Bacterial Infection Imaging with [^{18}F]Fluoropropyl-trimethoprim

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Materials and Methods

Mammalian cell culture

HCT116 cells (American Type Culture Collection) carrying the *dhfr* transgene were made as described previously,(1) and were cultured in complete media: DMEM with 10% fetal bovine serum (Invitrogen), 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin (all from Gibco). Cells were maintained in a humidified incubator at 37 ˚C.

Bacterial cell culture

Staphylococcus aureus (8325-4; P2) lux::Cm (chloramphenicol resistant) and *Pseudomonas aeruginosa* Xen5 (American Type Culture Collection 19660) lux::Tc (tetracycline resistant) were gifts of the Chris Contag Lab. *E. coli* (GR12) EM7-lux::Km (kanamycin resistant) was also a generous gift of the Contag Lab and made in similar manner to Lane et al.(2) Individual colonies were picked on LB plates with appropriate antibiotics. All bacteria were auto-bioluminescent and liquid cultured in non-selective Luria-Bertani broth. Bioluminescence is not known to affect pathogenicity or drug uptake.(3)

In vitro assays

Bmax, saturation, and mammalian cellular uptake studies. HCT116 *dhfr* cells were plated in a 96-well plate (35K cells/well) 24h prior to assay, incubated with [^{18}F]FPTMP (~2 million CPM, saline, <1% ethanol) in Opti-Mem (Gibco) for 60 minutes at 37 ˚C. Excess unlabeled TMP (10 μM) was used to define non-specific binding. Protein was quantified using the Lowry method and uptake assayed on a gamma counter (Perkin Elmer).

Bacterial minimum inhibitory concentration. All three bioluminescent species were grown overnight to saturation. 1 × E5 colony forming units (CFU) were added a 96 well plate and grown in various concentrations of TMP or FPTMP overnight using a standard procedure. (4) The bioluminescent signal was quantified the next day using a luminescent plate reader (Enspire, Perkin Elmer) (n=4).

Mouse models

Imaging with *E. coli, S. aureus,* and *P. aeruginosa*. Infection models were performed similar to previously described.(5, 6) Balb/c mice (Charles River) received injections of 1 × E8 CFU of live bacteria as measured by OD_{600} (*E. coli and S. aureus*) in the muscle belly of the gastrocnemius of the lower hind limb the day prior to FPTMP imaging. *P. aeruginosa* could not be administered greater than 1 × E7 CFU due to toxicity/sepsis at higher concentrations. Turpentine (30 μL) was injected on the contralateral hindlimb 2-3 days prior to imaging to induce chemical inflammation. 4T1(Luc+) mammary carcinoma cells (1 million), were injected into Balb/c mice (syngeneic) in the shoulder regions. Animals were anesthetized (2% isoflurane), given a tail vein injection of [^{18}F]FPTMP (~200 μCi /mouse) and placed on a warmed stage for small animal A-PET imaging (Philips MOSAIC HP) and CT images were acquired with a microCT (ImTek).(7) Uptake was measured from elliptical ROI and mean SUV was calculated using AMIDE software (Amide version 1.0.4 (http://www. amide.sourceforge.net).

Sensitivity Experiment. Balb/c mice were subcutaneously infected with *E. coli* 1 × E8 (hind limb), 1 × E7 (forelimb), and 1 × E6 (ear pinna) CFU and equivalent numbers of heat-killed bacterial cells on the contralateral side of the animal in 100 μL PBS (Corning). The next day animals were administered [^{18}F]FPTMP IV (~200 μCi /mouse) and imaged with PET as above. Mice were sacrificed and tissues harvested for autoradiography. All animal studies were completed with University of Pennsylvania's Institutional Animal Care and Use Committee, IACUC approval.

Statistical analysis was performed with Prism (Graphpad) and statistical significance established by an unpaired Student's t-test with $p<0.05$.

Autoradiography and tissue histology.

Mice were sacrificed after FPTMP injection and imaging. Infected and control tissues were dissected and embedded with in OCT. Sections (20-30 micron) were cut and

exposed to a phosphor plate overnight (GE) and developed on a Typhoon digital autoradiograph (GE). Gram staining of contiguous frozen sections was performed (Fisher) and micrographs were taken at 40x and 63x (Zeiss).

Estimated Human Dosimetry. Biodistribution data from female Balb/c mice were used to estimate human dosimetry in an adult female human model that was predefined in OLINDA/EMX 1.1 (VU e-Innovations). Kinetic data from time points (15, 60, and 120 min) were fitted as percent-injected dose/organ over time. By fitting the kinetic data using %ID/organ we assume the [^{18}F]FPTMP distribution would be relative to human and thus did not apply a scaling factor accounting for organ weight to subject total body weight between mouse and human.(8)

Primate imaging

Prior to the scan, rhesus monkeys were fasted overnight and anesthetized, initially with ketamine (4 mg/kg) and dexmedetomidine (0.05 mg/kg), for transport to the scanner facility. The monkey was intubated; inhalation anesthesia is maintained using 1-2% isoflurane and oxygen. Vital signs were monitored during the procedure using a pulse oximeter, ECG monitor and temperature probe. Body temperature was maintained during the procedure by the use of a circulating water heating pad and blankets. Images were obtained on a high sensitivity, high resolution PET scanner, G-PET (Philips Medical Systems). The bed was positioned with the subject's chest and upper abdomen centered in the scanner gantry and a 20 min transmission scan was performed. Subjects were given a bolus injection of the radiotracer (~1-2 mCi) followed by a saline flush with the concurrent start of dynamic list mode data acquisition for 1.5-2 hours (n=2). Imaging was performed with two different monkeys. The studies were performed in accordance with IACUC approval.

