Supplementary Information for

A Reactivity-Based $[^{18}F]$FDG Probe for In Vivo Formaldehyde Imaging Using Positron Emission Tomography

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**General Methods**

2-deoxy-2-F-fluorodeoxyglucose was purchased from Carbosynth LLC (San Diego, CA). All other compounds were purchased from Sigma-Aldrich. $^1$H NMR and $^{13}$C NMR spectra were collected in D$_2$O (Cambridge Isotope Laboratories, Cambridge, MA) at 25 °C on Bruker AVB-400 with $^{13}$C operating frequencies of 101 MHz, at the College of Chemistry NMR Facility at the University of California, Berkeley. All chemical shifts are reported in the standard notation of parts per million relative to residual solvent peak at 4.80 ppm (D$_2$O) as an internal reference. Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets. Electrospray mass spectral analyses were carried out using a LC-MS (Agilent Technology 6130, Quadrupole LC/MS).

**Cell Uptake Studies**

For cell uptake experiments, 0.5 million PC3 or U87 cells in 0.5 mL PBS with given amount of FA were placed in 12-well plates. ~2 µCi of the PET probe in 2 µLPBS was added to each well. Following incubation at 37 °C, media was removed and the cells were washed twice with ice cold PBS. The cells were then lysed with 1ml of 1M NaOH and collected (cell associated fraction). The cell-associated fractions were counted in a gamma counter and expressed as a percentage of the total activity added per equal relative number of cells (as determined using a MTT Cell Proliferation Assay). Experiments were performed in triplicate.

**Xenograft Mice**

All mice were handled according to the Guide for the Care and Use of Laboratory Animals and accredited by the Association for Assessment and
Accreditation of Laboratory Animal Care. Mouse studies were carried out following the procedures approved by the Laboratory Animal Resource Center facilities of UCSF. For inoculation into nude mice, PC3 cells were washed with PBS, digested with trypsin, resuspended in DMEM containing fetal bovine serum, and pooled. After centrifugation, cells were resuspended in Matrigel (BD Biosciences Discovery Labware, Bedford, MA)-PBS (1:1) at a concentration of $5 \times 10^6$ cells per 100 µL. Cell/Matrigel mixture of 100 µL was injected subcutaneously into 6-week-old male nu/nu mice (Charles River, Wilmington, Massachusetts) on the right flank surface. Tumor volumes and body weights were monitored the day of the imaging experiments. Mice were sacrificed after the imaging and tumors and several essential organs were removed for biodistribution analysis.

**PET Imaging**

All animals were fasted 4-5 hours before PET imaging exams. Under isofluorane anesthesia, a tail vein catheter was placed. Approximately 200 µCi of FDG, FAC-FDG-1, or Ctrl-FAC-FDG-1 was injected via the tail vein catheter. The animals were then placed in original cage. At one hour post-injection, the animals were transferred to a Siemens Inveon micro PET-CT system (Siemens, Erlangen, Germany), and imaged using a single static time frame of 10 minutes. A micro-CT scan was then obtained for attenuation correction and anatomical coregistration. No adverse events were observed during or after injection of any compound. Anesthesia was maintained during imaging using isofluorane. All spectra were viewed using open source Amide software (amide.sourceforge.net).

**Probe Synthesis**

$^{18}$F Probe Synthesis (FAC-FDG-1)
[^18F]FDG (~15 mCi) was dried by evaporation at 110 °C under vacuum and nitrogen stream. To the dried[^18F]FDG was added a solution of 25 µL adamantanemethylamine (0.14 mmol) in 400 µL 1:1 EtOH/H₂O. The resulting solution was stirred at room temperature for 15 min. 25 µL pinacol allylboronate (0.14 mmol) was then added and the reaction mixture was heated at 80 °C for 30 min. The resulting mixture was then purified using semi-preparative HPLC on a gradient of 0-100% B over 40 minutes, where A = water with 0.1% formic acid, and B = acetonitrile with 0.1% formic acid. The collected solution was dried by evaporation at 80 °C under vacuum and nitrogen stream (~3 mCi final product).

**Control[^18F] Probe Synthesis (ctrl-FAC-FDG-1)**

[^18F]FAC-FAG-1 was redissolved in 200 µL ethanol. To the corresponding solution was added 10 µL acetaldehyde and 2 mg sodium cyanoborohydride. The reaction mixture was heated at 50 °C for 1h and was then purified using semi-preparative HPLC on a gradient of 0-100% B over 40 minutes, where A = water with 0.1% formic acid, and B = acetonitrile with 0.1% formic acid. The collected solution was dried by evaporation at 80 °C under vacuum and nitrogen stream.

