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TITLE:

^{18}F-Labeling of Radiotracers Functionalized with a Silicon Fluoride Acceptor (SiFA) for Positron Emission Tomography

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KEYWORDS:

^{18}F-radiolabeling, radiotracer, in vivo imaging, positron emission tomography (PET), fluorine-18, silicon-fluoride acceptor (SiFA)

SUMMARY:

The synthesis of fluorine-18 (^{18}F) labeled radiopharmaceuticals for positron emission tomography typically requires months of experience. When incorporated into a radiotracer, the silicon-fluoride acceptor (SiFA) motif enables a simple ^{18}F-labeling protocol that is independent of costly equipment and preparatory training, while reducing precursor quantity needed and utilizing milder reaction conditions.

ABSTRACT:

The para-substituted di-*tert*-butylfluorosilylbenzene structural motif known as the silicon-fluoride acceptor (SiFA) is a useful tag in the radiochemist's toolkit for incorporating radioactive [^{18}F]fluoride into tracers for use in positron emission tomography. In comparison to conventional radiolabeling strategies, isotopic exchange of fluorine-19 from SiFA with [^{18}F]fluoride is carried out at room temperature and requires minimal reaction participants. The formation of by-products is thus negligible, and purification is greatly simplified. However, while the precursor molecule used for labeling and the final radiolabeled product are isotopically discrete, they are chemically identical and are thus inseparable during purification procedures. The SiFA tag is also susceptible to degradation under the basic conditions arising from the processing and drying of [^{18}F]fluoride. The '4 drop method', wherein only the first 4 drops of eluted [^{18}F]fluoride are used from the solid-phase extraction, reduces the amount of base in the reaction, facilitates lower molar amounts of precursor, and reduces degradation.

INTRODUCTION:

Fluorine-18 (109-minute half-life, 97% positron emission) is among the most important radionuclides for positron emission tomography (PET), a noninvasive imaging method that visualizes and quantifies the bio-distribution of radiolabeled tracers for various diseases[1]. Peptides and proteins are especially difficult to label with [^{18}F]fluoride because they require building blocks formed by multi-step syntheses[2]. To reduce the complexity of ^{18}F-radiolabeling, silicon-fluoride acceptor (SiFA) was recently introduced as reliable tools[3]. The SiFA group consists of a central silicon atom connected to two tertiary butyl groups, a derivatized phenyl moiety, and a non-radioactive fluorine atom. The tertiary butyl groups impart hydrolytic stability to the silicon-fluoride bond, which is a critical feature for in vivo applications of SiFA conjugates as imaging agents.

When attached to a small molecule or biomolecule, the SiFA building blocks bind radioactive [^{18}F]fluoride anions by exchanging fluorine-19 for fluorine-18 at nanomolar concentrations without forming significant amounts of radioactive side products[4]. Moreover, a high radiochemical yield is quickly achieved by labeling the SiFA moiety in dipolar aprotic solvents at low temperatures. This is in stark contrast to classical isotopic exchange reactions, which produce radiotracers of low specific activity[5]. In these cases, large amounts of precursor (in the range of milligrams) must be used to obtain reasonable incorporation of [^{18}F]fluoride. Isotopic exchange reactions using SiFAs are far more efficient, as confirmed by kinetic studies and density functional theory calculations[6,7]. Labeled SiFAs are easily purified by solid-phase extraction since both the labeled and unlabeled SiFA compounds are chemically identical. This differs from traditional radiolabeled tracers, where the precursor molecule and the labeled product are two different chemical species and must be separated after radiolabeling by high-performance liquid chromatography (HPLC). Using SiFA building blocks, small-molecules, proteins, and peptides can be successfully labeled with [^{18}F]fluoride by one- and two-step labeling protocols devoid of complicated purification procedures (**Figure 1**)[4,8,9]. Moreover, some SiFA-labeled compounds are reliable in vivo imaging agents for blood flow and tumors[10]. The simplicity of SiFA chemistry enables even untrained investigators to use [^{18}F]fluoride for radiotracer synthesis and development.