Chemical Synthesis

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Alfa Aesar (Ward Hill, MA, USA). ^{1}H NMR spectra were obtained using a Bruker DMX 360 (360 MHz) spectrometer (Rheinstetten, Germany), and chemical shifts (δ) were reported as the ppm downfield of the internal tetramethylsilane. For purification and analysis of radioligands, HPLC analysis was conducted using the Agilent 1100 (Agilent Technologies, Santa Clara, CA, USA) equipped with a semi-preparative column (Phenomenex, Luna 5μ Phenyl-Hexyl New Column 250 x 10 mm; Kinetex C18 5μ 150 x 10 mm) or Waters Alliance e2695 HPLC (Waters Corporations, Milford, MA, USA) equipped with an analytical column (Agilent ZORBAX Eclipse XDB-C18, 5 μm, 4.6 x 150 mm). The eluent was monitored simultaneously using UV (281 nm) and NaI(T1) radioactivity detectors. TLC was performed on Merck F_{254} silica plates and analyzed on a Bioscan Mini-Scan TLC Imaging Scanner (Hopkinson, MA, USA).

[^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F reaction using an IBA Cyclone® 18 (Louvain-la-Neuve, Belgium). Radioactivity was measured in a dose calibrator (Capintec, Ramsey, NJ, USA). All animal experiments were performed under IACUC-approved protocols in compliance with the guidelines for the care and use of research animals established by the University of Pennsylvania.

[*^{18}F*]*FPTMP Synthesis*

Synthesis of 4-((2,4-diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol (**2**)
TMP (3.00 g, 10.3 mmol) was dissolved in HBr (37.4 mL, 48% in H_2O), and stirred at 95 °C for 20 min. The reaction mixture was cooled down to the ambient temperature, NaOH (8.91 mL, 50% w/w) added, and kept at 4 °C for overnight. The precipitate was filtered and washed with H_2O. The collected precipitate was re-dissolved in boiling H_2O, 1 N NaOH added to pH~7.0, recrystallized, and washed with H_2O. 4-((2,4-diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol was obtained as a pink solid (2.04 g, 71.6%). ^{1}H

NMR (DMSO-d_6) δ 8.06 (s, -OH), 7.45 (s, 1H), 6.48 (s, 2H), 5.99 (s, -NH_2), 5.63 (s, -NH_2), 3.71 (s, 6H), 3.47 (s, 2H).

Synthesis of 5-(4-(3-fluoropropoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine (**1**)

To a solution of **2** (500 mg, 1.81 mmol) and Cs_2CO_3 (1.18 g, 3.62 mmol) in DMF (25.0 mL) was added 1-bromo-3-fluoropropane (510 mg, 3.62 mmol), and stirred at 80 °C for 4 h. After the reaction, DMF was removed *in vacuo*, purified by flash column chromatography (CH_2Cl_2:MeOH = 15:1) gave 5-(4-(3-fluoropropoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine (**1**) as a yellow solid (167 mg, 27.4%). ^{1}H NMR (DMSO-d_6) δ 7.51 (s, 1H), 6.54 (s, 2H), 6.05 (s, -NH_2), 5.66 (s, -NH_2), 4.62 (dt, J = 46.8 and 7.2 Hz, 2H), 3.89 (t, J = 7.2 Hz, 2H), 3.71 (s, 6H), 3.52 (s, 2H), 1.94 (dq, J = 25.2 and 7.2 Hz, 2H).

Synthesis of 5-(4-(3-((tert-butyldimethylsilyl)oxy)propoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine (**3**)

To a solution of **2** (43.0 mg, 0.15 mmol) and Cs_2CO_3 (101 mg, 0.31 mmol) in DMF (2.14 mL) was added 3-bromopropoxy-tert-butyldimethyl silate (78.8 mg, 0.31 mmol), and stirred at 80 °C for 7 h. After the reaction, DMF was removed *in vacuo*, purified by flash column chromatography (CH_2Cl_2:MeOH = 20:1) gave 5-(4-(3-((tert-butyldimethylsilyl)oxy)propoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine (**3**) as a light yellow solid (167 mg, 28.7%). ^{1}H NMR ($CDCl_3$) δ 7.78 (s, 2H), 6.38 (s, 2H), 4.73 (-NH_2, 2H), 4.55 (-NH_2, 2H), 4.05 (t, J = 7.2 Hz, 2H), 3.83-3.79 (m, 2H), 3.78 (s, 6H), 3.65 (s, 2H), 1.94 (t, J = 7.2 Hz, 2H), 0.88 (s, 9H), 0.06 (s, 6H).