**[^18F] FAC-FDG-1 Synthesis**
30 mg (0.16 mmol) FDG and 28 mg adamantanemethylamine (0.17 mmol, 1.06 eq.) was dissolved in 400 µL 1:1 EtOH/H₂O. The solution was stirred at room temperature for 1h. 29 mg pinacol allylboronate (0.17 mmol, 1.06 eq.) was then added and the reaction mixture was heated at 80°C for 60 min. The resulting mixture was then purified on Waters mass directed UHPLC system with 80% A and 20% B, where A = water with 0.05% formic acid, and B = acetonitrile with 0.05% formic acid. Pure fractions were combined and lyophilized to yield the title product as a white solid. 25 mg (36% yield). Isolated as a mixture of diastereomers. ¹H NMR (400 MHz, D₂O): δ 8.44 (s, 1H), 5.80 (m, 1H), 1.99 (m, 2H), 4.31 – 3.40 (m, 6H), 3.08 – 2.46 (m, 4H), 1.86 – 1.52 (m, 16 H). note: hydroxyl protons not observed. ¹³C NMR (101 MHz, D₂O), 131.41, 120.62, 91.68, 70.61, 68.47, 62.60, 57.53, 50.69, 38.95, 38.76, 35.89, 37.79, 31.96, 27.62, 23.73. HRESI/MS m/z C₂₀H₃₅FNO₄ (m+H)⁺ found 372.2561, cal’d 372.2550.

**¹⁸F-ctrl-FAC-FDG-1 Synthesis**

5 mg FAC-FDG-1 (0.016 mmol) was dissolved in 200 µL ethanol. To the corresponding solution was added 30 µL acetaldehyde (0.53 mmol) and 5 mg sodium cyanoborohydride (0.08 mmol). The solution was stirred at room temperature for 2h. The resulting mixture was then purified on Waters mass directed UHPLC system with 85% A and 15% B, where A = water with 0.05% formic acid, B = acetonitrile with 0.05% formic acid. Pure fractions were
combined and lyophilized to yield the title product as a white solid. 1.1 mg (19% yield). Isolated as a mixture of diastereomers. \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta\) 5.94 (m, 1H), 5.32 (m, 2H), 4.25 (m, 1H), 3.80 (m, 3H), 3.63 (m, 2H), 3.44 (m, 1H), 3.19 (m, 1H), 3.01 – 2.59 (m, 3H), 2.05 (s, 1H), 2.02 (s, 3H), 1.80 – 1.60 (m, 13 H), 1.35 (m, 3H). note: hydroxyl protons not observed. \(^{13}\)C NMR \(\delta\) 137.63, 114.56, 99.94, 71.65, 69.50, 69.38, 65.82, 64.56, 64.39, 63.54, 41.23, 36.82, 35.74, 28.62, 28.01, 13.69; HRESI/MS m/z C\(_{20}\)H\(_{39}\)FNO\(_4\) (m+H)\(^+\) found 400.2870, cal’d 400.2863

**Selectivity Tests**

990 \(\mu\)L of a 50 \(\mu\)Ci \([^{18}\text{F}]\)FAC-FDG-1 solution in PBS was incubated with corresponding analytes at 37°C. At indicated time, 100 \(\mu\)L of the solution was analyzed by radio-HPLC.

**FA:** 10 \(\mu\)L of 100 mM stock solution of FA in PBS buffer (freshly prepared by diluting 7.52 \(\mu\)L commercial 37 wt. % FA solution to 1mL with PBS) was added to 990 \(\mu\)L of \([^{18}\text{F}]\)FAC-FDG-1 in PBS.

**Acetaldehyde:** 10 \(\mu\)L of 100 mM stock solution of acetaldehyde in PBS buffer (freshly prepared by diluting 5.6 \(\mu\)L neat acetaldehyde to 1mL with Milli-Q water) was added to 990 \(\mu\)L of \([^{18}\text{F}]\)FAC-FDG-1 in PBS.

**Glucose:** 10 \(\mu\)L of 100 mM stock solution of glucose in PBS buffer (freshly prepared by dissolving 180 mg of glucose in 1 mL PBS) was added to 990 \(\mu\)L of \([^{18}\text{F}]\)FAC-FDG-1 in PBS.

**Methylglyoxal (1mM):** 10 \(\mu\)L of 100 mM stock solution of methylglyoxal in PBS buffer (freshly prepared by diluting 15.4 \(\mu\)L of neat methylglyoxal to 1 mL with PBS) was added to 990 \(\mu\)L \([^{18}\text{F}]\)FAC-FDG-1 in PBS.
Methylglyoxal (10 µM): 10 µL of 1 mM stock solution of methylglyoxal in PBS buffer (freshly prepared by diluting 10 µL of 100 mM stock solution of methylglyoxal in PBS to 1 mL with PBS) was added to 990 µL of [\(^{18}\)F]FAC-FDG-1 in PBS.

