Regiospecific radiolabeling of Nanofitin on Ni Magnetic Beads with [^{18}F]-FBEM and in vivo PET-MRI studies

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Introduction

The formation of a C-^{18}F bond requiring hard conditions, an alternative method developed few decades ago consists in incorporating the ^{18}F on a prosthetic group and then coupling it to the biomolecule by bioorthogonal reactions.[1-2] Following a similar strategy, a single cysteine has been incorporated in a Nanotifin(NF) model and its regioselective radiolabeling has been performed with [^{18}F]-4-fluorobenzamido-N-ethylamino-maleimide ([^{18}F]-FBEM).
Coupling with the [^{18}F]-FBEM has been achieved on Ni magnetic beads system. PET-MRI studies on mice were conduct after injection of [^{18}F]-FBEM-Cys-NF in order to know the biodistribution of this NF model.

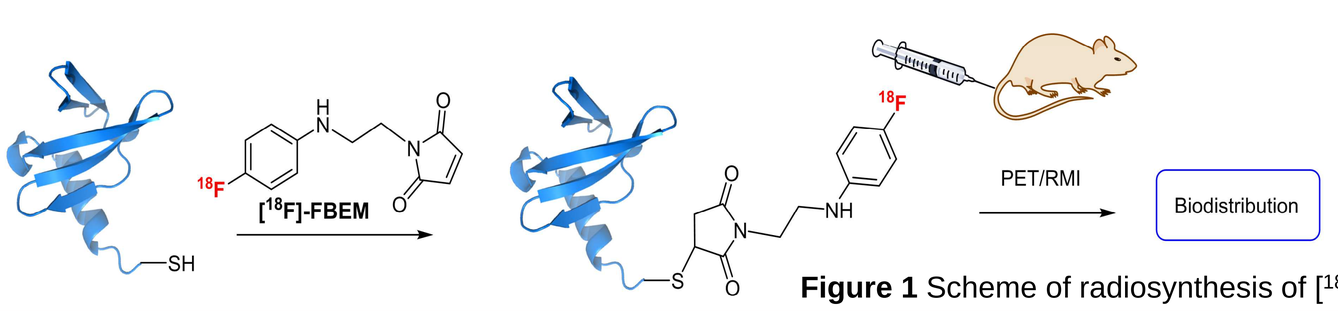
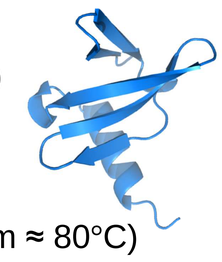


Figure 1 Scheme of radiosynthesis of [^{18}F]-FBEM-Cys-NF

Nanofitins

- Small protein (+/-10kDa)
- pH stability (0-13)
- Temperature stability (Tm ≈ 80°C)
- Produced by Sac7D bacteria
- Alternative to antibodies
- Cysteine-free protein



Methods

We first synthetized the labeling precursor **1** (**Figure 2**) as previously reported in literature.[3] The three-step synthetic pathway synthesis was implemented on a GE Healthcare FastLab™.

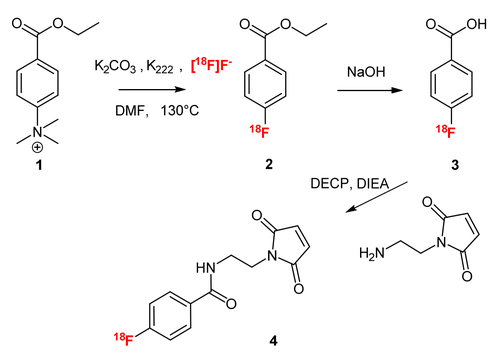


Figure 2 Radiosynthesis of [^{18}F]-FBEM

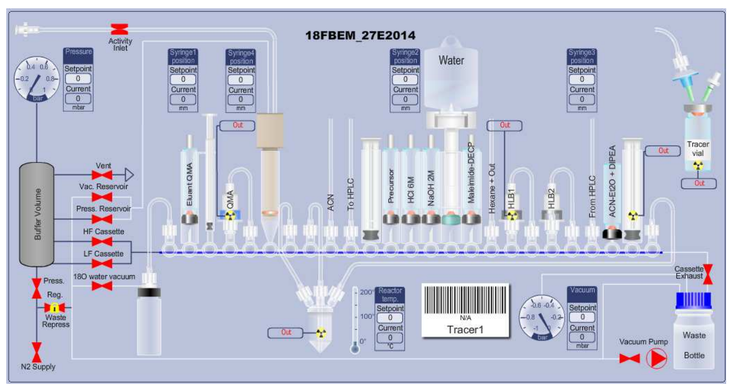


Figure 3 Cassette layout for [^{18}F]-FBEM preparation

The labeling was then done on a selected model histagged NF that has been reengineered to bear a unique cysteine. This cysteine leads to the dimerization of the NF via a disulfide bridge which needs to be reduced before the coupling. To overcome the reduction of the maleimide moiety of the [^{18}F]-FBEM in presence of reducing agent, the NF was first trapped on Ni-NTA magnetic beads and then reduction and labeling were proceeded successively on-bead (**Figure 4**).