Synthesis of di-tert-butyl (5-(4-(3-((tert-butyldimethylsilyl)oxy)propoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diyl)bis((tert-butoxycarbonyl)carbamate) (**4**)

To a solution of **3** (400 mg, 0.89 mmol) in THF (12.0 mL) was added di-*tert*-butyl dicarbonate (0.66 mL, 2.67 mmol), Et_3N (0.497 mL, 2.67 mmol), and dimethylaminopyridine (75.6 mg, 0.26 mmol). The reaction mixture was stirred at the ambient temperature for 20 h. After the reaction, the reaction mixture was diluted with H_2O (50.0 mL), extracted with EtOAc (50.0 mL x 2), washed with brine (50.0 mL), and

then dried over anhydrous Na_2SO_4, concentrated. Flash column chromatography (Hexane:EtOAc = 5:1) gave (5-(4-(3-((tert-butyldimethylsilyl)oxy)propoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diyl)bis((tert-butoxycarbonyl)carbamate) (**4**) as a white solid (196 mg, 25.8%). ^{1}H NMR ($CDCl_3$) δ 8.56 (s, 1H), 6.37 (s, 2H), 4.04 (t, *J* = 7.2 Hz, 2H), 3.83-3.82 (m, 4H), 3.78 (s, 6H), 1.95 (q, *J* = 7.2 Hz, 2H), 1.45 (s, 18H), 1.39 (s, 18H), 0.89 (s, 9H), 0.58 (s, 6H).

Synthesis of di-tert-butyl (5-(4-(3-hydroxypropoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diyl)bis((tert-butoxycarbonyl)carbamate) (**5**)

To a solution of **4** (196 mg, 0.23 mmol) in THF (6.90 mL) was added 1 M TBAF in THF (0.69 mL, 0.69 mmol), and stirred at the ambient temperature for 1 h. After the reaction, the reaction mixture was diluted with H_2O (50.0 mL), extracted with EtOAc (50.0 mL x 2), washed with brine (50.0 mL), and then dried over anhydrous Na_2SO_4, concentrated. Flash column chromatography (Hexane:EtOAc = 2:3) gave (5-(4-(3-hydroxypropoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diyl)bis((tert-butoxycarbonyl)carbamate) (**5**) as a colorless solid (147 mg, 86.7%). ^{1}H NMR ($CDCl_3$) δ 8.56 (s, 1H), 6.39 (s, 2H), 4.13 (t, *J* = 7.2 Hz, 2H), 3.90 (t, *J* = 7.2 Hz, 2H), 3.82 (s, 2H), 3.81 (s, 6H), 3.68 (t, *J* = 3.6 Hz, 2H), 1.96 (q, *J* = 7.2 Hz, 2H), 1.54 (s, 18H), 1.45 (s, 9H), 1.39 (s, 9H).

Synthesis of 3-(4-((2,4-bis(bis(tert-butoxycarbonyl)amino)pyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)propyl methanesulfonate (**6**)

To a solution of **5** (138 mg, 0.18 mmol) in CH_2Cl_2 (20.0 mL) was added Et_3N (78.3 μL, 0.56 mmol) at 0 °C, and stirred for 5 min. The reaction mixture was added methane sulfonyl chloride (43.5 μL, 0.56 mmol) dropwise at at 0 °C, and stirred at the ambient temperature for 2 h. After the reaction, the reaction mixture was diluted with H_2O (50.0 mL), extracted with CH_2Cl_2 (50.0 mL x 2), washed with brine (50.0 mL), and then dried over anhydrous Na_2SO_4, concentrated. Flash column chromatography (Hexane:EtOAc = 1:1) gave 3-(4-((2,4-bis(bis(tert-butoxycarbonyl)amino)pyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)propyl methanesulfonate (**6**) as a white solid (134 mg, 87.8%). ^{1}H NMR ($CDCl_3$) δ 8.55 (s, 1H), 6.38 (s, 2H), 4.54 (t, J = 7.2 Hz, 2H), 4.05 (t, *J* = 3.6 Hz, 2H), 3.81 (s, 2H), 3.79 (s, 6H), 3.03 (s, 3H), 1.45 (s, 18H), 1.39 (s, 18H).

Radiolabeling of 5-(4-(3-[^{18}F]fluoropropoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine ([^{18}F]**1**).

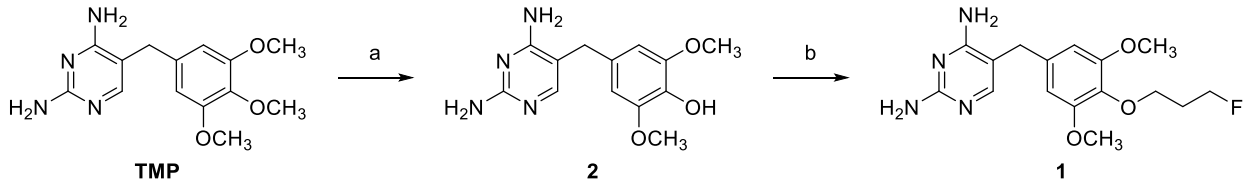
[^{18}F]Fluoride (1.8 – 2.0 GBq) was trapped on a Sep-pak® QMA-bicarb cartridge from the cyclotron, and eluted to the reaction vial using 7.0 mg of Kryptofix 222 and 3.0 mg of K_2CO_3 solution. Three azeotropic distillations were then performed using 0.5-1.0 mL aliquots of anhydrous acetonitrile at 100 °C (oil bath) under a gentle stream of N_2. The precursor **6** (2.0 mg) in CH_3CN (200 μL) was added to the resulting K[^{18}F]F in the reaction vial. The reaction mixture was heated at 100 °C for 10 min. At the end of the reaction, the reaction mixture was cooled down to the ambient temperature, 1.0 mL of 1 N HCl added for deprotection of Boc group, and heated at 100 °C for 10 min. 1.5 mL of the HPLC mobile phase was added to the reaction vial, after passing through Sep-pak® Alumina light cartridge, the crude product was then purified by HPLC using a semi-preparative column eluted with a 80:20 mixture of 20 mM ammonium bicarbonate aqueous solution and CH_3CN at a flow rate of 4 mL/min. The desired product ([^{18}F]**1)** eluted between 23 and 25 min. The collected product was diluted with 40 mL of H_2O, trapped Sep-pak® C-18 plus cartridge, and formulated 10% EtOH-saline for the biology study. Specific activity was determined by comparing the UV peak area of the desired radioactive peak and the UV peak areas of different concentrations of unlabeled standard **1** on HPLC. Identification of radioligand [^{18}F]**1** was determined by co-injecting the radioligand with the corresponding unlabeled standard into the HPLC system.

