Supporting Information

Materials and Methods

Reagents were purchase from Sigma-Aldrich Co. and used without further purification unless otherwise noted. [^{18}F]-Fluoride (n.c.a.) in [^{18}O]-enriched water was purchased from PETNET (Woburn, MA). Solid phase extraction cartridges were purchased from Thermo (HYPERSEP C18, 500 mg, 3 mL). Proton and carbon nuclear magnetic resonance (1H & 13C NMR) spectra were recorded on a Varian AS-400 (400MHz) spectrometer. [^{1}H] chemical shifts are referenced to residual deuterated dimethyl sulfoxide (DMSO-d6; 2.50 ppm), and 13C chemical shifts are referenced to DMSO-d6 (39.52 ppm). Silica Gel 60 (40-63 µm) was used for purification. High performance liquid chromatography (HPLC) analysis was performed using a Waters XTerra RP C8 5 µm, column (95:5:0.1% v/v/v water/ acetonitrile/formic acid). Preparative HPLC purification (HPLC Method A) was achieved using a Machery-Nagel Nucleodur C18 Pyramid 250 x 10 mm Vario-Prep column (80:20 v/v, in water:acetonitrile (MeCN) with 0.1 % trifluoroacetic acid at 5.5 mL·min^{-1}). Analytical HPLC (HPLC Method B) was performed using a Grace Vydac (218TP510) C18 reversed-phase column (eluents: A = 0.1% trifluoroacetic acid (v/v) in water and B = MeCN; gradient: 0-17 minutes, 5-60% B; 17-21 minutes, 60-95% B; 21-24 minutes, 95% B; 24-25 minutes, 95-5% B; 25-30 minutes, 5% B; 2 mL·min^{-1}). Size-exclusion chromatography (SEC) employed a Superdex 200 column (eluent: 50 mM phosphate buffer, 150 mM NaCl, pH 7.0; 0.5 mL·min^{-1}) with a dual-wavelength UV-Visible detector followed by a flow-through gamma detector, connected in series. Preparative and analytical HPLC and SEC analyses of [^{18}F]-labeled compounds were calibrated with their corresponding [^{19}F] analogs. Radio-thin layer chromatography (radio-TLC) was performed on silica impregnated glass sheets (ITLC) (Pall Life Sciences) and analyzed using a Bioscan AR-2000 scanner operated by WinScan V3 software package. Microwave synthesis was conducted in a CEM (Matthews, North Carolina) Microwave Synthesizer, operated with a Discover® software package. [^{18}F]-Fluoride (n.c.a.) in [^{18}O]-enriched water was dried using a Synthra RN Plus automated synthesizer (Synthra GmbH, Hamburg, Germany) operated by SynthraView software in an average time of 18 minutes. The target well was charged with [^{18}F]-F^-, n.c.a., (1110 MBq, 30 ± 10 mCi) in [^{18}O]-water (150 µL), 250 µL of a 75 mM tetrabutylammonium bicarbonate (TBAB) solution in water, and 200 µL of MeCN. The synthesizer reagent vials were filled as follows: A2 with 350 µL MeCN, A3 with 400 µL MeCN, and A5 with 50 µL MeCN. The [^{18}F]-F^-/TBAB solution was then transferred to Reaction Vessel #1 and dried via azeotropic distillation of the acetonitrile/water solution by first heating to 60°C (under reduced pressure and a flow of argon to achieve 310 mbar) for 2 minutes, followed by heating to 98°C (and 270 mbar) for 4 minutes and finally to 98°C (and 90 mbar) for 1 minute. The dried [^{18}F]/TBAB mixture was reconstituted in MeCN (400 µL) at 90°C for 5 minutes and eluted into a collection vial to be aliquoted for subsequent labeling experiments.

1,3,5,7,8-Pentamethyl BODIPY **2,** 1,3,5,7,8-Pentamethyl DMAP-BODIPY triflate **3** and 1,3,5,7-Tetramethyl-8-propionic BODIPY succinimidyl ester **5** were synthesized following literature reported procedures. (T. W. Hudnall, F. P. Gabbaï, *Chemical Communications* **2008**, 4596.; D. Wang, J. Fan, X. Gao, B. Wang, S. Sun, X. Peng, *J. Org. Chem.* **2009**, *74*, 7675.)