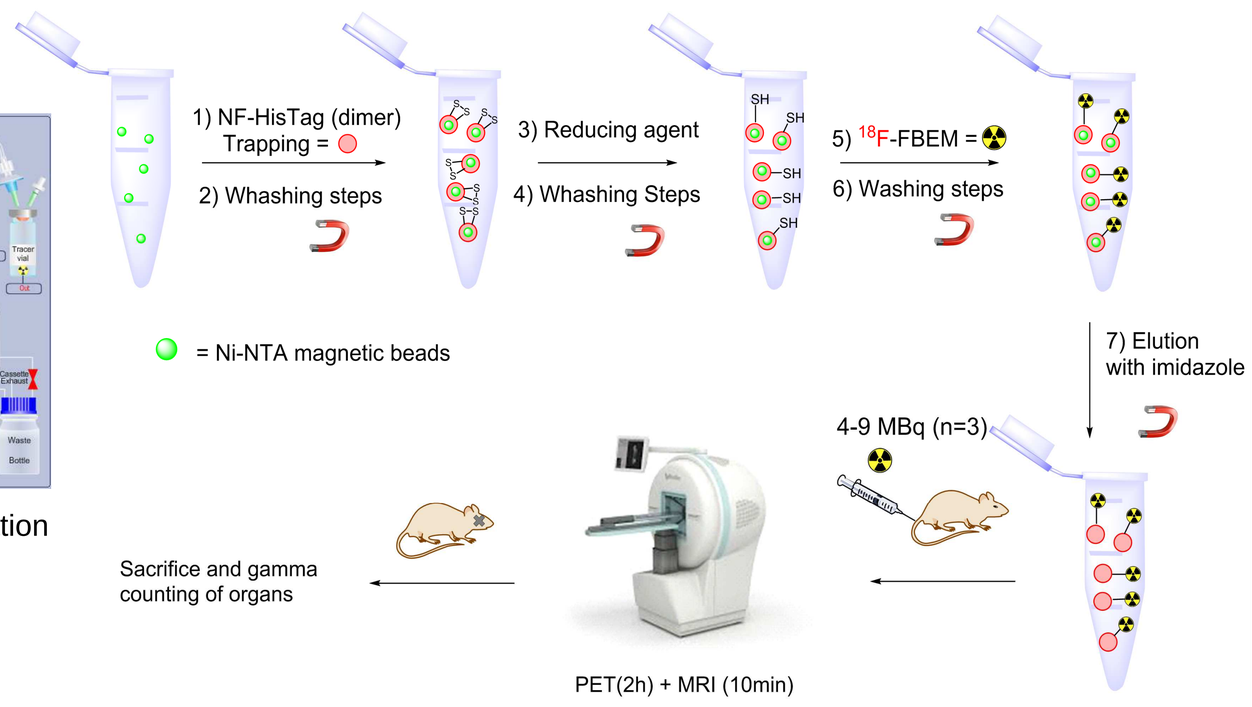


Figure 4 Radiolabeling of Nanofitin with [^{18}F]-FBEM on Ni-NTA magnetic beads

Results

Radiochemical yield(RCY) of [^{18}F]-FBEM synthesis was 28.7±3.2% (n=10) and lasted 90 min including purification.
The RCY obtained for NF labeling is 53.8±7.3% (n=9) with a radiochemical purity >95%.

