Electronic Supplementary Material

Selective Imaging of Matrix Metalloproteinase-13 to Detect Extracellular Matrix Remodeling in Atherosclerotic Lesions

Ariel Buchler[1,3], Maxime Munch[2,3], Gedaliah Farber[2,3], Xiaoling Zhao[3], Rami Al-Haddad[2,3], Eadan Farber[3], and Benjamin H. Rotstein[1,2,3]

(1) Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5

(2) Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5

(3) University of Ottawa Heart Institute, Ottawa, Ontario, Canada, K1Y 4W7

Corresponding author:

Benjamin Rotstein, PhD

40 Ruskin St, H-5219, Ottawa, ON, K1Y 4W7

613-696-7324

benjamin.rotstein@uottawa.ca

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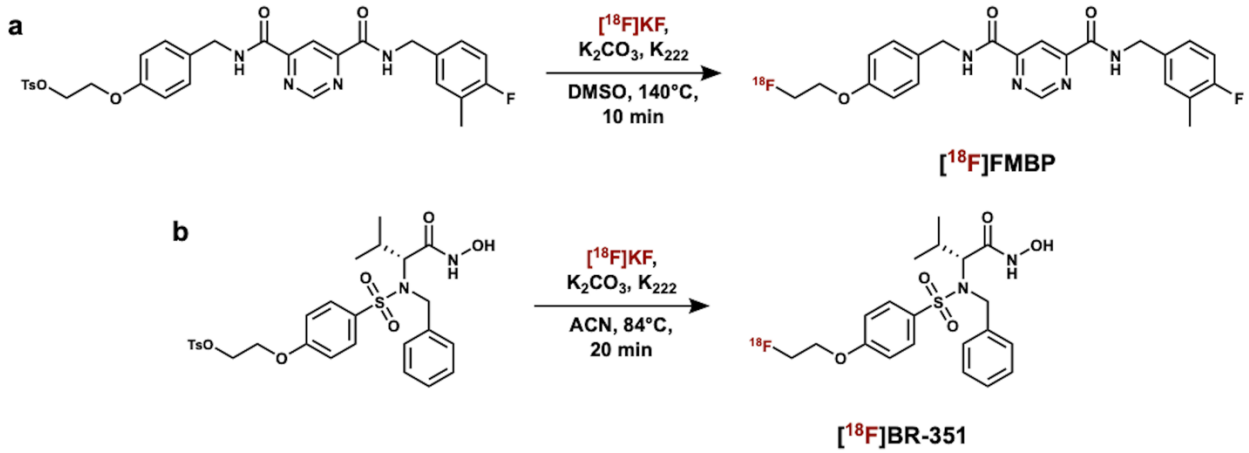
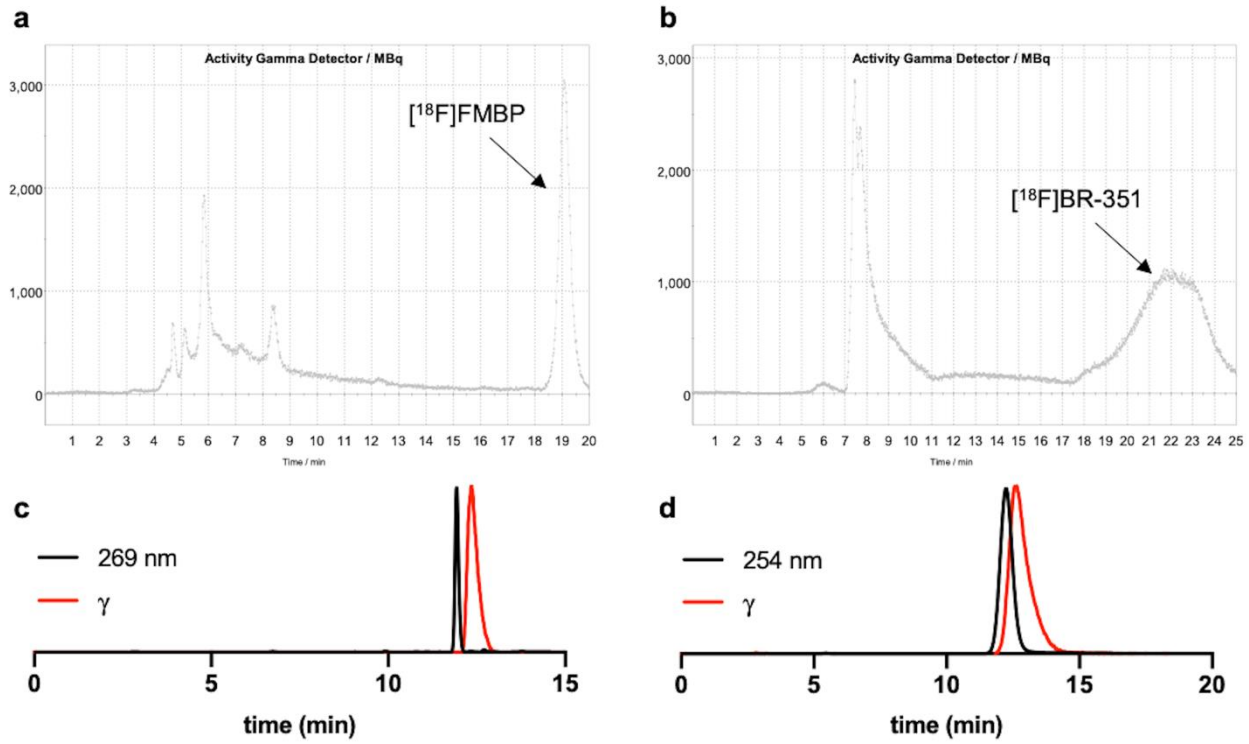
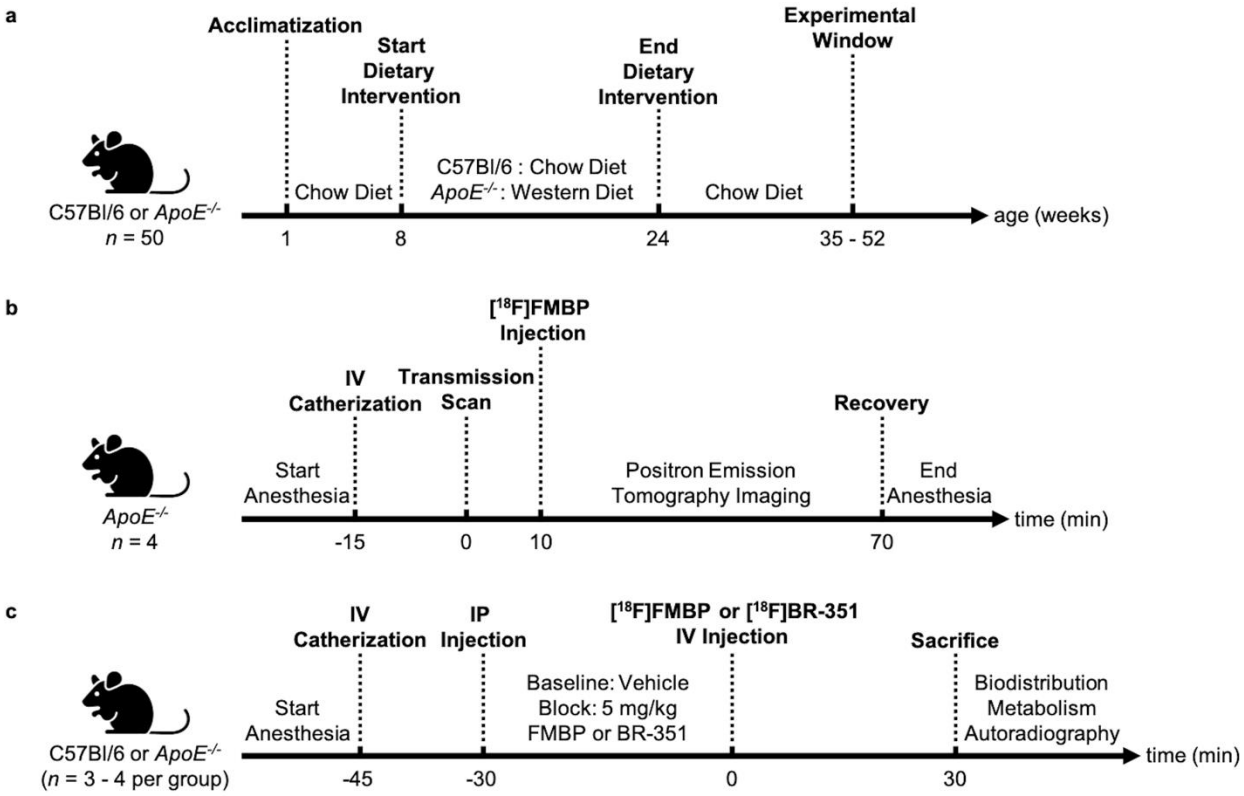
Supplemental Figures