PROTOCOL:

CAUTION: One must keep in mind that ^{18}F is a radioactive isotope, and therefore it is necessary to carry out all procedures behind adequate shielding. Lead shielding is appropriate for this type of radiation. Be sure to wear radiation detection badges throughout the entirety of this procedure. Additionally, immediately dispose of gloves before touching anything after the synthesis, as they may be contaminated with radioactive activity. Utilize hand-foot monitors as well as pancake Geiger counters to check for contamination of sleeves, hands, and feet.

1. Azeotropic drying of ^{18}F-anion

NOTE: **Figure 2A** shows a workflow graph of this procedure, which takes ~10 min.

1.1. Precondition a quaternary methyl ammonium (QMA) anion exchange cartridge (**Table of Materials**) by passing 0.5 M K_2CO_3 (10 mL) through the cartridge, followed by deionized water (10 mL).

1.2. Pass an aqueous solution of $[^{18}F]F^-/[^{18}O]H_2O$ (100−500 MBq) through the preconditioned QMA cartridge in reverse, using a male to male adapter. Discard the $[^{18}O]H_2O$.

NOTE: These steps can be performed using an automated synthesis module, or by using additional shielding on the syringe.

1.3. Elute the first four drops of the fixed $[^{18}F]$fluoride anions from the QMA cartridge into a prepared solution of [2.2.2]cryptand (**Table of Materials**) (10 mg), 0.2 M K_2CO_3 (50 µL, 10 µmol), and acetonitrile (1 mL) in a thick-walled v-vial, and seal the vial.

NOTE: Only the first four drops are used as the majority of the radioactive $[^{18}F]$fluoride is eluted off the QMA in these drops. This reduces the amount of base carried forward in the $[^{18}F]$fluoride stock solution, which is necessary to avoid degradation of the SiFA moiety.

1.4. Seal the vial and place in a 90 °C mineral oil bath positioned on a hot plate. Insert a vent needle and a needle connected to a stream of argon gas into the septum of the vial cap. Wait 5 min to evaporate the solvents under the gentle stream of argon. Remove any remaining traces of water by adding 1 mL of acetonitrile to facilitate azeotropic co-evaporation. Repeat this step 2x to ensure dryness.

1.5. Once the solvent is visibly removed, stop the argon flow, and remove the syringes from the vial cap, and remove the vial from the oil bath.

1.6. Resuspend the dried $[^{18}F]$fluoride in the reaction solvent of choice.

NOTE: In this case, acetonitrile (1 mL) is added to create a stock solution of highly reactive $[^{18}F^-]F^-$ (100−500 MBq). This solution can now be used for labeling.

2. One-step SiFA-ligand labeling

NOTE: **Figure 2B** shows a workflow graph of this procedure, which takes ~15 min.

2.1. Precondition a C-18 cartridge (**Table of Materials**) by rinsing it with ethanol (10 mL) and distilled water (10 mL).

2.2. Add the [$^{18}F^-$]fluoride stock solution to a reaction vial containing a SiFA-labeled precursor (100 µL, 20−100 nmol). Allow the labeling reaction to proceed for 5 min at room temperature without stirring the solution.

NOTE: The entire stock solution can be added or an aliquot, depending on how much activity is desired for the reaction.

2.3. Draw up the reaction mixture in a 20 mL syringe containing 0.1 M phosphate buffer (9 mL) and pass the solution through the preconditioned C-18 cartridge to trap the labeled tracer.

2.4. Wash the cartridge with distilled water (5 mL), then elute trapped tracer from the C-18 cartridge with ethanol (300 µL), and dilute with sterile phosphate buffer for injection (3 mL).

2.5. Pass the purified [^{18}F]SiFA-tracer through a sterile filter.

NOTE: To obtain a clear PET imagine for small animal imaging, the partitioned patient dose should be between 5−8 MBq. For human use, the partitioned patient dose should be between 200−300 MBq.

