Video Article

Silicon-Fluoride-Receptors (SiFAs) for the 18F-labeling of peptides and proteins for Positron-Emission-Tomography

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Abstract

Fluorine-18 (109 minute half-life, 97% positron emission ^{18}F) 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 because they require building blocks formed by multi-step syntheses [2]. Otherwise, macromolecules would constitute a large proportion of available PET tracers. To reduce the complexity of ^{18}F-radiolabeling, silicon fluoride acceptors (SiFAs) were 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 two 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 bio-molecule, the SiFA building blocks bind radioactive ^{18}F anions by exchanging ^{19}F for ^{18}F at nanomolar concentrations without forming significant amounts of radioactive side products [4] (**Figs. 1,2**). 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 a reasonable incorporation of ^{18}F. 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; normally, the labeling precursor and the labeled product are two different chemical species that must be separated by high-performance liquid chromatography (HPLC). Using SiFA building blocks, peptides and proteins are successfully labeled with ^{18}F by one- and two-step labeling protocols devoid of complicated purification procedures [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 for radiotracer synthesis and development.

Video Link

The video component of this article can be found at http://www.jove.com/video/3222/

Protocol

1. Azeotropic Drying of ^{18}F-Anion

1. Precondition a QMA SepPak Light cartridge (Waters) with 0.5 M K_2CO_3 (10 mL) followed by deionized water (10 mL).
2. Pass aqueous [^{18}F]F-/H_2[^{18}O]O (5-50 mCi) through the preconditioned QMA cartridge. Discard the H_2[^{18}O]O.
3. Elute the fixed ^{18}F- from the QMA cartridge into a Wheaton vial with a premade solution of Kryptofix 2.2.2 (10 mg), 0.2 M $K_2C_2O_4$ (50 µL, 10 µmol), and acetonitrile (1 mL). Place the vial in a mineral oil bath positioned on a hot plate.
4. Remove the solvents under a gentle vacuum (Heidolph pump, 500 mbar) and a sweep stream of argon at 90°C. Remove any remaining traces of water by azeotropic co-evaporation with acetonitrile (1 mL). Complete this step twice to ensure dryness.
5. Once the solvents are visibly removed, stop the argon flow, set the vacuum to maximum (10 mbar), and dry for another two minutes.
6. Add acetonitrile (1 mL) to the dry residue to make a solution of highly reactive ^{18}F- (5-50 mCi).

2. One-Step SiFA-Peptide Labeling

1. Precondition a C-18 SepPak Light cartridge (Waters) by subsequently rinsing it with ethanol (10 mL) and distilled water (10 mL).
2. Add the ^{18}F- (450-500 µL, 5-50 mCi) to a solution containing a SiFA-peptide (**Fig. 1A**). Allow the labeling reaction to proceed for five minutes at room temperature without stirring the solution.

3. Add 0.1 M phosphate buffer (9 mL, pH 4) to the solution, and load the mixture onto the preconditioned C-18 cartridge.
4. Wash the cartridge with distilled water (5 mL), elute trapped [^{18}F]SiFA-peptide from the C-18 cartridge with ethanol (300 µL), and dilute with sterile phosphate buffer for injections (3 mL).
5. Pass the purified [^{18}F]SiFA-peptide through a sterile filter. To obtain a clear PET image, the final product's concentration should be between 0.5-2 mCi/mL (**Fig. 1A**).
6. Inject a small aliquot (40 µL) of the purified [^{18}F]SiFA-peptide onto an HPLC system (Agilent Technologies) equipped with a reversed phase C-18 column (LiChrosorb RP-select) to confirm that the radiochemical purity is greater than 95% (**Fig. 1B**).

