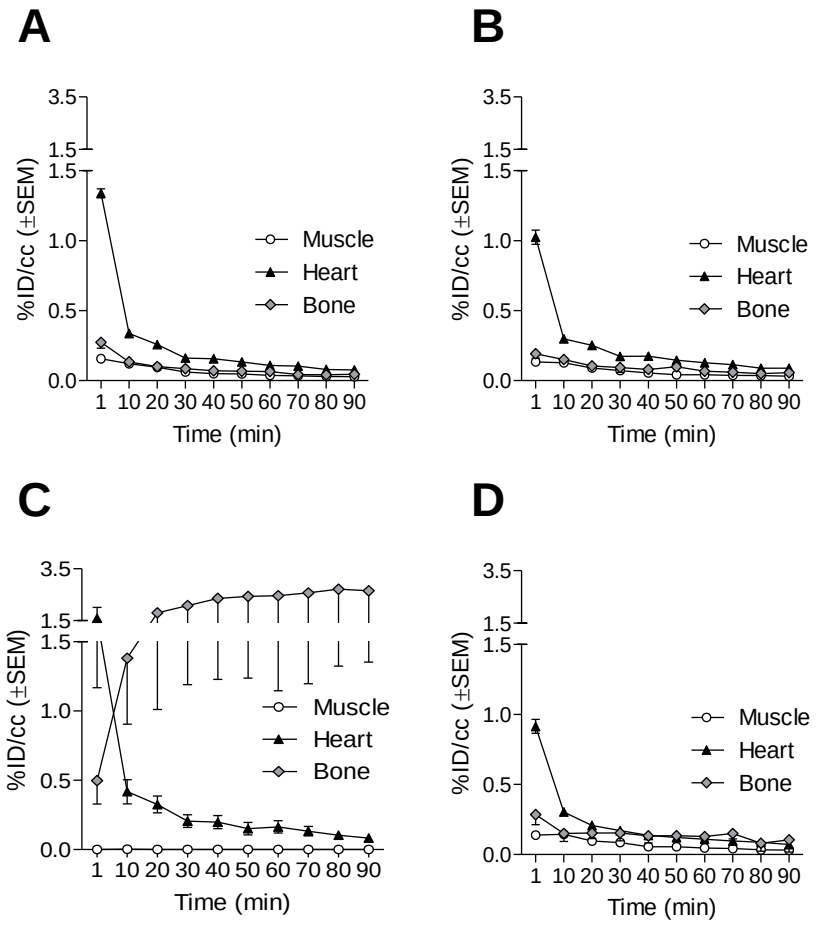
Tissue	FBP8	FBP9	FBP10	FBP11
Thrombus	0.795 ± 0.050	0.814 ± 0.178	0.428 ± 0.077	0.592 ± 0.139
Left Carotid	0.144 ± 0.021	0.166 ± 0.034	0.097 ± 0.007	0.198 ± 0.032
Liver	0.399 ± 0.066	0.296 ± 0.083	0.116 ± 0.032	0.558 ± 0.121
Kidney	5.732 ± 0.482	4.912 ± 0.965	3.246 ± 0.298	2.543 ± 0.340
Heart	0.039 ± 0.004	0.051 ± 0.010	0.018 ± 0.002	0.024 ± 0.004
Lungs	0.063 ± 0.002	0.102 ± 0.016	0.041 ± 0.002	0.082 ± 0.010
Spleen	0.126 ± 0.016	0.089 ± 0.011	0.035 ± 0.006	0.204 ± 0.053
Intestine	0.102 ± 0.032	0.187 ± 0.038	0.056 ± 0.008	0.480 ± 0.051
Muscle	0.065 ± 0.021	0.088 ± 0.032	0.056 ± 0.037	0.094 ± 0.035
Bone	0.042 ± 0.005	0.039 ± 0.010	1.435 ± 0.302	0.138 ± 0.015
Brain	0.005 ± 0.001	0.007 ± 0.001	0.012 ± 0.006	0.005 ± 0.0005
Blood	0.058 ± 0.004	0.085 ± 0.005	0.038 ± 0.002	0.048 ± 0.009
n	7	5	3	5

Supplemental Table 6. Biodistribution data (%ID/g ± SEM) at 300 min post injection.