2.6. Inject a small aliquot (~4 MBq) of the purified [^{18}F]SiFA-tracer onto an HPLC system equipped with a reversed-phase C-18 column to confirm that the radiochemical purity is greater than 95%.

REPRESENTATIVE RESULTS:

The simplistic SiFA isotopic exchange can achieve high a degree of radiochemical incorporation of [^{18}F]fluoride (60−90%) with a minimum amount of synthetic complexity (**Figure 1**). Most molecules can be radiolabeled with [^{18}F]fluoride in one step without involving HPLC for purification (**Figure 2**). Radio-HPLC can be used for quality control purposes, wherein the ultraviolet (UV) absorbance peak of the final product should coincide with its radio peak greater than 95% total peak area (**Figure 3**). If radio-HPLC chromatogram reveals a significant formation of UV active or radioactive impurities, the precursor may not be stable under the mildly basic radiolabeling conditions. A dilute solution of oxalic acid dissolved in an organic solvent may be added to the [^{18}F]fluoride stock solution prior to the addition to SiFA-precursor in an effort to lower the basicity; however, lowering the basicity too much will lessen the nucleophilicity of the [^{18}F]fluoride anion. Thus, the molar amount of oxalic acid needed will have to be experimentally determined beforehand. Alternatively, the labeled SiFA-ligand can be purified by HPLC after step 2.3 if the formation of impurities is significant but manageable. Steps 2.4 and beyond will still be

needed after HPLC purification in order to remove HPLC solvent from the purified labeled SiFA-ligand.

If the [^{18}F]fluoride is not readily incorporated into SiFA-ligand, there may be issues with solubility and another aprotic solvent of choice may be used in place of acetonitrile for the reaction. Protic solvents, such as ethanol, have been used successfully but may require heating. Monitoring the reaction by radio-thin-layer chromatography (radio-TLC) can quickly aide in identifying the outcome of any changes made to the labeling protocol as unincorporated [^{18}F]fluoride will adhere to the baseline on a standard silica TLC plate.

If the labeled SiFA-ligand passes through the C18 cartridge in step 2.3, as indicated by the bulk of the activity appearing in the eluted solution and not in the cartridge, then the phase of the cartridge used may have to be changed. Polar SiFA-ligands may need a larger C18 cartridge or a dual-phase cartridge containing a C18 resin with some hydrophilic characteristics.

Labeled SiFA-ligands can also be used for in vivo applications such as PET. For instance, the labeled small molecule [^{18}F]SiFA-PSMA (**Figure 4A**) was used to image an implanted tumor xenograft in a mouse model (**Figure 4B**). The SiFA-tracer displayed favorable tumor uptake over 60 min, which could be blocked by a competitive inhibitor (**Figure 4C**). More impressively, the ^{18}F-labeled peptide [^{18}F]SiFA*lin*-TATE (**Figure 5A**) was used to image metastatic neuroendocrine tumors in a cancer patient via PET (**Figure 5B**) and PET/CT (**Figure 5C**)[11].

FIGURE LEGENDS:

Figure 1: Overview of the SiFA ^{18}F-radiolabeling workflow.

Figure 2: Typical SiFA ^{18}F-radiolabeling protocol. (**A**) Azeotropic drying of [^{18}F]fluoride. (**B**) SiFA labeling reaction and purification via solid-phase extraction.

Figure 3: Final radio-HPLC chromatogram after solid-phase extraction purification of [^{18}F]SiFA-PSMA, taken for quality control.

Figure 4: Application of labeled SiFA-ligands. (**A**) Structure of the [^{18}F]SiFA-PSMA radiotracer. (**B**) Reconstructed image of a mouse carrying an LNCaP xenograft tumor over left shoulder, 60 min post injection (p.i.) with [^{18}F]SiFA-PSMA. (**C**) Time-activity curves for [^{18}F]SiFA-PSMA uptake in tumor and muscle tissues over 60 min, with or without prior administration of 300 µg DCFPyL as a blocking agent.

