Supplementary Material (ESI) for Chemical Communications

Supplementary information:

Fluorinase mediated C-^{18}F bond formation, an enzymatic tool for PET labelling

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Materials

L-Amino acid oxidase (*Crotalus adamanteus*, type I, E.C. 1.4.3.2. 0.3 unit/mg), 5'-adenylic acid deaminase (*Aspergillus sp.*, 0.1 unit/mg), phytase (alkaline, Type VII-S, 2,000-4,000 DEA units/mg protein), L-methionine and S-adenosyl-L-methionine (SAM) were purchased from the Sigma Chemical Co, UK. Immobilised purine nucleotide phosphorylase beads were kindly donated by GlaxoSmithKline (Ulverston, UK). Recombinant 5'-FDAS was purified as previously described (*Nature*., **2004**, *547*, 111-114)

Typical fluorinase reaction with [^{18}F]-fluoride

A typical reaction mixture containing the fluorinase (8mg/ml at 100µL = 1.85x10^{-3} unit), SAM 20mM (20 µl), [^{18}F]HF (200-350 MBq in ^{18}O-water, 100 µl), L-amino acid oxidase (0.15 unit) or 5'-adenylic acid deaminase (0.05 unit) (5'-FDI production) or immobilised PNP enzyme (~10 mg) and phytase (1.75 unit) was incubated at room temperature or 35 ^{O}C. The reaction product mixture was applied to the top of the packing bed of an anion exchange column (Alltech, SAX, 50mg), which had been pre-washed with deionized water (10ml). This resulted in an eluent containing the radioactive products.

Enzymatic radiolabelling assays for [^{18}F]-5'-FDA and [^{18}F]-5'-FDI

The ratio of the total radioactivity of eluent against the radioactivity retained (unreacted [^{18}F]-fluoride) on the column was used to calculate the total yield of radioactive product. This fraction was then loaded onto a reverse phase Spheraclone 5µ ODS(2) column (Phenomenex, 250 x 4.6 mm) and the column eluted (1 ml/min) isocratically with NaH_2PO_4 (70mM) solution and acetonitrile (90:10 v/v) over a 10 min period. The eluent was monitored simultaneously by UV (260nm, Beckman Coulter, System Gold 168 Detector) and by radioactivity using a Packard Flow Scintillation Analyzer. For the production of [^{18}F]-5'-FDA only two radiochemical activities were detected, the predominant one corresponded to [^{18}F]-5'-FDA and the minor one (retention time = 2.5 min) equated to some [^{18}F]-fluoride bound to protein leaked from the anion exchange column (see Figure 1). For the production of [^{18}F]-5'-FDI, three radiochemical products were detected, two corresponded to [^{18}F]-5'-FDI and [^{18}F]-5'-FDA and the other was again [^{18}F]-fluoride bound to protein leaked from the anion exchange column (see Figure 2). The retention times of [^{18}F]-5'-FDA and [^{18}F]-5'-FDI were 5.6 min and 3.6 min respectively.

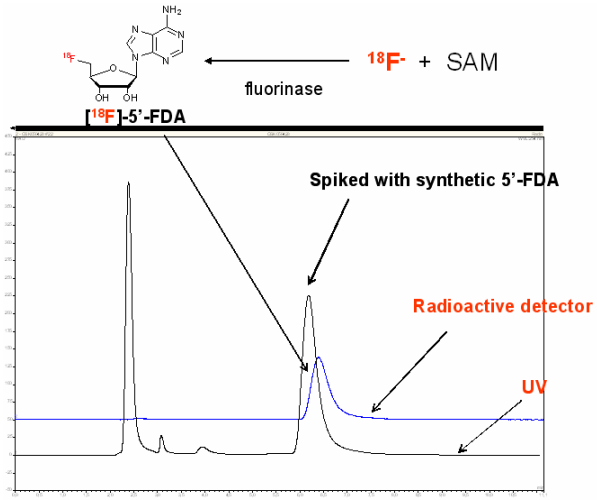

Fig1. A HPLC chromatograph showing both UV and radioactivity detector outputs for the production of [^{18}F]-5'-FDA (RT = 5.6 min) after fluorinase incubation.