3. Two-Step Protein Labeling via succinimidyl 3-(di-tert-butyl[^{18}F]fluorosilyl)benzoate ([^{18}F]SiFB)

1. Precondition a C-18 SepPak Light cartridge (Waters) with ethanol (10 mL) followed by distilled water (10 mL).
2. Add a solution of 0.1 M $H_2C_2O_4$ (50 µL, 5 µmol) in anhydrous acetonitrile to the ^{18}F- solution (1 mL, 30-50 mCi).
3. Add succinimidyl 3-(di-*tert*-butylfluorosilyl)benzoate (SiFB) (50 µL, 130 nmol, 1 µg/µL in acetonitrile) to the ^{18}F- solution. Keep the reaction mixture at room temperature for five minutes.
4. Dilute the mixture with distilled water (10 mL), pass through the preconditioned C-18 cartridge, and wash the C-18 cartridge with water (10 mL). Elute the [^{18}F]SiFB from the cartridge with diethyl ether (3 mL).
5. Evaporate the diethyl ether under a stream of argon.
6. Use radio-thin layer chromatography (Raytest) to confirm that the radiochemical purity is greater than 98% (**Fig. 3**).
7. Re-dissolve the [^{18}F]SiFB in borate buffer (0.5 mL, pH 9), and add to a solution of protein (0.5 mL). In this example, we used rat serum albumin (RSA) dissolved in borate buffer (0.5 mL, 2 mg/mL).
8. After 30 minutes at room temperature, inject 5 mCi of the solution onto an HPLC system equipped with a size exclusion column (BIOSEP-SEC-S4000) to purify the labeled protein. Use the collected [^{18}F]SiFB-labeled RSA protein fraction (**Fig. 4**) in animal PET studies to visualize the blood pool.

4. Applying SiFA-Protein Labeling *In Vivo* using Positron Emission Tomography

1. Inject [^{18}F]SiFB-RSA (1 mCi) into a healthy, anesthetized rat that is positioned in a micro-PET scanner (Siemens).
2. Acquire dynamic PET data for one hour. Reconstruct the PET image using two-dimensional filtered backprojection to create a time-series of three-dimensional images.
3. Define volumes of interest within each time frame to determine the amount of activity in a specific region over a period of time.

5. Representative Results

The SiFA technique is an easy approach to labeling peptides and proteins with a minimum amount of complexity. Peptides can be labeled with ^{18}F in one step without involving HPLC purification. After solid phase extraction, the labeled peptide has a radiochemical purity greater than 95% (**Fig. 1B**) and can be easily formulated for *in vivo* PET applications. Labeling larger molecules, like proteins, requires the synthesis of small SiFA-bearing labeling synthons, such as [^{18}F]SiFB, followed by active ester labeling of free amino groups in the lysine side-chains of a protein. After purification by size-exclusion chromatography, labeled proteins have a radiochemical purity greater than 98% (**Fig. 3**). Labeled proteins can also be used for *in vivo* applications; in this example, the labeled rat serum albumin was a valuable tool to measure the blood pool in a healthy rat using PET (**Fig. 5**).