Radiolabeling of 5-(4-(3-[^{18}F]fluoropropoxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine ([^{18}F]**1**) in AllinOne automated synthesis module.

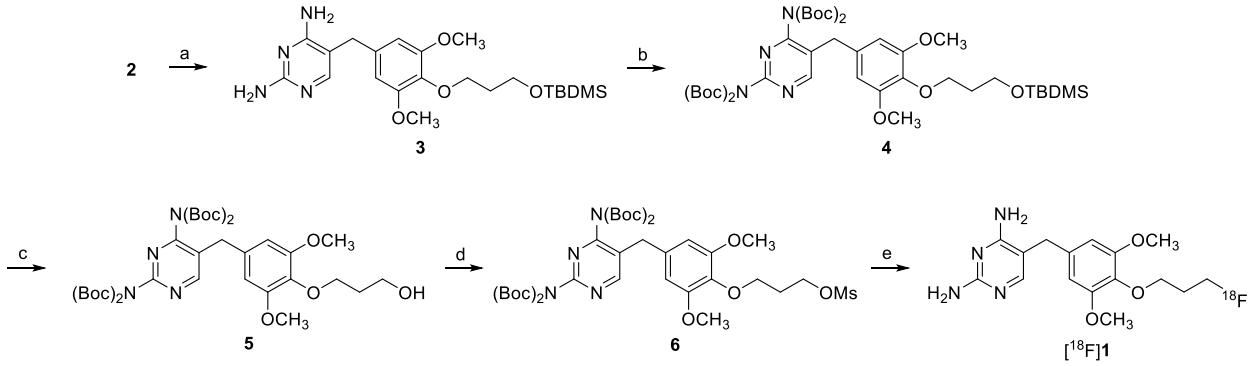
With modification of manual radiolabeling procedure, the automation of [^{18}F]**1** was achieved in AllinOne module . [^{18}F]F^- in $H_2{}^{18}O$ was delivered from cyclotron to the module and trapped by passing the solution through a preconditioned QMA Carb cartridge. Meanwhile $H_2{}^{18}O$ was collected in a recovery vial. Release of [^{18}F]F^- from QMA Carb cartridge to the reaction vessel was achieved by passing 1 mL basic eluent: 0.85 mL CH_3CN and 0.15 mL H_2O containing 7 mg K222 and 2 mg K_2CO_3. Subsequently drying of the solution was conducted at 100 °C for 4 min under vacuum.

Anhydrous acetonitrile (1 mL) was then added to the reaction vessel to further azeotropically removing water residue.

Then 4.0 mg precursor **6** in 1.0 mL MeCN was added into the reaction vessel containing dried [^{18}F] fluoride/potassium carbonate and Kryptofix 222 (K222). The reaction mixture was heated at 100°C for 10 min. After the reaction, 1 N HCl solution (1 mL, aq) was added and allowed for 10 min to deprotect. After cooling down to 50°C, the reaction mixture was neutralized with 1 N NaOH solution (1.0 mL) and then quenched with 3.0 mL mobile phase. The reaction mixture was passed through an Al-N light cartridge before transferred to HPLC loop. The cartridge was washed with additional 3.0 mL of water. Unreacted [^{18}F]F- was trapped on Al-N Light cartridge and the crude mixture was purified by a semi-preparative HPLC with a Phenomenex Kinetex® C18 column (5µm, 150 x 10 mm). The mobile phase was 80:20 mixture of 20 mM ammonium bicarbonate aqueous solution/CH_3CN and flow rate was 5 mL/min. The desired product was eluted around 20.0 min and the fraction (around 8 mL) was collected in 20 mL syringe and then diluted into 21.0 mL with water. The product was enriched on a C18 Plus cartridge and rinsed with 10.0 mL water. Finally the product was eluted out with 1 mL ethanol and passed through a 0.2µm sterile Millex® FG filter into final production vial. The final formulation was done with adding 9 mL saline into the vial.



Scheme 1. Reagents and conditions: a) 48% HBr, 95 °C, 20 min; b) Cs_2CO_3, 1-bromo-3-fluoropropane, DMF, 80 °C, 4 h.