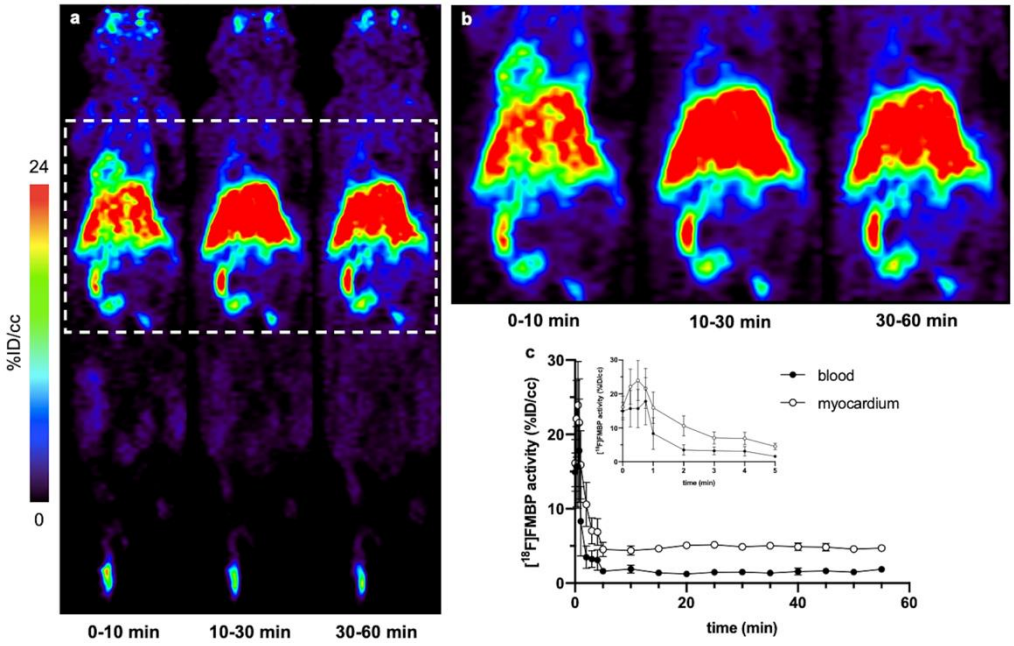
Supplemental Figure 1. Radiolabeling Schematic. (a) [^{18}F]FMBP and (b) [^{18}F]BR-351