\( \text{H}_2\text{O}_2 \): 10 µL of 100 mM stock solution of \( \text{H}_2\text{O}_2 \) in PBS (freshly prepared by diluting 10.2 µL of commercial 9.8 M \( \text{H}_2\text{O}_2 \) to 1 mL with PBS) was added to 990 µL of [\(^{18}\)F]FAC-FDG-1 in PBS.

Benzaldehyde: 1 µL of 100 mM stock solution of benzoaldehyde in \( \text{CH}_3\text{CN} \) (freshly prepared by dissolving 10.2 µL benzoaldehyde in 1 mL \( \text{CH}_3\text{CN} \)) was added to 999 µL of [\(^{18}\)F]FAC-FDG-1 in PBS.

Sodium pyruvate: 10 µL of 100 mM stock solution of sodium pyruvate in PBS buffer (freshly prepared by dissolving 11 mg of sodium pyruvate in 1 mL Milli-Q water) was added to 990 µL of [\(^{18}\)F]FAC-FDG-1 in PBS.

Dehydroascorbate: 10 µL of 100 mM stock solution of dehydroascorbic acid in Milli-Q water (freshly prepared by dissolving 3.5 mg of dehydroascorbic acid in 200 µL Milli-Q water) was added to 990 µL of [\(^{18}\)F]FAC-FDG-1 in PBS.

Glucosone: 10 µL of 100 mM stock solution of glucosone in PBS buffer (freshly prepared by dissolving 3.6 mg of glucosone in 200 µL Milli-Q water) was added to 990 µL of a [\(^{18}\)F]FAC-FDG-1 in PBS.

**MTT Cell Proliferation Assay**

The viability of PC3 and U87 cells was assessed using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) dye reduction assay (Sigma-Aldrich). The cells were seeded in a 96-well plate at a density of \( 1 \times 10^4 \) cells/well, cultured for 24 hours, then treated with different
concentration of formaldehyde. At the end of the treatment, MTT, 50 µg/10 µL, was added, and the cells were incubated for another 3 hours. MTT stock solution (5 mg/ml) is added to each culture being assayed to equal one-tenth the original culture volume and incubated for 3 to 4 hr. At the end of the incubation period the resultant formazan crystals were dissolved in dimethyl sulfoxide (100 µl) and the absorbance intensity measured by a microplate reader (TECAN Infinite M200) at 570 nm with a reference wavelength of 620 nm; all measurements were performed six times. The cell viability is typically higher than 85% in the presence of 1mM formaldehyde and the cell-associated activity is corrected by the cell viability.

**Biodistribution Assay**

After tumors grew to approximately 200-300 mm³, a biodistribution assay was performed. All animals were fasted starting the 5 hours before PET biodistribution to minimize muscle uptake and optimize tumor to background signal. Under isofluorane anesthesia, a tail vein catheter was placed. ~ 200 µCi of the probe was injected via the tail vein catheter. The animals were then placed on in the original cage. At one hour post injection, the animals were sacrificed under isofluorane anesthesia. The indicated organs and blood were harvested and weighed, and the activity counted using a gamma counter. Calibration with known amounts of ^18F was performed to determine the amount of activity in each organ. The activity in each organ was decay-corrected, and the percent injected dose per gram of tissue was calculated and reported.
Supplementary Figures

**Figure S1.** Radio-HPLC traces of FDG generation from FAC-FDG-1 in the presence of 1000 µM FA at 30 min (top trace), 60, 120, and 180 min (bottom trace). FAC-FDG-1 shows two peaks because of the formation of diastereomers.

**Figure S2.** Cellular uptake of FAC-FDG-1 (light grey) and Ctrl-FAC-FDG-1 (dark grey) in U87 cells in the presence of 0, 200, 500, and 1000 µM FA.
Figure S3. Effect of cytochalasin B on the cellular uptake of $[^{18}\text{F}]$FAC-FDG-1 and $[^{18}\text{F}]$FDG in U87 cells.

Figure S4. Cellular uptake of FAC-FDG-1 (light grey) and Ctrl-FAC-FDG-1 (dark grey) in the PC3 cell line in the presence of 0, 200, 500 and 1000 μM FA.
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Figure S11. $^1$H NMR spectra of $[^{19}$F$]$Ctrl-FAC-FDG-1.
Figure S12. $^{13}$C NMR spectra of $[^{19}F]$Ctrl-FAC-FDG-1.