Scheme 2. Reagents and conditions: a) 3-bromopropoxy-tert-butyldimethyl silate, Cs_2CO_3, DMF, 80 °C, 7 h; b) di-*tert*-butyl dicarbonate, Et_3N, DMAP, THF, rt, 20 h; c) 1 M TBAF in THF, rt, 3 h; d) MsCl, Et_3N, CH_2Cl_2, rt, 3 h; e) i) $K^{18}F$, CH_3CN, 100 °C, 10 min, ii) 1 N HCl, 100 °C, 10 min

Supplemental Figures

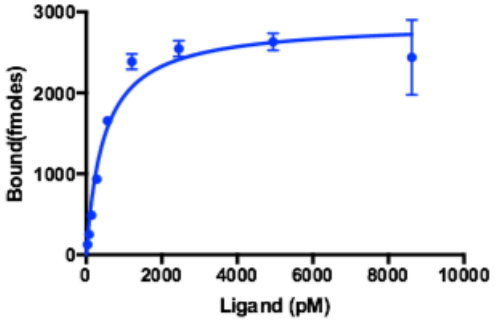
Organism (strain)	Uniprot	Gene Name	% Identity (*E. coli*)	Identical	Similar	TMP Susceptible
Escherichia coli (K12)	P0ABQ4	folA	100	NA	NA	Yes
Klebsiella pneumoniae subsp. pneumoniae (ATCC 700721)	A6T4I4	folA KPN_00045	92.4	147	9	Yes
Proteus mirabilis (ATCC 29906)	A0A0V9GZW7	folA APT96_09150	75.2	121	26	Yes
Haemophilus influenzae (ATCC 51907)	P43791	folA folH, HI_0899	52.5	47	84	Yes
Pseudomonas aeruginosa (ATCC10145)	A0A081HD68	folA	43.1	48	73	No
Enterococcus faecalis (ATCC 700802 / V583)	Q834R2	folA EF_1577	35.4	58	58	Yes
Staphylococcus aureus (ATCC12600)	P0A017	folA	33.3	54	61	Yes
Streptococcus pneumoniae serotype 4 (ATCC BAA-334)	Q54801	dhfR	32.7	55	57	Yes
Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)	P9WNX1	folA	30.5	46	51	Mixed evidence
Candida albicans (1006)	P22906	DHFR1	25.5	49	56	No
Toxoplasma gondii (ATCC 50859)	C9DIG7	dhfr	21.3	52	56	Yes

Figure S1. Organism search for *dhfr* homology using the Uniprot database and clustal alignment. Percent identity represents the number of identical amino acids compared to *E. coli* dhfr. Susceptibility data from the package insert of trimethoprim. (9)

Bmax: 2870 ± 106.0 fmol/mg
Kd: 0.465 ± 0.07 nM

	HCT-116 DHFR
One site binding (hyperbola)	
Best-fit values	
Bmax	2870
Kd	465.8
Std. Error	
Bmax	106.0
Kd	66.40
95% Confidence Intervals	
Bmax	2652 to 3089
Kd	329.0 to 602.5
Goodness of Fit	
Degrees of Freedom	25
R square	0.9499

-35k cells/well/100µL plated 24 hr prior
-Incubation time 60 min @ 37C
-Cold TMP (10µM) used to define NS binding
-Protein quantified by Lowry Method



Bmax: 2870 ± 106.0 fmol/mg
Kd: 0.465 ± 0.07 nM

Figure S2. In vitro binding studies with [^{18}F]FPTMP in HCT116 *E. coli dhfr* cells. *E. coli dhfr* cells were generated using retroviral transduction and selection with YFP as previously described.(1) Bmax and Kd were calculated as indicated.

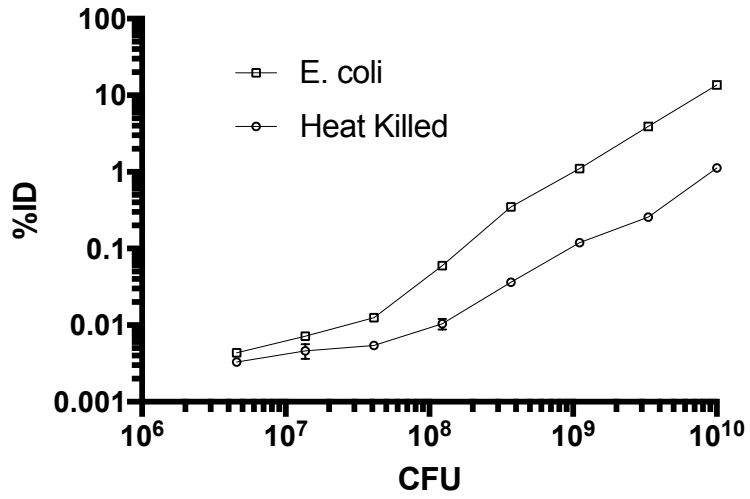


Figure S3. *E. coli* dilution series and uptake compared to heat-killed control. *E. coli* (up to 10^{10} CFU) and control heat killed *E. coli* (95 degrees Celsius for 20 minutes) were incubated with [18F]FPTMP for 30 minutes at room temperature, washed in PBS twice and assayed for uptake with a gamma counter (n=3). Error bars represent the standard deviation.