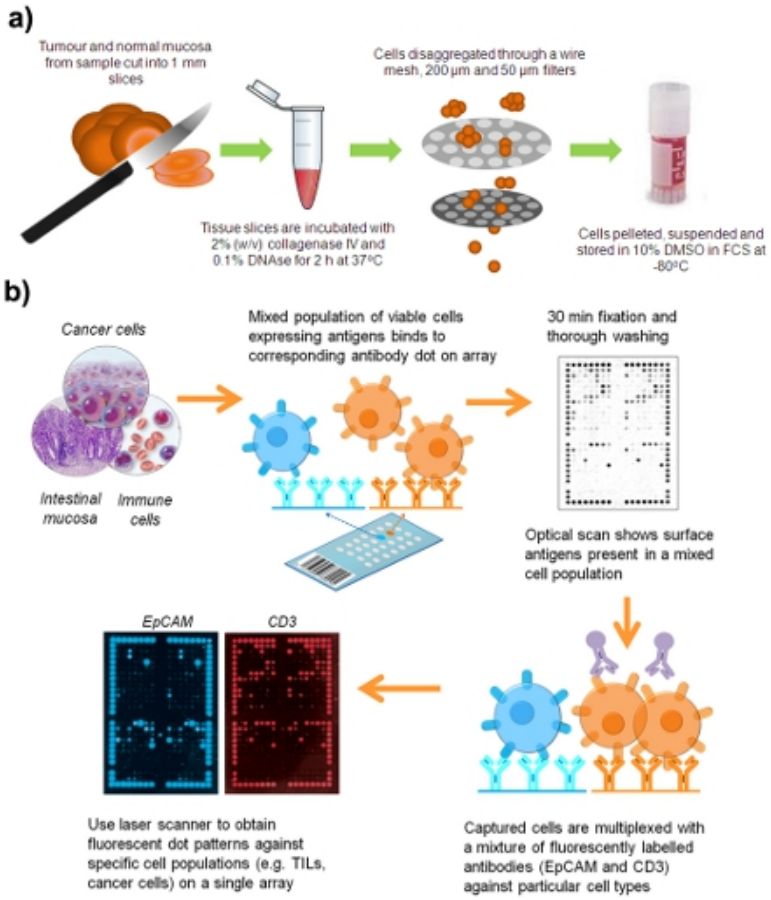

Figure 1. Synthesis of [^{18}F]SiFA+-maleimido-Tyr3-octreotate ([^{18}F]SiFA+-MI-TATE): **A)** Solution phase conjugation of SiFA+-SH to MI-TATE at pH 9 and radioactive labeling of [^{18}F]SiFA+-MI-TATE via simple isotopic exchange (^{19}F-^{18}F); **B)** HPLC quality control of solid phase cartridge purified [^{18}F]SiFA+-MI-TATE showing 99.6% purity of the labeled peptide and total absence of un-reacted ^{18}F-

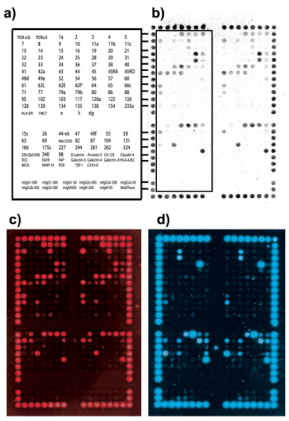

Figure 2. Succinimidyl 3-(di-tert-butyl[^{18}F]fluorosilyl)benzoate ([^{18}F]SiFB): **A)** Synthesis and **B)** Bio-conjugation to a protein

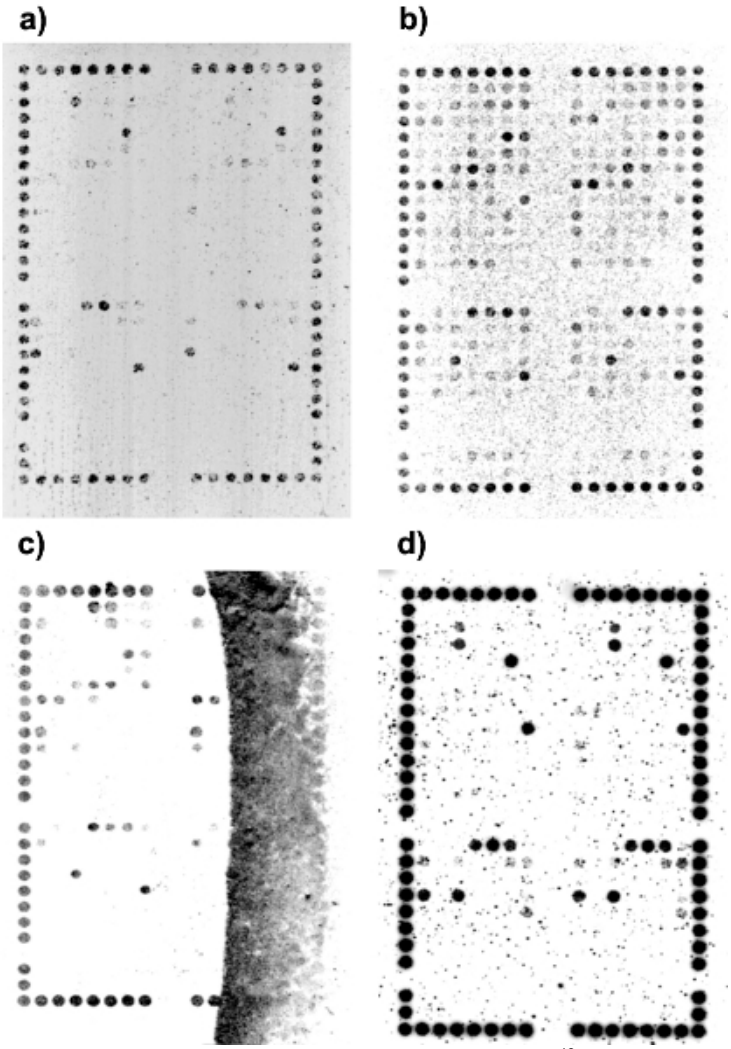

Figure 3. Radio-TLC quality control of the labeling synthon [^{18}F]SiFB after solid phase purification