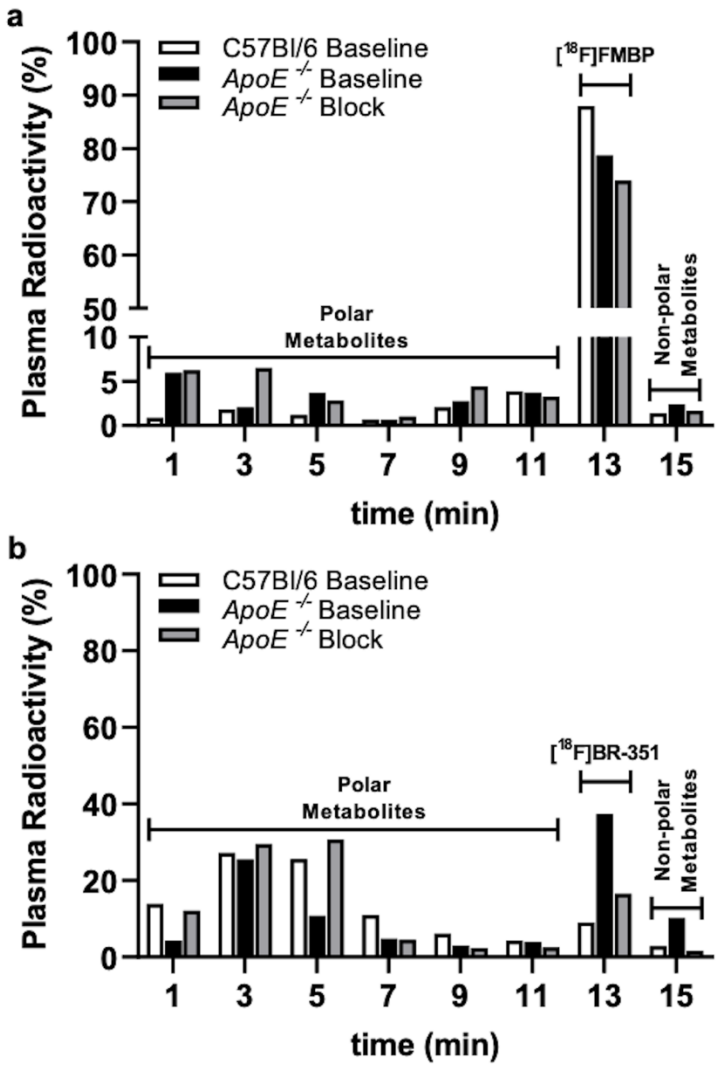
Supplemental Figure 2. Radiotracer purification and quality control. (a/b) Crude radio-HPLC chromatogram of radiolabeling reaction. (c/d) Coinjection with non-radioactive standard following isolation of (a) [^{18}]FMBP and (b) [^{18}F]BR-351. UV and radiation detectors are connected in series.



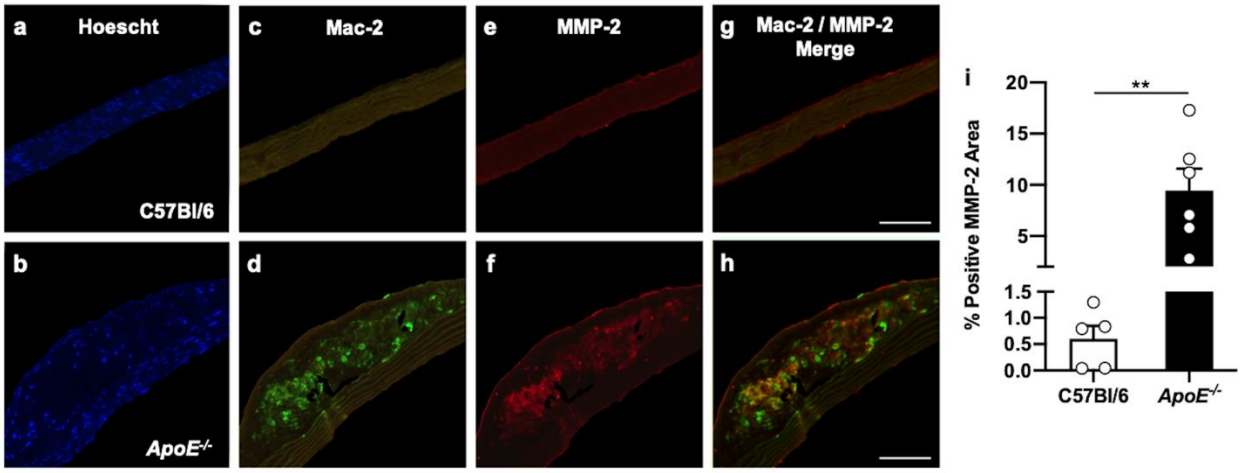
Supplemental Figure 3. Timeline of experiments. (a) Animal model dietary intervention (b) *In vivo* PET imaging (c) *Ex vivo* analyses.