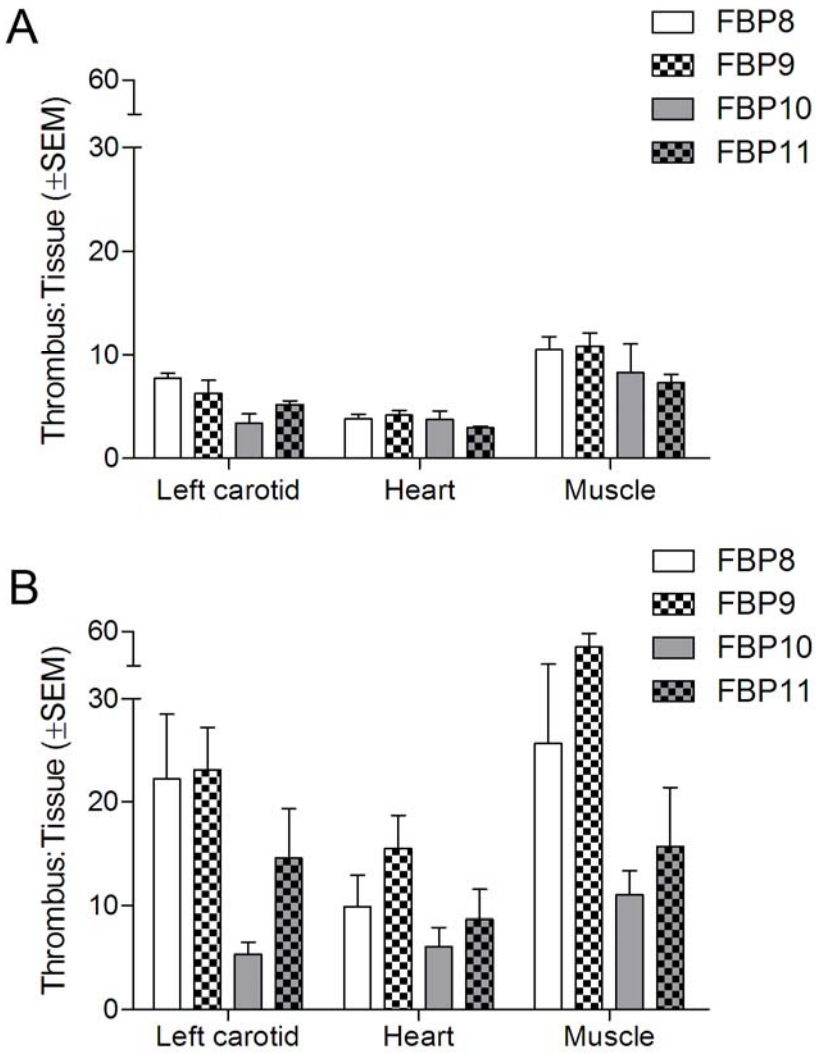
Tissue	FBP8	FBP9	FBP10	FBP11
Thrombus	0.415 ± 0.101	0.498 ± 0.056	0.298 ± 0.081	0.208 ± 0.069
Left Carotid	0.091 ± 0.029	0.040 ± 0.011	0.057 ± 0.015	0.036 ± 0.011
Liver	0.238 ± 0.040	0.144 ± 0.040	0.094 ± 0.031	0.277 ± 0.032
Kidney	6.113 ± 0.688	7.258 ± 1.297	3.446 ± 0.517	1.848 ± 0.133
Heart	0.030 ± 0.005	0.021 ± 0.006	0.014 ± 0.003	0.007 ± 0.001
Lungs	0.038 ± 0.007	0.046 ± 0.013	0.034 ± 0.009	0.028 ± 0.006
Spleen	0.071 ± 0.013	0.037 ± 0.012	0.046 ± 0.012	0.086 ± 0.014
Intestine	0.082 ± 0.031	0.082 ± 0.039	0.097 ± 0.062	0.067 ± 0.024
Muscle	0.035 ± 0.021	0.038 ± 0.028	0.097 ± 0.045	0.061 ± 0.033
Bone	0.036 ± 0.006	0.023 ± 0.009	1.553 ± 0.257	0.128 ± 0.018
Brain	0.003 ± 0.0004	0.003 ± 0.001	0.006 ± 0.001	0.002 ± 0.0003
Blood	0.040 ± 0.007	0.035 ± 0.012	0.027 ± 0.009	0.012 ± 0.003
n	5	5	3	4