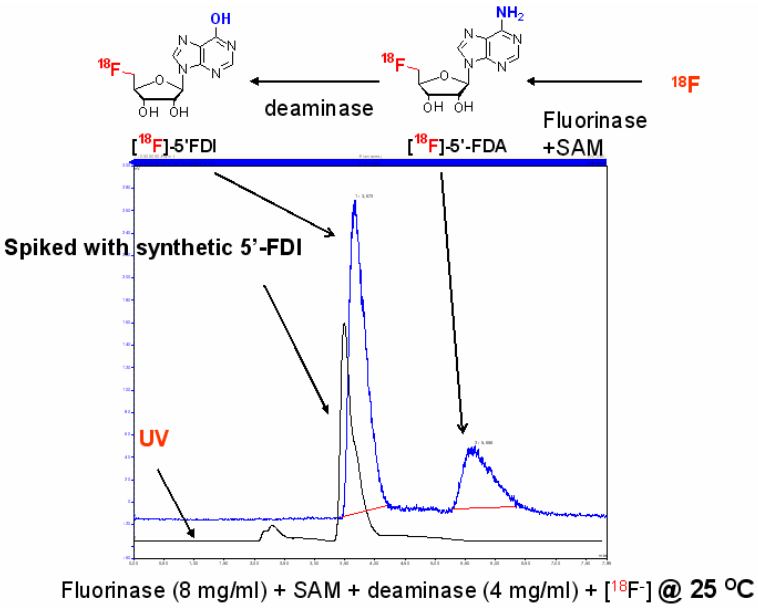

Fig 2. A HPLC chromatograph showing both UV and radioactivity detector outputs for the production of [^{18}F]-5'-FDI and [^{18}F]-5'-FDA (RT = 5.6 and 3.6 min, respectively) in a coupled enzyme system (fluorinase + deaminase).

Enzymatic radiolabelling assay for [^{18}F]-5-FDR

The radioactive fraction eluted from the anion exchange column was injected onto a Dionex Carbopac column (PA 100) and eluted (0.5 ml/min) using an isocratic mobile phase of NaOH (0.5 M) for 10 min. Only two radiochemical activities were detected as illustrated in Figure 3. One corresponded to [^{18}F]-5-FDR (retention time of 2.8min). The other at a retention time of 2.3 min was a combination of [^{18}F]-5'-FDA and [^{18}F]-fluoride attached to the protein which had leaked from the anion exchange column.

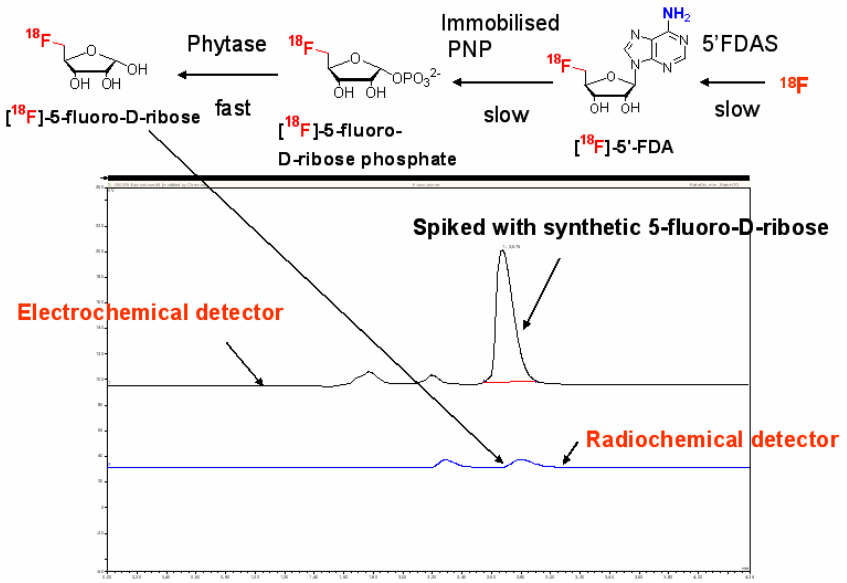

Fig. 3 A HPLC chromatography coupled with electrochemical and radioactivity detectors for the production of [^{18}F]-5-FDR (RT = 2.8 min). Two radiochemical peaks were evident one of which is [^{18}F]-5-FDR and the other is polar [^{18}F]-fluoride/enzyme material.