Synthesis of 4-[^{18}F]-1,3,5,7,8-Pentamethyl BODIPY [^{18}F]2

Method A: 4-Fluoro-4-dimethylaminopyridine-1,3,5,7,9-pentamethyl-3a,4a-diaza-4-bora-*s*-indacene (0.2 µmol) in 10 µL dimethylformamide was treated with 20-30 µL of aqueous [^{18}F]-F$^-$ (n.c.a., 5.4 (199.8 MBq) ± 3.8 mCi, n = 3) and heated (microwave) to 100°C for 10 minutes. A small aliquot was removed for HPLC analysis (HPLC Method B); this demonstrated 67.6 ± 22.9 % radiochemical yield of 4-[^{18}F]-1,3,5,7,8-pentamethyl BODIPY; t_R ([^{18}F]-F$^-$) = 4.4 minutes; t_R (4-[^{18}F]-1,3,5,7,8-pentamethyl BODIPY) = 17.5 minutes. The decay-corrected radiochemical yield following HPLC purification was 18.3 ± 2.5%.

Method B: In separate reactions, 4,4-difluoro-1,3,5,7,8-pentamethyl-BODIPY (0.25 µmol) in 25 µL 2:1 MeCN:dichloromethane (DCM) was treated with 5 µL of 100, 150, 200, 250, and 500 mM solutions of TMS-OTf (2, 3, 4, 5, and 10 mol equivalents, respectively) in MeCN. These mixtures were allowed to react at 0°C for 10 minutes before being treated with 15 µL azeotropically dried ^{18}F /TBAB (1.8 (66.6 MBq) ± 0.2 mCi) solution in MeCN. After stirring at room temperature for 4 minutes, each reaction was analyzed by radio-TLC (10:1 MeCN:Toluene, ITLC); R_f ([^{18}F]-F$^-$) = 0.0; R_f (4-[^{18}F]-1,3,5,7,8-pentamethyl BODIPY) = 0.91.

Table 1: Radiochemical yields as function of trimethylsilyl triflate concentration

#	BODIPY µmol	TMS-OTf µmol	^{18}F mCi	TBAB µmol	RCY %
1	0.25	2.50	2.00	0.70	41.1
2	0.25	1.25	1.97	0.70	53.2
3	0.25	1.00	1.72	0.70	58.0
4	0.25	0.75	1.70	0.70	21.2
5	0.25	0.50	1.61	0.70	6.3

Method C: : At room temperature 5 µL of a 250 mM solution (1.25 µmol, 5 mol equivalents) trimethylsilyl triflate in acetonitrile were added to 0.25 µmol 4,4-Difluoro-1,3,5,7,8-pentamethyl-bodipy **2** in 25 µL acetonitrile/ dichloromethane (2:1). After 1 min *tert*-butanol (5 µL, 250 mM in acetonitrile) was added to quench residual trimethylsilyl triflate, followed by 2,6-lutidine (5 µL, 250 mM in acetonitrile) to stabilize the BODIPY-triflate **4** intermediate.
An aliquot of this solution (see table 2) was combined with a solution of azeotropically dried ^{18}F/TBAB (15 µL, 1.8 (66.6 MBq) ± 0.3 mCi) in acetontitrile, that had been pretreated with triflic anhydride (10 µL, 100 mM in acetonitrile) to remove residual water, followed by *tert*-butanol (5 µL, 250 mM in acetonitrile) to quench excess triflic anhydride and to acidify the reaction mixture (as result of the generated triflic acid). The reaction was complete after stirring for 1 min at room temperature. Radiochemical yield was determined by radio-HPLC and radio-TLC (10:1 acetonitrile:toluene, ITLC; R_f (^{18}F$^-$) = 0.0; R_f (4-[^{18}F]-1,3,5,7,8-pentamethyl bodipy **[^{18}F]2**) = 0.91)