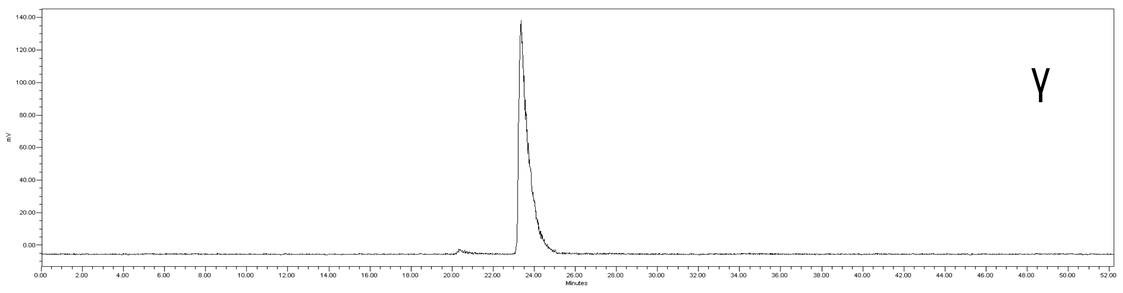


Figure 5 Analytical C8-HPLC of the [^{18}F]-NF

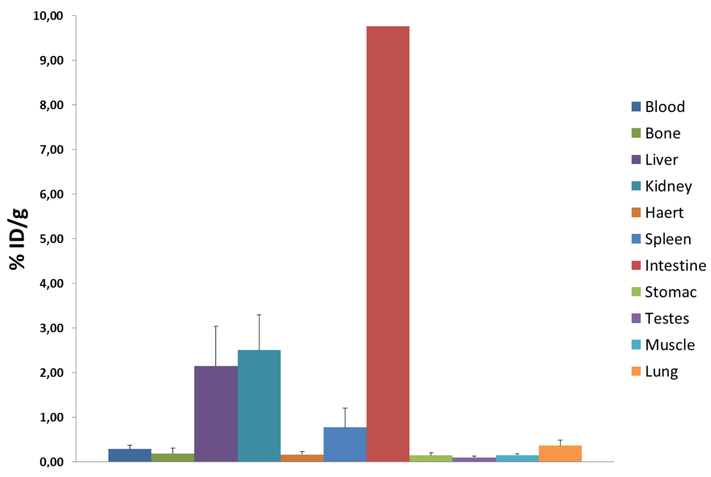


Figure 6 %ID/g for each organ 3h after injection

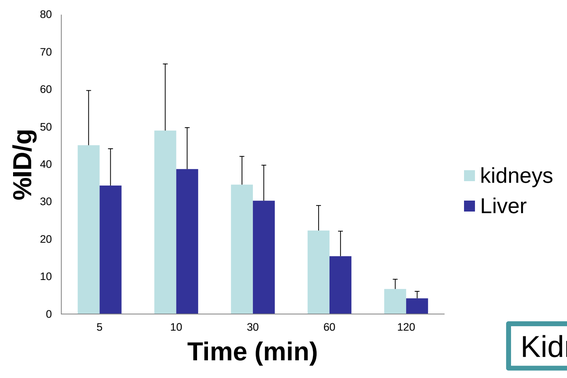


Figure 7 Evolution of the %ID/g for the kidneys and the liver

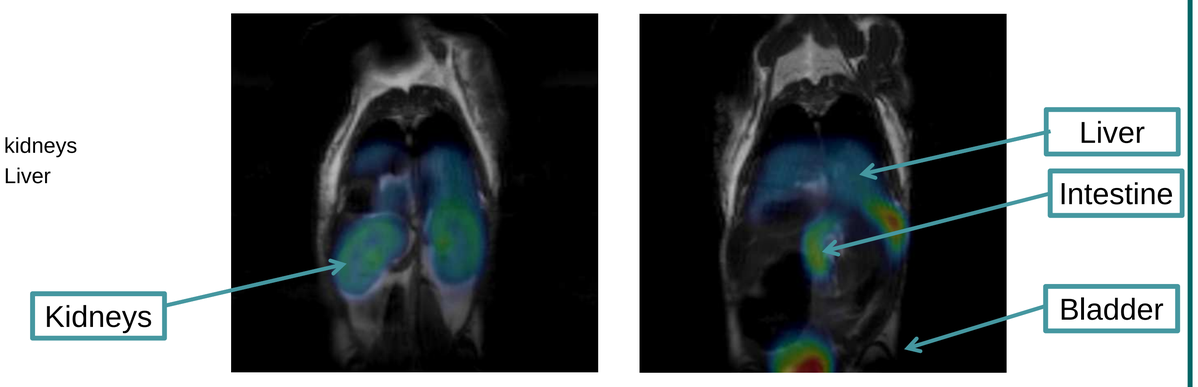


Figure 8 PET-MRI images of the biodistribution of the NF

Conclusions

We successfully radiolabeled a Cys-tagged Nanofitin with [^{18}F]-FBEM automated synthetized at high activity. A simple and new method for the radiolabeling of biomolecule with high yield (up to 61%) on Ni magnetic beads was developed. PET-MRI studies on mice were conduct after injection of [^{18}F]-NF in order to know the biodistribution of this Nanofitin model. The results show a fast renal clearance and an uptake in the liver. The metabolites are excreted by kidneys through urine and by the liver via biliary excretion to the intestine.

References

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Acknowledgements

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