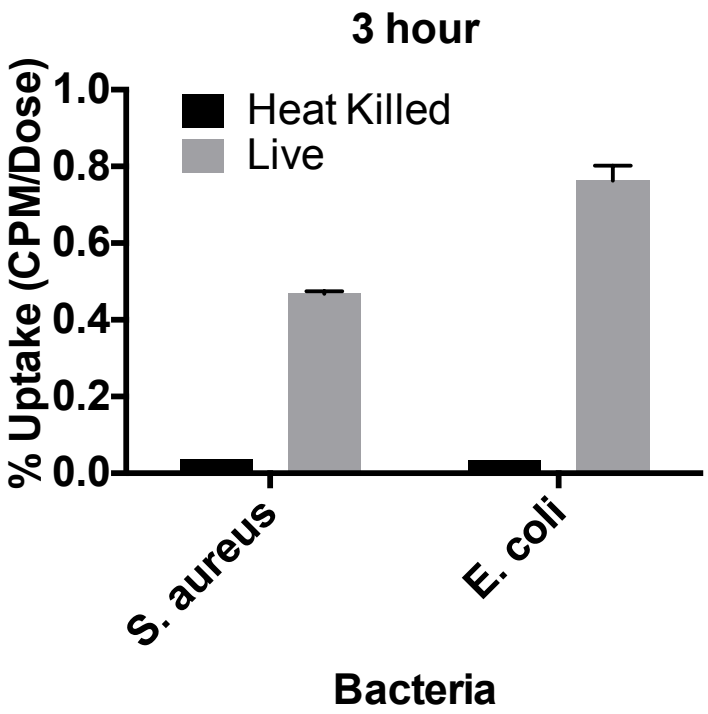


Figure S4. Uptake of live bacteria compared to heat killed bacteria after 3h incubation. Bacteria 10^8 CFU were incubated with [^{18}F]FPTMP (~2 million CPM) at 37 degrees Celsius. Equal numbers of heat killed bacteria were incubated with [^{18}F]FPTMP. Counts were recorded on a gamma counter (n=3). Error bars represent the standard deviation.

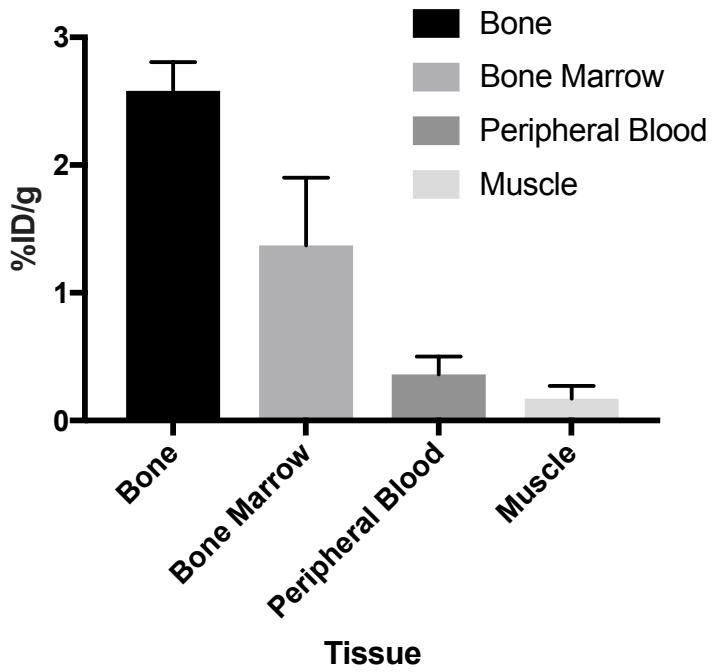


Figure S5. Limited biodistribution in mouse at 90 minutes after injection. Cortical bone shows approximately 2.5%ID/g whereas bone marrow (red marrow) shows 1.5 %/ID per gram. Error bars represent the standard deviation (n=6). Briefly, mice were injected with [^{18}F]FPTMP ~100 μCi TV and sacrificed at 90 minutes. The femurs were harvested, bone marrow explanted and uptake per gram measured in a gamma counter.

Organ	Total (mSv)
Brain	0.02
Gallbladder wall (non-metabolic)	1.08
Large intestine	0.94
Small intestine	3.12
Kidneys	0.90
Liver	0.26
Lungs	0.09
Muscle	0.01
Pancreas	0.50
Red marrow	0.32
Heart	1.37
Ovaries	0.12
Spleen	0.20
Thyroid	0.08
Bladder	0.28
Total Body	0.43

Figure S6. Estimated Human Dosimetry. Biodistribution data from female Balb/c mice were used to estimate human dosimetry of [^{18}F]FPTMP (10 mCi) in an adult female human model that was predefined in OLINDA/EXM 1.1 software. Kinetic data from time points (15, 60, and 120 min) were fitted as percent-injected dose/organ over time. By fitting the kinetic data using %ID/organ we assume the [^{18}F]FPTMP distribution would be relative to human and thus did not apply a scaling factor accounting for organ weight to subject total body weight between mouse and human.