Table 2

	Bodipy Activation					Fluorination				Analysis		
#	BODIPY [µmol]	TMS-OTf [µmol]	Time [min]	tBuOH [µmol]	2,6-Lutidine [µmol]	^{18}F$^-$ [mCi]	Tf_2O [µmol]	tBuOH [µmol]	Time [min]	%RCY (HPLC)	%RCY (TLC)	Spec. Activity [Ci/µmol]
1	0.25	1.25	1	1.25	1.25	2.07	1	1.25	1	37	42.1	0.07
2	0.05	0.25	1	0.25	0.25	1.76	1	1.25	1	37.3	40.6	0.34
3	0.01	0.05	1	0.05	0.05	1.51	1	1.25	1	66.7	67.9	0.96

Monitoring of BODIPY activation

To monitor the extend of activation of BODIPY **2** and to determine the purity of the activation reaction 10µL aliquots of the respective reaction mixture were taken, quenched in 200 µL methanol (containing 1% of diisopropylethyl amine) and analyzed by LC/MS. The BODIPY triflate **4** reacts instantaneously with methanol to form the corresponding methoxy-BODIPY analog, which served as surrogate for the amount of **4**. The extend of activation was determined based on the integrated peaks (at 490nm) of the BODIPY starting material and the methoxy-BODIPY analog (see Figure S1).

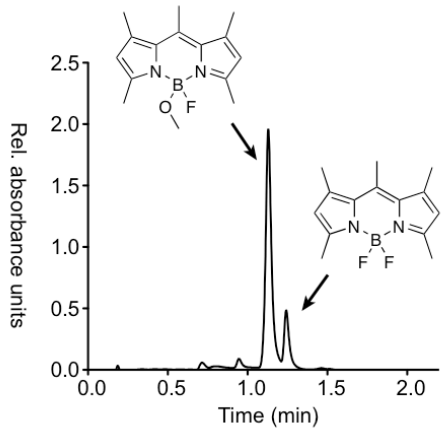

Fig. S1: Analytical HPLC trace (490nm).

Hydrolysis testing of 4-[^{18}F]-1,3,5,7,8-Pentamethyl BODIPY [^{18}F]2

4-[^{18}F]-1,3,5,7,8-Pentamethyl BODIPY prepared by Method A was HPLC purified (HPLC Method A) and isolated by C18 SPE cartridge followed by elution with DCM (2 x 1 mL). DCM was removed by heating (35°C) under an argon stream and 4-[^{18}F]-1,3,5,7,8-pentamethyl BODIPY (202 µCi) was dissolved in 250 µL of 1:1 MeCN:1xPBS. This solution was then heated to 37°C. At 0, 0.5, 1.0 and 2.0 hours, 40 µL aliquots were removed and analyzed by HPLC (HPLC Method B). HPLC analyses decay, corrected to time = 0.

Synthesis of 4-[^{18}F]-1,3,5,7-Tetramethyl-8-propionic BODIPY succinimidyl ester [^{18}F]5

4,4-Difluoro-1,3,5,7-tetramethyl-8-propionic BODIPY succinimidyl ester **5** (10 mM in 2:1 MeCN:DCM) was treated with 5 mol equivalents of TMS-OTf (250 mM in MeCN) and stirred at 0°C for 10 minutes before being treated with 15 µL azeotropically dried ^{18}F/TBAB (0.9 (33.3 MBq) ± 0.1 mCi) solution in MeCN. After stirring at room temperature for 4 minutes, the reaction was analyzed by radio-TLC (10:1 MeCN:Toluene, ITLC); R_f ([^{18}F]-F^-) = 0.0; R_f (4-[^{18}F]-1,3,5,7-tetramethyl-8-propionic BODIPY succinimidyl ester) = 0.80.

Table 3

#	BODIPY µmol	Solvent	TMS-OTf µmol	^{18}F mCi	TBAB µmol	RCY %
1	0.50 (10 mM Sol'n) 50 µL	MeCN:DCM (2:1)	2.50 (250 mM sol'n) 10 µL	0.89	0.88	64.18
2	0.75 (10 mM Sol'n) 75 µL	MeCN:DCM (2:1)	3.75 (250 mM sol'n) 15 µL	0.89	0.88	73.18
3	1.00 (10 mM Sol'n) 100 µL	MeCN:DCM (2:1)	5.00 (250 mM sol'n) 20 µL	0.94	0.88	62.73