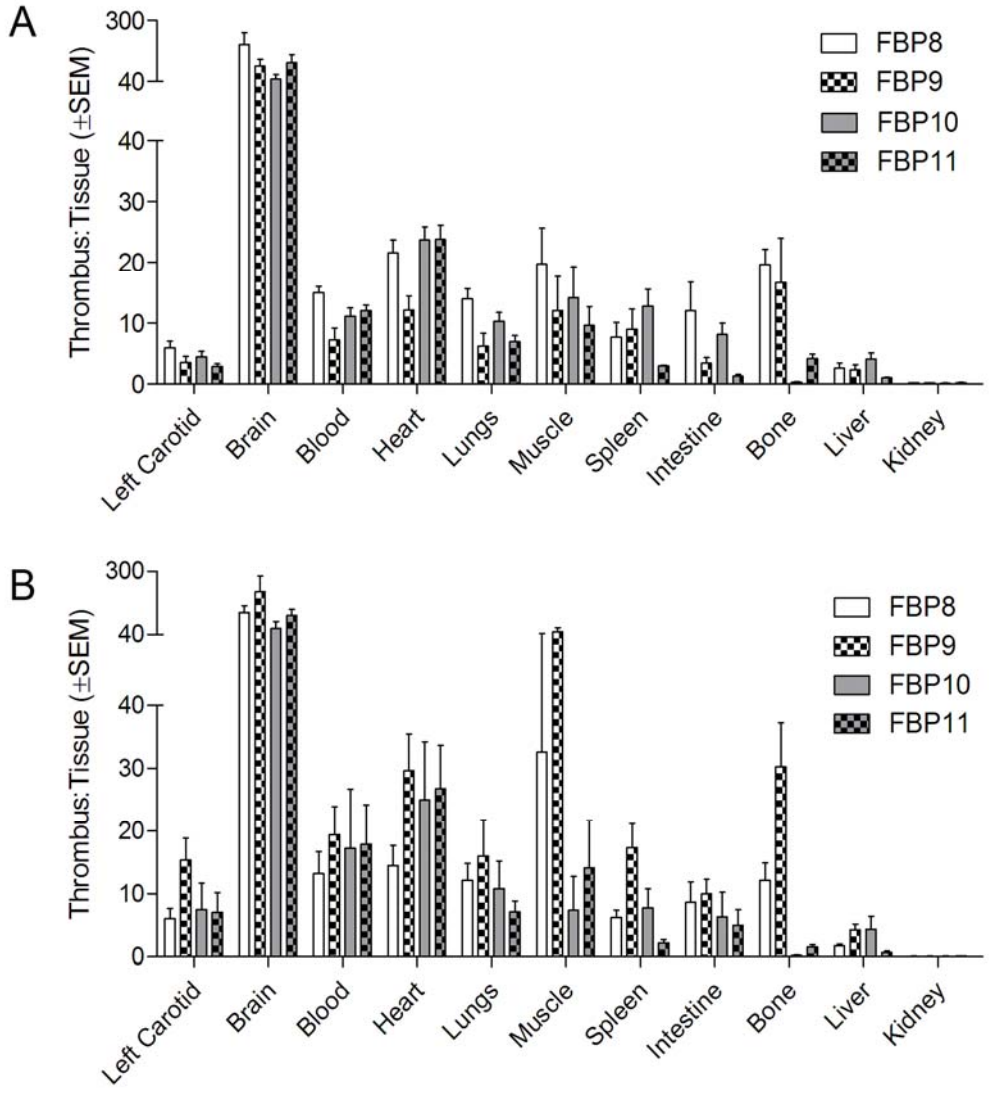
Supplemental Fig. I. Side-by-side comparison of imaging properties of FBP8 (A, n=5), FBP9 (B, n=5), FBP10 (C, n=3) and FBP11 (D, n=4) and relative PET activity values (%ID/cc) in the thrombus, left carotid, heart, bone, muscle and brain (E-H, data from 240-285 min post-injection). All images are on the same scale. Bone is off scale. Arrow, thrombus; arrowhead, bone uptake. * $p < 0.001$, vs. Thrombus.



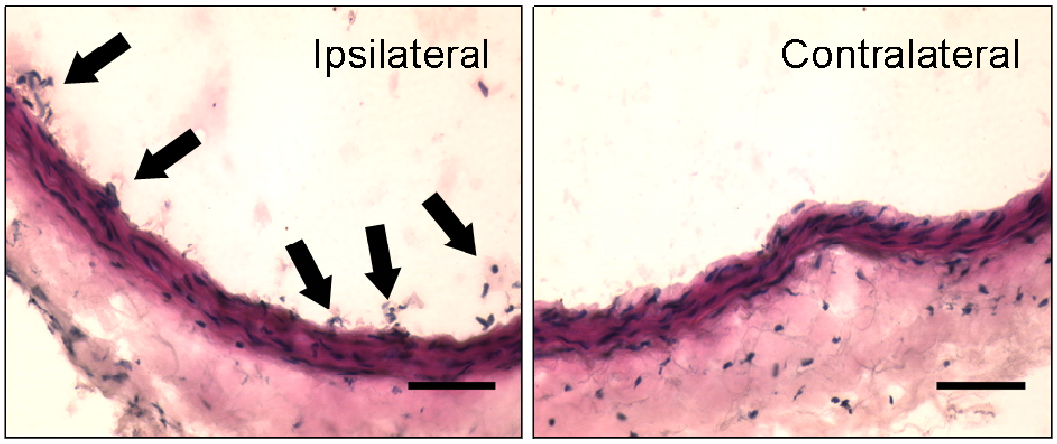
Supplemental Fig. II. Muscle, heart and bone time-activity curves FBP8 (A, n=7), FBP9 (B, n=5), FBP10 (C, n=3) and FBP11 (D, n=5) at 30-90 min post-injection. The bone time-activity curves for these radiotracers showed a sustained washout of the radioactivity in the case of FBP8, FBP9 and FBP11, but an increase in the bone radioactivity for FBP10, indicative of some degree of defluorination.