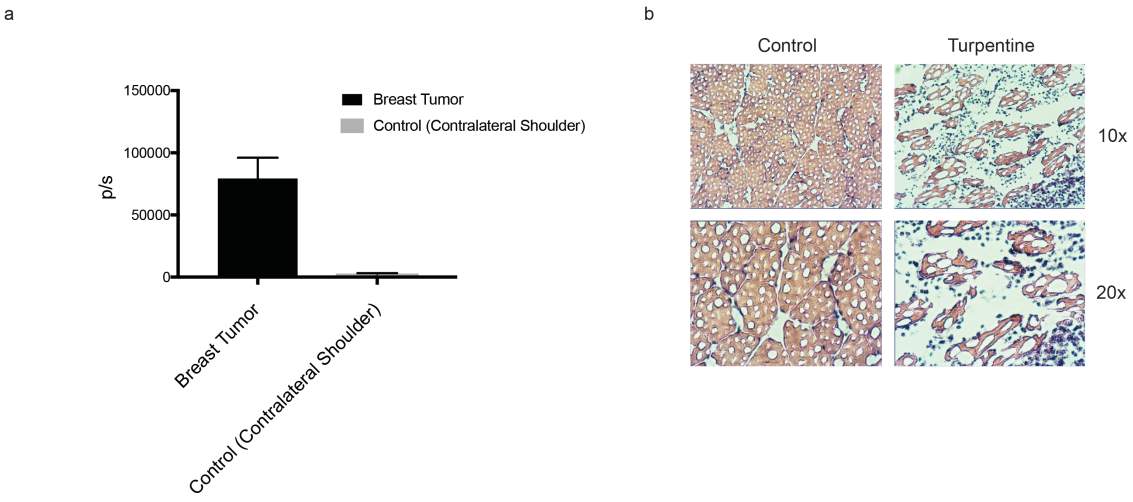


Figure S7. Tumor bioluminescence and histology showing typical turpentine inflammation. a) Regions of interest were drawn around the 4T1 breast carcinoma on the shoulder of balb/c mice and luminescence was compared to the contralateral shoulder. **b)** Histology with H&E staining using a standard protocol from turpentine injected hindlimb of a representative mouse compared to contra-lateral control.

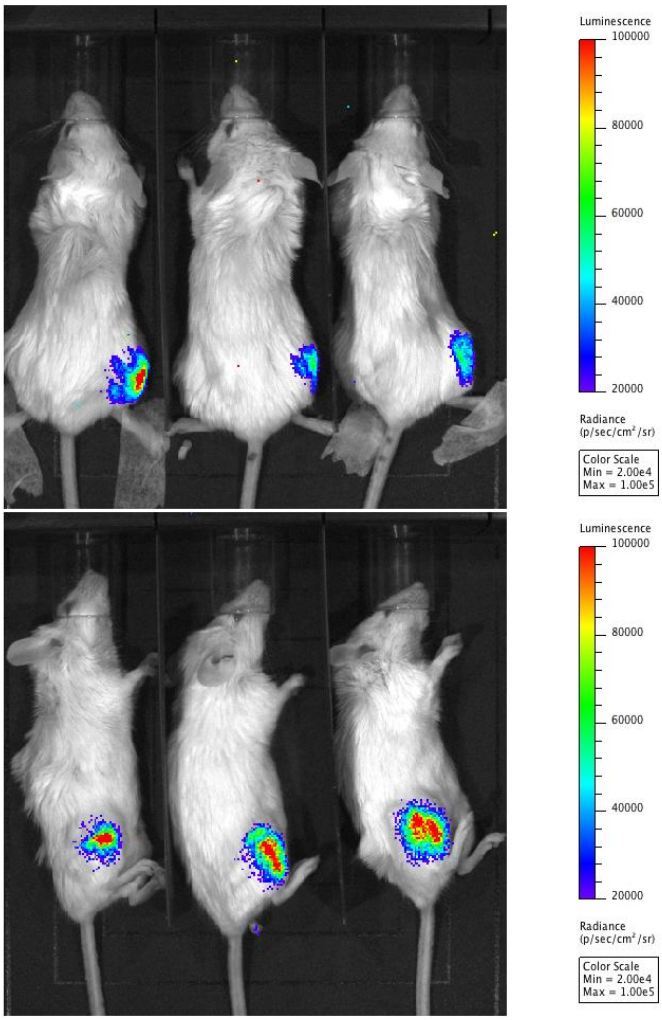


Figure S8. Bioluminescent images of *S. aureus* infection (1xE8) in the hindlimb of mice. Mice are shown in dorsal and left lateral decubitus positioning.