Figure 5: Application of labeled SiFA-ligands. (**A**) Structure of the [^{18}F]SiFA-TATE radiotracer. (**B**) Reconstructed PET image of a human cancer patient with metastatic endocrine tumors with [^{18}F]SiFA-TATE. (**C**) PET/CT images of the same patient in the transverse and sagittal planes.

DISCUSSION:

SiFA labeling chemistry represents one of the first ^{18}F-labeling methods employing an extraordinarily efficient isotopic exchange reaction that can be performed at room temperature. A typical radiochemical reaction relies on the formation of a carbon-fluorine bond via reaction of [^{18}F]fluoride with a fluoride-reactive functionality through an elimination or substitution pathway. These reaction conditions are often harsh, performed at extreme pH or high temperature, and are laden with byproducts or reaction participants that must be removed using laborious and time-consuming techniques such as HPLC. With the SiFA-labeling technique, the labeling precursor and ^{18}F-labeled compound are chemically identical. Moreover, no side products are usually observed since the reaction proceeds under very mild conditions. These features make it possible to label more complicated molecules (i.e., proteins, free radical generators, metal-chelates, fluorophores, bioluminescent precursors) that may normally decompose or epimerize under more reactive conditions or elevated temperatures. Additionally, ^{18}F-labeled SiFA-containing compounds can be purified quickly using simple solid-phase extraction techniques.

This labeling methodology utilizes the '4 drop method', wherein only the first 4 drops of the basic elution solution are used when eluting trapped [^{18}F]fluoride off of a QMA cartridge. This modification was made to reduce the amount of base in the [^{18}F]fluoride stock solution as it would degrade the SiFA moiety if the [^{18}F]fluoride stock contained all of the base from the elution solution. Previously, oxalic acid was added to the [^{18}F]fluoride stock solution to reduce basicity, or a small aliquot of the stock was used instead of the entire solution, which was wasteful. The '4 drop method' represents the latest iteration of the SiFA-labeling protocol.

As the precursor molecule and the labeled final product are chemically identical, they cannot be separated from each other during purification and the molar activity of the final injectable is thus entirely dependent on the quantity of precursor used for the isotopic exchange. Having too high of a fraction of unlabeled precursor in the final solution will decrease the opportunity of the labeled SiFA-ligand to bind its intended molecular target due to competition with the unlabeled precursor. Thus, the molar activity is entirely dependent on the amount of precursor that is used for labeling. Typically, 20−100 nmol of precursor is required for reproducible labeling reactions, and as little as 5 nmol of precursor has been labeled successfully to achieve molar activities of 80 GBq/μmol and higher.

Small molecules and peptides derivatized with the SiFA building block (e.g., SiFA-octreotate) can be labeled with [^{18}F]fluoride in one step; however, SiFA labeling of proteins requires a two-step protocol. A small, highly reactive SiFA-prosthetic group (e.g., [^{18}F]SiFB) has to be prepared and reacted with the given protein, and the labeled protein must then be purified by HPLC.

The SiFA-labeling methodology lends itself well to radiopharmaceutical kit syntheses as HPLC purification and extensive reaction manipulation are not typically needed. Simple 'shake and bake' style kits with single patient dose quantities of a SiFA-ligand could be easily handled by radiopharmacy technicians—requiring a much smaller learning curve and time/labor cost than with an automated synthesis unit.

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The authors have no acknowledgements.

DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

Figure 2

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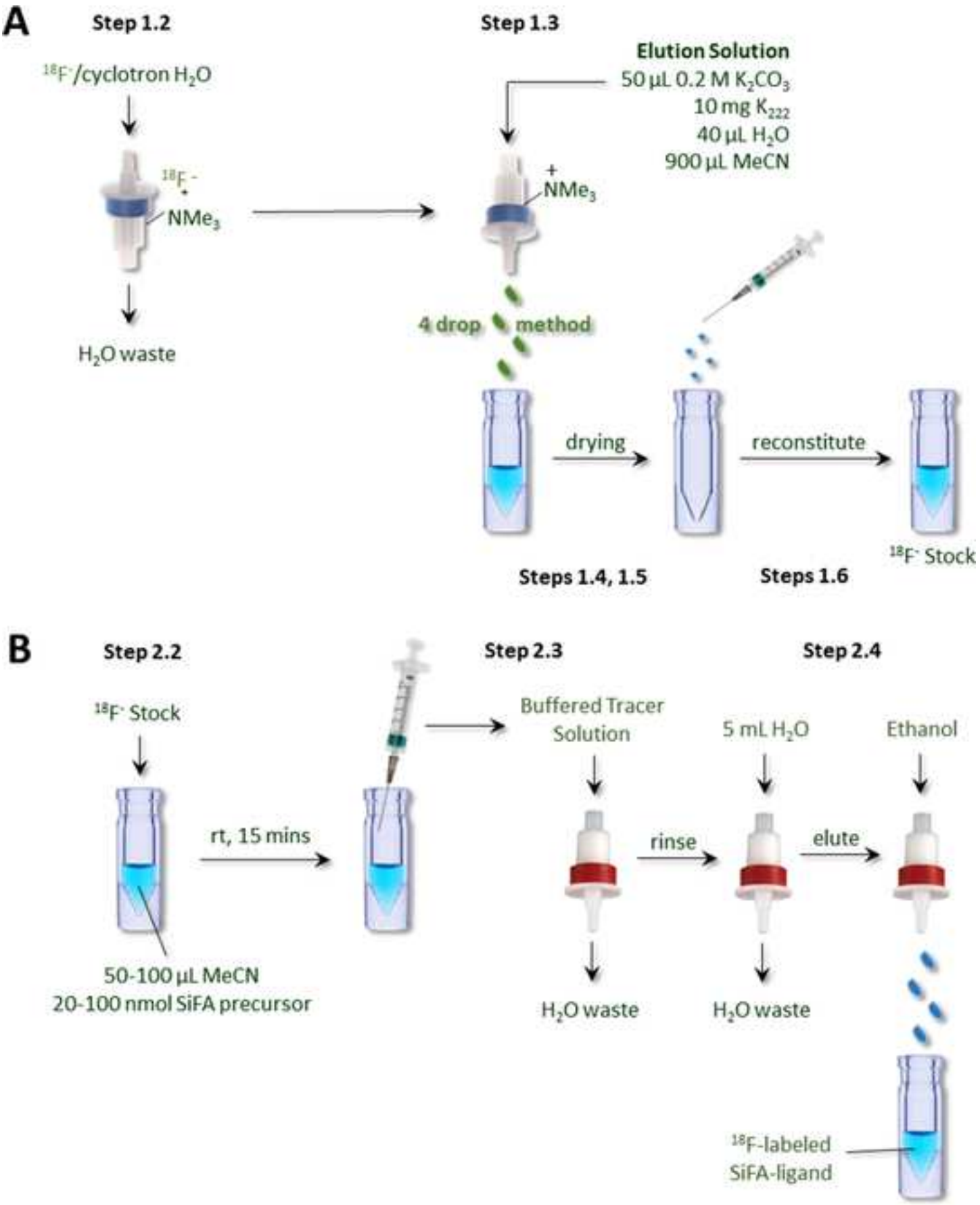