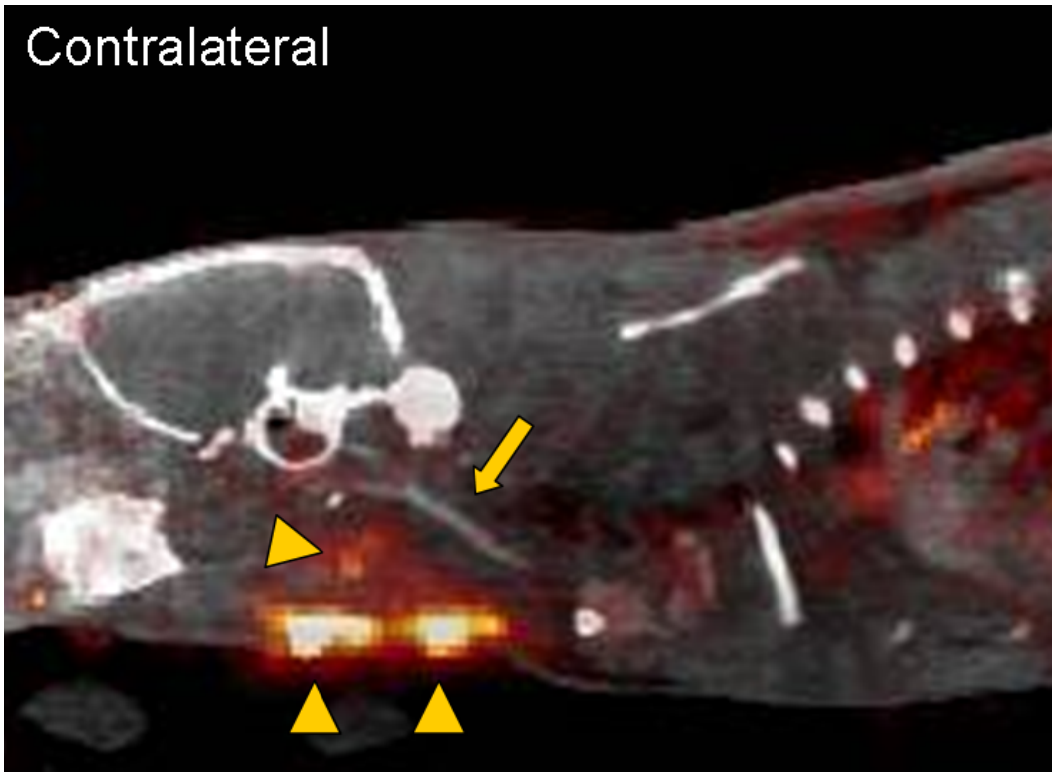
Supplemental Fig. III. Thrombus:tissue ratios from PET data at 30-90 min (A, n=3-7/group) and 240-285 min (B, n=3-5/group) post-injection.



Supplemental Fig. IV. Thrombus:tissue ratios from biodistribution data at 120 min (A, n=3-7/group) and 300 min (B, n=3-5/group) post-injection.



Supplemental Fig. V. Hematoxylin and Eosin staining (n=2) reveals mural thrombi at the level of endothelial cells layer after crush injury, but not in contralateral artery. This finding was consistent with previously reported models of mural thrombosis (*5*). Arrows, mural thrombi. Scale bars, 50 μm.



Supplemental Fig. VI. Fused PET-CT sagittal view of an animal injected with FBP8. The contralateral common carotid artery and the surgical wound are easily distinguishable, although closely located. The wound uptake did not affect the quantification at the level of the carotid artery. Arrow, contralateral, uninjured, common carotid artery; arrowheads, surgical wound.

SUPPLEMENTAL REFERENCES

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