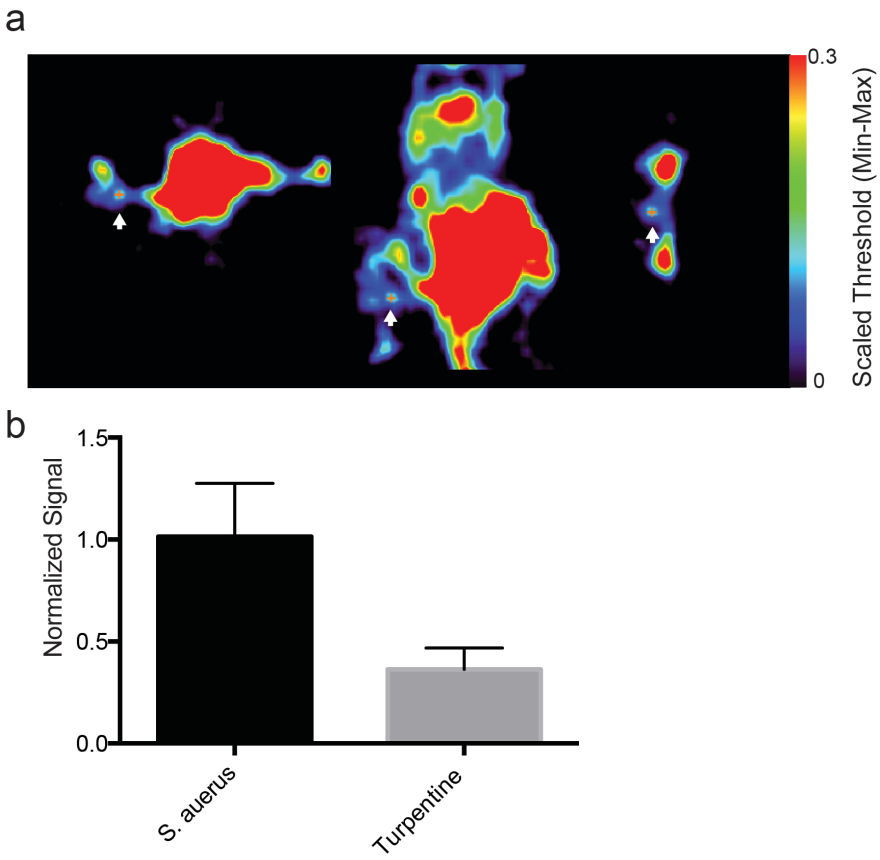


Figure S9. PET images of *S. aureus* infection v. turpentine in the hindlimbs of mice after injection of ~200 μCi of [^{18}F]FPTMP. a) MicroPET imaging showing axial, coronal, and sagittal views of a Balb/c mouse (left to right). The white arrow indicates the site of live infection. **b)** Quantification of the data from a). Error bars represent the standard deviation (n=3).

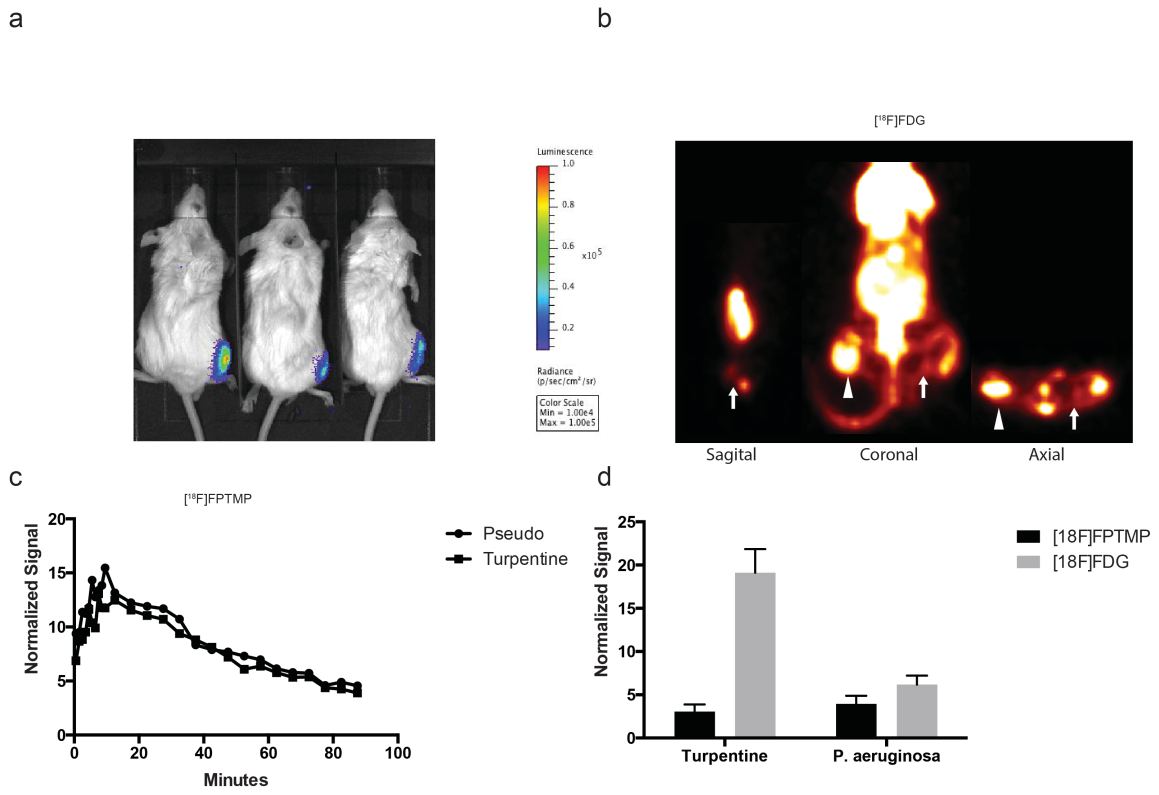


Figure S10. PET uptake of *P. aeruginosa* infection v. turpentine. a) BLI showing active *P. aeruginosa* infection in the right hindlimb. **b)** MicroPET of [^{18}F]FDG imaging of a representative mouse. The arrow indicates the site of *P. aeruginosa* infection and the arrowhead indicates turpentine. High FDG uptake is evident from the turpentine injection. **c)** Time activity quantification of a representative animal after [^{18}F]FPTMP, ~200 μCi IV, with ROI placed over the tissues of interest. **d)** Quantification of the data from [^{18}F]FDG and [^{18}F]FPTMP PET imaging. Error bars represent the standard deviation (n=3).

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