Synthesis and purification of hexadecyl-4-[^{18}F]fluorobenzoate using the cassette-based Synthera® module for labeling exosomes and macromolecules

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Objectives: Hexadecyl-4-[^{18}F]fluorobenzoate ([^{18}F]HFB) (Figure 1) is a long chain lipophilic radiotracer that is retained within macromolecules such as biomaterials, exosomes, or cell membranes [1]. Exosomes are 50-120 nm extracellular vesicles that can transfer their cytoplasmic contents between cells. However, understanding where exosomes traffic in the body remains a challenge [2]. The aim of this work was to automate the radiosynthesis of [^{18}F]HFB for PET imaging exosomes and biomaterials.
Methods: The radiosynthesis and purification of [^{18}F]HFB was done using the IBA Synthera® chemistry synthesiser with the R&D IFP cassette and HPLC module. [^{18}F]HFB was prepared by [^{18}F]F$^-$ (IBA Cyclone® 18 MeV) substitution of the triflate precursor in DMSO at 100˚C/20 mins, followed by HPLC. After removal of unreacted [^{18}F]F$^-$ and DMSO on a C18 light cartridge, [^{18}F]HFB was eluted with acetonitrile and purified by C18 HPLC. The solvent from the radioactive product peak was evaporated at 140˚C under nitrogen, and [^{18}F]HFB was reformulated in DMSO (10%), filtered, and diluted in sterile saline. Using exosome exclusive spin columns and size exclusion chromatography, work is currently underway to purify labeled exosomes and biomaterials from free [^{18}F]HFB.
Results: [^{18}F]HFB was obtained in RCY (isolated after HPLC and evaporation) ranging from 15 – 45% (decay corrected) for a total synthesis time of 60 mins with high radiochemical and chemical purities. HPLC separation from a non-radioactive by-product was improved by going from a C8 to C18 column. The HPLC solvent was evaporated rather than using a Sep-Pak cartridge to trap [^{18}F]HFB as this allowed to reformulate with DMSO, and not ethanol, which is more tolerated by exosomes and cells.
Conclusion: The automated production of [^{18}F]HFB has been completed using a R&D IFP cassette and Synthera® platform in high yield and purity. [^{18}F]HFB PET imaging of exosomes and biomaterials (with/without stem cells) presents a novel approach to determining their *in vivo* distribution.
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References: [1] Ahmadi, Ali, et al. *Biomaterials.* **2015**, *49*: 18-26. [2] Vader P, et. *Adv Drug Deliv Rev.* **2016**. *106 (Pt A)*: 148-156.

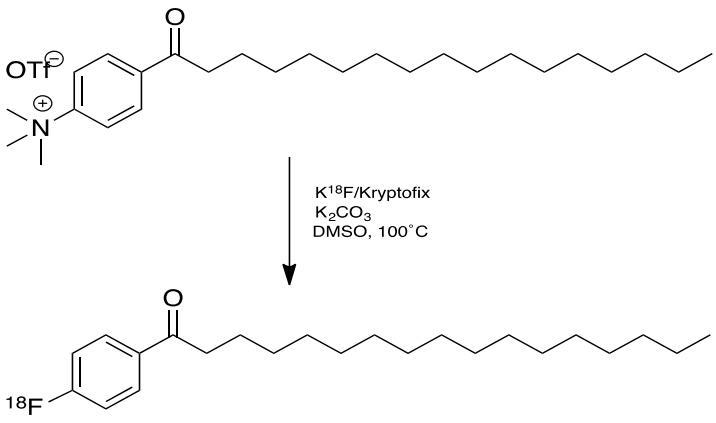

Figure 1. Synthesis of hexadecyl-4-[^{18}F]fluorobenzoate