Figure 3

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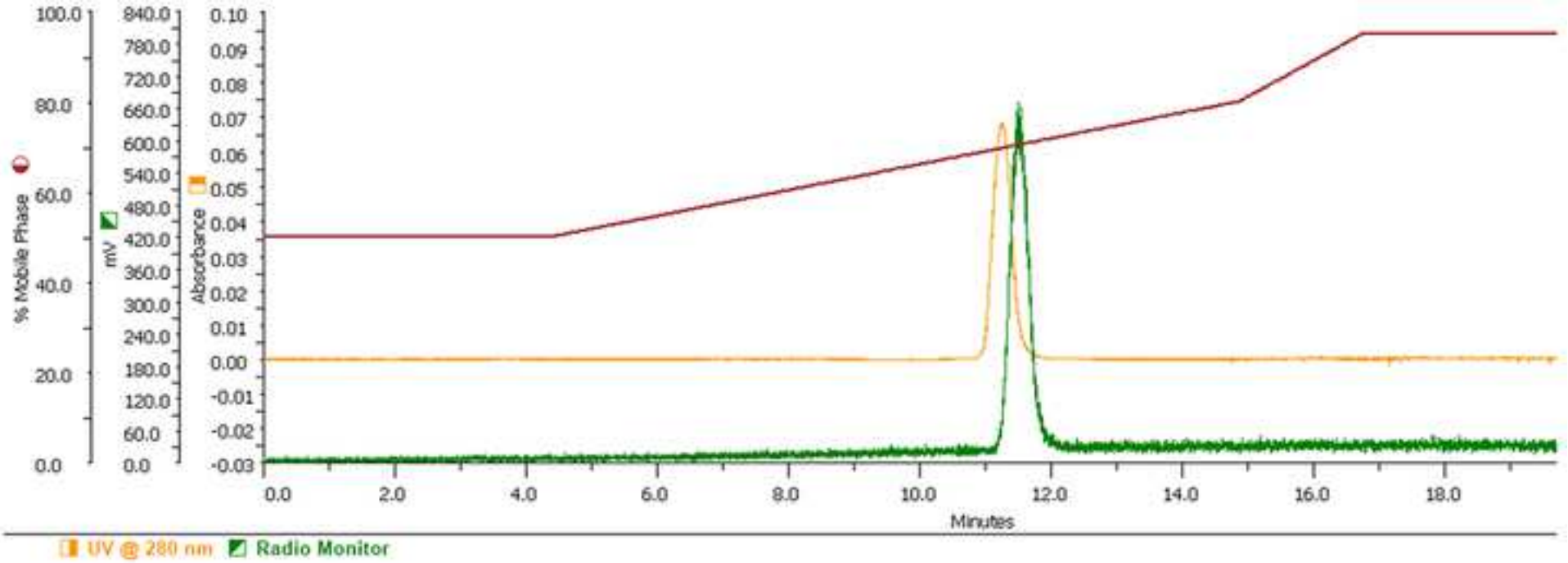