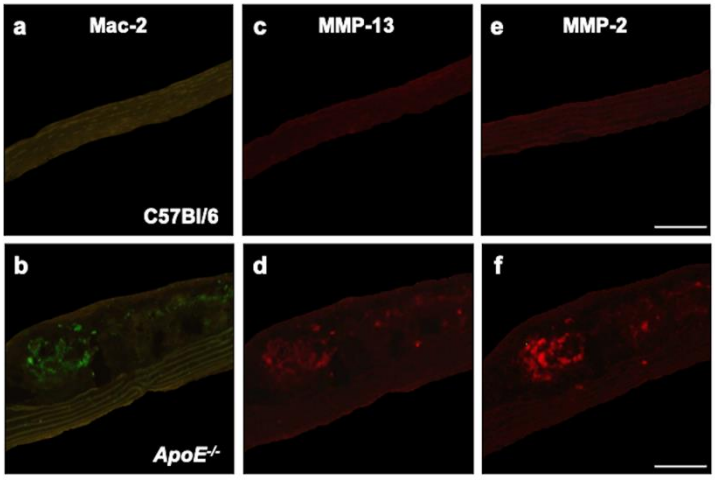
Supplemental Figure 4. *In vivo* [^{18}F]FMBP PET imaging in *ApoE*$^{-/-}$ mice. (a) Summed coronal whole-body PET images. (b) Magnified view within region of interest. (c) Mean [^{18}F]FMBP blood pool and myocardial time-activity curves derived from dynamic PET imaging (n = 4).



Supplemental Figure 5. Reconstructed fractional HPLC chromatograms of plasma radio-metabolites. Values represent the % of total plasma radioactivity obtained for pooled blood samples 30 min after intravenous administration of (a) [^{18}F]FMBP or (b) [^{18}F]BR-351 via the lateral tail vein (n = 2-3 per group).



Supplemental Figure 6. Immunofluorescent staining of atherosclerotic lesions detected by [^{18}F]FMBP *ex vivo* autoradiography. Corresponding composite images of C57Bl/6 and *ApoE*$^{-/-}$ aortic *en face* cross-sections following immunofluorescent staining for (a/b) Hoescht, (c/d) Mac-2, and (e/f) MMP-2. (g/h) Mac-2 and MMP-2 merge. Scale bar = 100 μm. (i) Quantification of percentage positive MMP-2 areas in *ApoE*$^{-/-}$ and C57Bl/6 mice. Unpaired t-test: $^{**}P = 0.0048$, $n = 5$-6.



Supplemental Figure 7. Immunofluorescence signal specificity. Representative composite images of C57Bl/6 and *ApoE*$^{-/-}$ aortic *en face* cross-sections following immunofluorescent staining for (a/b) Mac-2, (c/d) MMP-13, and (e/f) MMP-2 with the corresponding isotype control antibody at an equivalent working concentration to the primary testing antibody.

Supplementary Methods

Chemistry

Chemical reagents and solvents were obtained from commercial sources and used without further purification. Standards and precursors were prepared as previously described.[1,2] Compound characterization was completed by ^{1}H and ^{13}C NMR using a Bruker AVANCE III 400 or 600 MHz spectrometer and analyzed using MestReNova software.

General Radiochemical Methods

High molar activity no-carried-added aqueous [^{18}F]fluoride was prepared on a Siemens CTI Eclipse HP/RD Hybrid Cyclotron (11 MeV) via the $^{18}O(p,n)^{18}F$ nuclear reaction. Radiofluorination reactions were carried out on a GE TRACERlab FX2N automation system with TRACERlab FX software. In-line radio-HPLC purification was performed with a Phenomenex Synergi Hydro-RP (C18, 80 Å, 10 μm, 250 mm × 10 mm) column. Radioactivity was quantified using a Biodex Atomlab 500 Dose Calibrator.

Synthesis of [^{18}F]FMBP and [^{18}F]BR-351

Radiochemical syntheses were adapted from the literature with minor modifications. [1,2] [^{18}F]Fluoride was captured from the [^{18}O]H_2O target solution using a Waters Sep-Pak Light Accell Plus QMA Cartridge (preconditioned with 10 mL EtOH, 10 mL water, 10 mL 0.5 M $NaHCO_3$, 10 mL water, and 1 mL air). Following [^{18}F]fluoride elution with $K_2CO_{3\,(aq)}$ ([^{18}F]FMBP: 0.05 M, 530 μL, 27 μmol or [^{18}F]BR-351: 0.075 M, 530 μL, 40 μmol), anhydrous ACN (1 mL) containing Kryptofix 2.2.2 ([^{18}F]FMBP: 22.4 mg, 60 μmol or [^{18}F]BR-351: 19 mg, 50 μmol) was dispensed into the reactor. The aqueous [^{18}F]K(K_{222})F solution was evaporated to dryness *in vacuo*