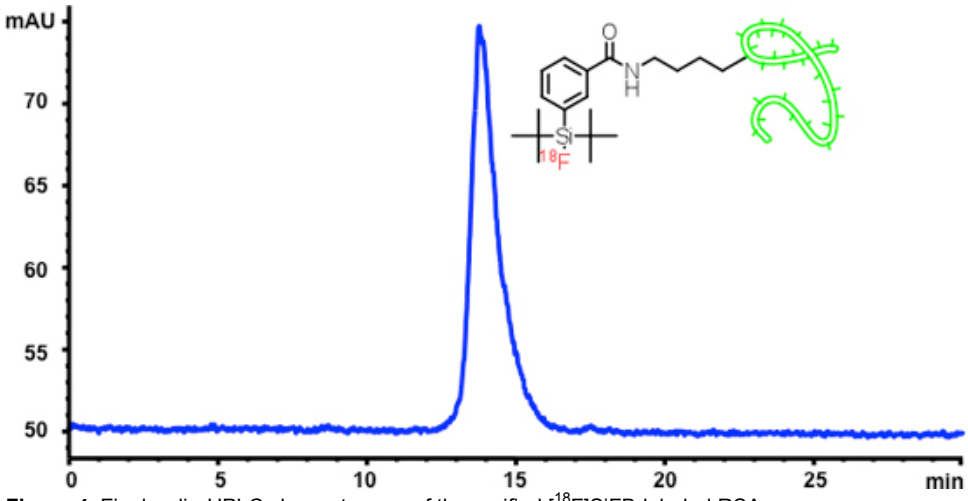

Figure 4. Final radio-HPLC chromatogram of the purified [^{18}F]SiFB-labeled RSA

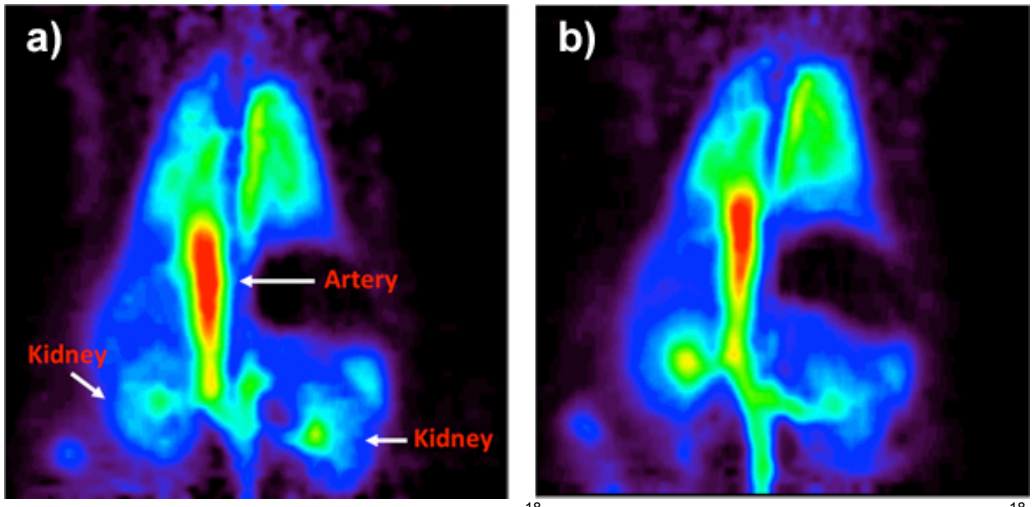

Figure 5. micro-PET data: **A)** Reconstructed image of [^{18}F]SiFB-labeled RSA; **B)** Reconstructed image of [^{18}F]SFB-labeled RSA; **C)** Time-Activity curve for [^{18}F]SiFB-labeled RSA

Discussion

SiFA labeling chemistry represents one of the first ^{18}F-labeling methods employing an extraordinarily efficient isotopic exchange reaction at room temperature or below. In common radiochemistry, which is based on the formation of a carbon-fluorine bond, final purification of the labeled compound from its precursor requires a laborious HPLC separation. With the SiFA 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 purify ^{18}F-labeled SiFA compounds using simple solid phase extraction.

Peptides derivatized from the SiFA building block (e.g. SiFA-octreotate) can be labeled with ^{18}F 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. Afterwards, the pure protein fraction can be injected into a rat and scanned using micro-PET to demonstrate the bio-distribution of the SiFA-labeled protein. In this example, a thorough analysis of the PET data revealed that [^{18}F]SiFB-labeled RSA is a reliable *in vivo* blood pool imaging agent.

Disclosures

No conflicts of interest declared.

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