Figure 4

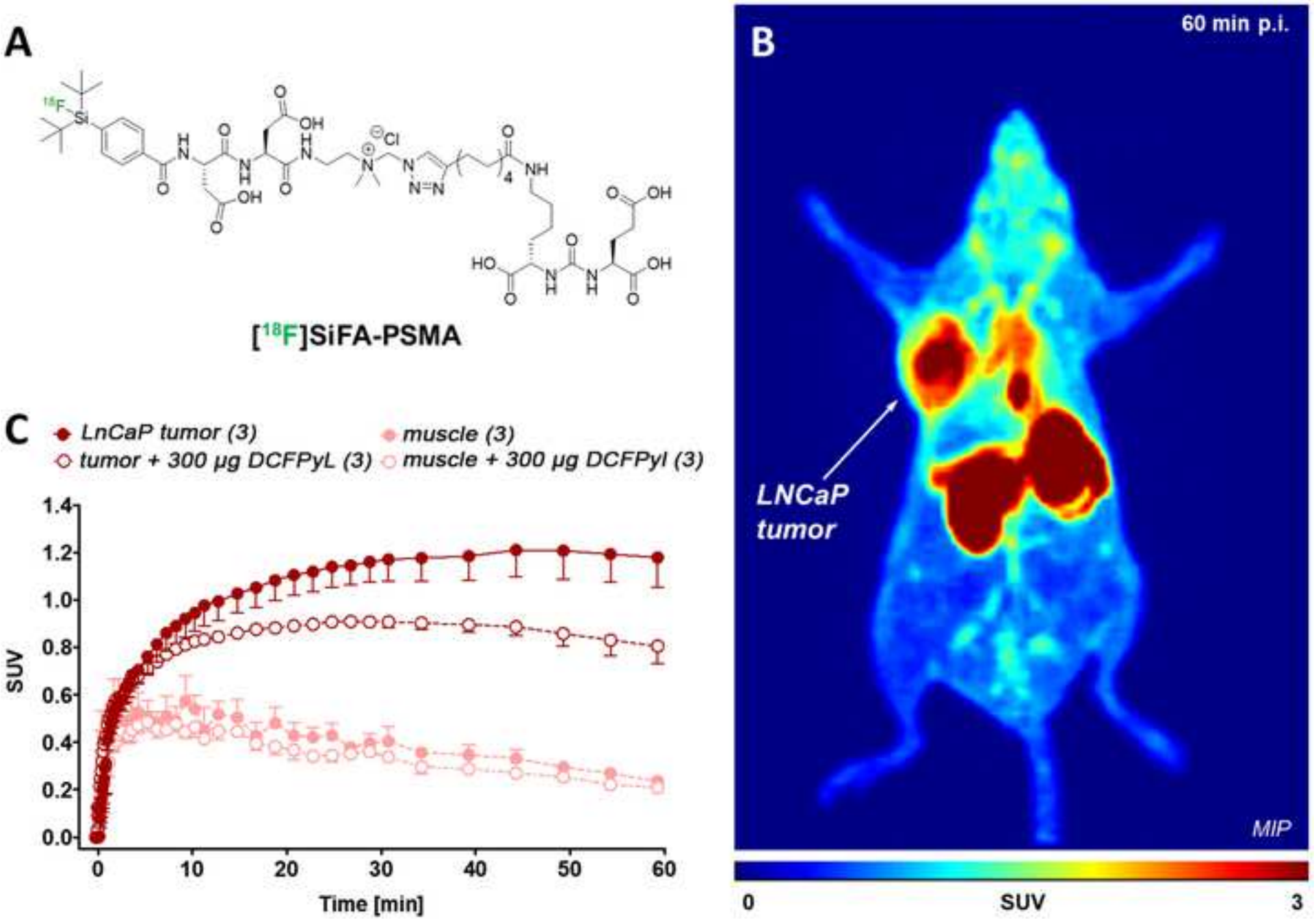
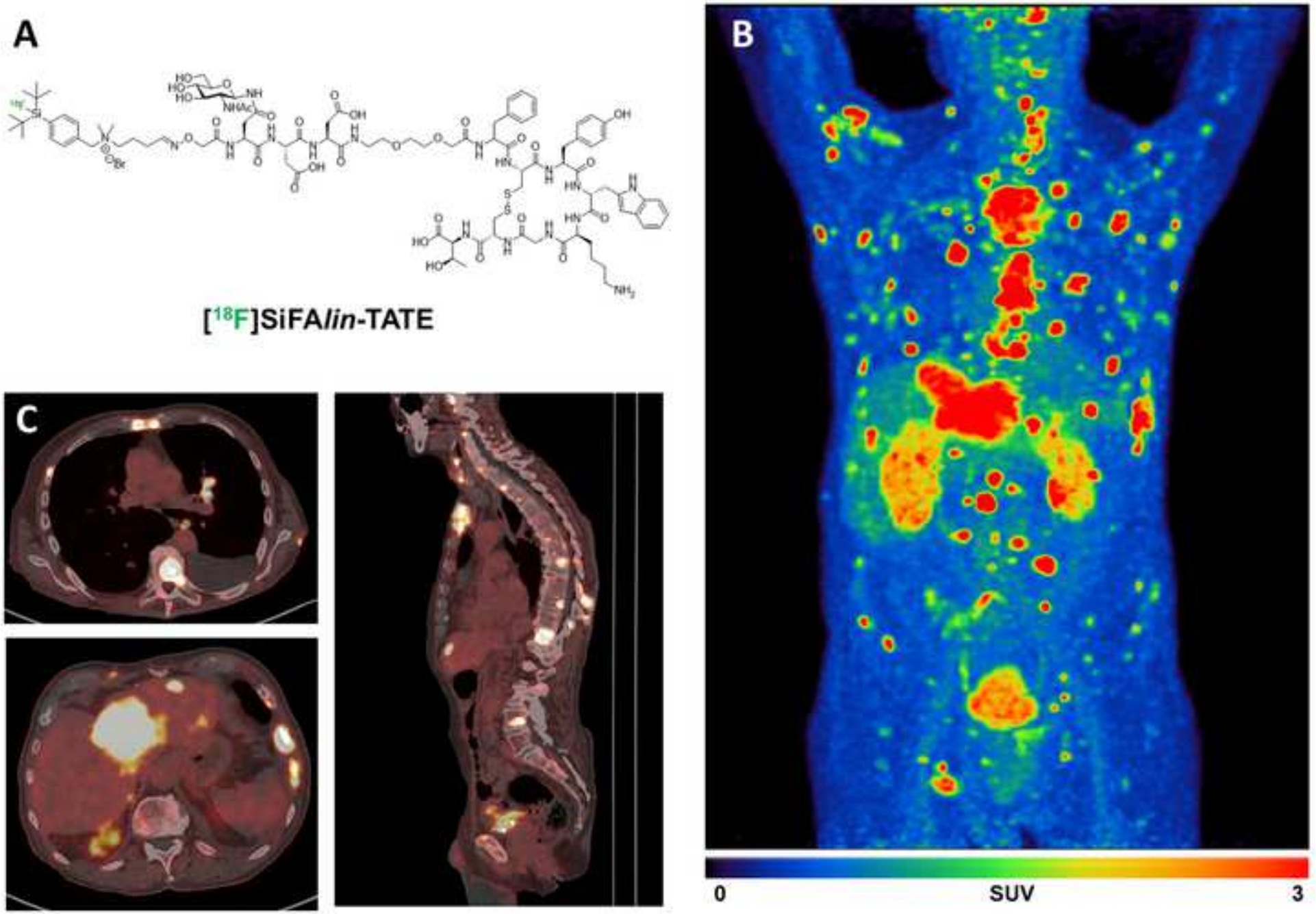