(4 min at 80 °C without helium, 4 min at 60 °C with helium, and cooling to 35 °C with helium). The tosylate precursor ([^{18}F]FMBP: 4 mg, 6.8 μmol or [^{18}F]BR-351: 3 mg, 5.2 μmol), dissolved in anhydrous solvent ([^{18}F]FMBP: DMSO, 500 μL or [^{18}F]BR-351: ACN 1 mL), was added to the [^{18}F]K(K_{222})F residue and the reaction mixture was heated ([^{18}F]FMBP: 140 °C, 10 min or [^{18}F]BR-351: 84 °C, 20 min). The reaction was cooled to 40 °C, quenched with HPLC mobile phase (2 mL), loaded onto the HPLC loop, and purified by isocratic elution ([^{18}F]FMBP: 50/50, v/v, 0.1 M AMF/ACN, flow = 5 mL·min^{-1} or [^{18}F]BR-351: 55/45, v/v, 0.05 M NaOAc pH 5.5/EtOH, flow = 3 mL·min^{-1}). The product fraction ([^{18}F]FMBP: ~19 min or [^{18}F]BR-351: ~22 min) was collected in a bulk vessel pre-loaded with water (25 mL). The contents of the flask were transferred over a Waters Sep-Pak Plus Short C18 Cartridge (preconditioned with 1 mL EtOH, 5 mL water, and 1 mL air) and subsequently flushed with water (10 mL). The product was eluted with EtOH (1 mL) and diluted with saline (9 mL).

Quality Control Protocol

Radiochemical purity was determined using a Waters 2695 Alliance HPLC equipped with a Phenomenex Luna C18(2) (100 Å, 5 μm, 250 mm × 4.6 mm) column, a 996 Photodiode Array Detector (PerkinElmer), and a Carroll & Ramsey Associates 105-S high-sensitivity radiation detector. The following conditions were utilized for [^{18}F]FMBP: 80/20 for 2 minutes, linear gradient to 10/90 over 8 minutes , 10/90 for 2 minutes, linear gradient to 80/20 over 1 minute, 80/20 for 2 minutes, v/v, 0.1 M AMF/ACN, flow = 1 mL·min^{-1}, retention time: ~12 min and [^{18}F]BR-351: isocratic, 0-20 min: 55/45, v/v, 0.1 M AMF/ACN, flow = 1 mL·min^{-1}, retention time: ~11.5 min. Radiochemical product identity was confirmed by coinjection of the labeled compound and corresponding non-radioactive standard. Molar activity was determined by measurement of

the UV absorbance of a known amount of radioactivity under identical HPLC conditions used to generate a calibration curve for the corresponding non-radioactive standard. All quality control was completed using Empower software.

PET Imaging Protocol

Mice (45 ± 2 g, $n = 4$) were anesthetized with 2-3% isoflurane, positioned in the PET scanner, and maintained under anesthesia during the imaging protocol. Following a transmission scan, animals were injected with [^{18}F]FMBP (5 MBq) as a bolus over 30 sec via intravenous lateral tail vein injection. The whole body was imaged for 60 min (4 × 15 sec frames; 4 × 1 min frames; 10 × 5 min frames). PET imaging was performed using a Siemens DPET scanner. Emission data were corrected for attenuation and scatter, then reconstructed using the 3D-OSEM/MAP algorithm. Volumetric regions of interest (ROIs) were drawn for the myocardium and cardiac blood pool. Uptake values obtained in nCi/cc were converted to %ID/cc using the injected dose and presented as time-activity curves.

References

1. Hugenberg V, Wagner S, Kopka K, et al (2017) Radiolabeled Selective Matrix Metalloproteinase 13 (MMP-13) Inhibitors: (Radio)Syntheses and in Vitro and First in Vivo Evaluation. J Med Chem 60:307–321.
2. Wagner S, Breyholz H-J, Law MP, et al (2007) Novel Fluorinated Derivatives of the Broad-Spectrum MMP Inhibitors *N* -Hydroxy-2(*R*)-[[(4-methoxyphenyl)sulfonyl](benzyl)- and (3-picolyl)-amino]-3-methyl-butanamide as Potential Tools for the Molecular Imaging of Activated MMPs with PET. J Med Chem 50:5752–5764.