The reaction mixture was loaded onto a C18 SPE cartridge (conditioned with 10ml ethyl acetate

followed by 10mL pentane) and washed with pentane (2 x 1 mL) following elution with ethyl acetate (3 x 1 mL). The combined ethyl acetate solution was concentrated at 60°C (microwave) under a stream of argon. Concentrated 4-[^{18}F]-1,3,5,7-tetramethyl-8-propionic BODIPY succinimidyl ester was subsequently dissolved in DMSO for further experiments. Radio-TLC (10:1 MeCN:Toluene, ITLC) analysis of this DMSO solution demonstrated 77.2% radiochemical purity of the 4-[^{18}F]-1,3,5,7-tetramethyl-8-propionic BODIPY succinimidyl ester.

Preparation of [^{18}F]-BODIPY Herceptin. To a solution of Herceptin in 1xPBS (30 µL of a 67 mg/mL solution) was added 1xPBS (90 µL) and 20.7 µCi of 4-[^{18}F]-1,3,5,7-tetramethyl-8-propionic BODIPY succinimidyl ester in DMSO (30 µL). After stirring for 15 minutes at room temperature, the mixture was loaded onto 50 kDa molecular weight cut off (MWCO) filters, and centrifuged for 5 minutes at 15k rpm. Material remaining in the filter was then reconstituted in 1xPBS and analyzed by size-exclusion chromatography; t_R ([^{18}F]-BODIPY Herceptin) = 25 minutes; t_R (4-[^{18}F]-1,3,5,7-tetramethyl-8-propionic BODIPY succinimidyl ester) = 40 minutes. (19.9% decay-corrected radiochemical yield)

Mice

All animal experiments were approved by the Massachusetts General Hospital's Institutional Review Committee. All mice were anesthetized (isoflurane 1.5%; O_2 2 L/minute) during pharmacokinetic and imaging studies with a gas delivery system. C57BL/6 mice received intravenous tail-vein injection of **[^{18}F]5** (100 µCi).

PET-CT imaging

All PET-CT images were acquired on a Siemens Inveon PET-CT system. Each PET acquisition took approximately 45 minutes. PET data were reconstructed from 600 million coincidental, 511 keV photon counts on a series of lutetium oxyorthosilicate scintillating crystal rings. Counts were rebinned in three-dimensions by registering photons spanning no more than 3 consecutive rings. These were then reconstructed into sinograms by utilizing a high resolution Fourier Rebin algorithm. A reconstruction of sinograms yielded a three-dimensional map of positron signals using a two-dimensional filtered back-projection algorithm, and a Ramp filter at a Nyquist cut-off of 0.5. Image pixel size was anisotropic, with dimensions of 0.796 mm in the z direction and 0.861 mm in both the x and y directions; this produced an image consisting of 128 x 128 x 159 pixels. Calibration of the PET signal preceded all scans and was done by scanning an 8.0 cm cylindrical phantom containing a known amount of ^{18}F isotope. Data are expressed as standard uptake values (SUV), which normalizes activity for both body weight and injected activity.

CT images were reconstructed from 360 X-ray projections with a cone beam angle of 9.3 degrees over 360 degrees, perpendicular to the animal bed. 80 keV X-rays were transmitted from a 500 µA anode source, 347 mm from the center of rotation, and recorded using a CCD detector containing 2048 transaxial and 3072 axial pixels. Projections were calibrated using 70 dark and 70 light images; these were interpolated bi-linearly, processed through a Shepp-Logan filter, and then reconstructed using a filtered back projection algorithm. The isotropic CT pixel size was 110.6 µm, and the image consisted of a total of 512 x 512 x 768 pixels. Scaling to Hounsfield Units, calibration was done using an 8.0 cm cylindrical phantom, containing water (prior to CT acquisition). During CT acquisition, iodine contrast was infused into the tail vein at a rate of 35 µl/minute to enhance intravascular contrast. Projections were acquired at the end of expiration using a BioVet gating system (M2M Imaging, Cleveland, OH) and the CT acquisition time was ~10 minutes. Reconstruction of data sets, PET-CT fusion and image analysis were done using IRW software (Siemens). Two-dimensional visualizations were produced with the DICOM viewer OsiriX (The OsiriX foundation, Geneva, Switzerland).