Figure 5



Material	Company	Catalog Number	Comments/Description
$[^{18}F]F^-/H_2[^{18}O]O$	(Cyclotron produced)	-	-
[2.2.2]Cryptand	Aldrich	291110	Kryptofix 2.2.2
Acetonitrile anhydrous	Aldrich	271004	-
Deionized water	Baxter	JF7623	-
Ethanol, anhydrous	Commercial Alcohols		-
Potassium carbonate	Aldrich	209619	-
QMA cartridge	Waters	186004540	QMA SepPak Light (46 mg) cartridge
Equipment			
C-18 cartridge	Waters	WAT023501	C-18 SepPak Light cartridge
C18 column	Phenomenex	00G-4041-N0	HPLC Luna C18 250 x 10 mm, 5 µm
HPLC	Agilent Technologies	-	HPLC 1200 series
micro-PET Scanner	Siemens	-	micro-PET R4 Scanner
Radio-TLC plate reader	Raytest	-	Radio-TLC Mini Gita
Sterile filter 0.22µm	Millipore	SLGP033RS	-

Rebuttal Letter

To Whom It May Concern,

In this rebuttal letter we hope to address the editorial comments we received.

Editorial Comments

1. We have addressed the five comments made and submitted the updated manuscript.
2. The summary is now 50 words.
3. We have removed the citation from the video.
4. The updated video has been uploaded.

Sincerely,

David Connolly & Justin James Bailey