Accelerating the Development of Peptide-Based NET Tracers with 'Next-Gen' ^{18}F Chemistry

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- This early-stage research explores a technique where ^{18}F-labeled PET tracers that have an affinity for certain GEP-NETs might be synthesized in a far simpler fashion than current methods.

^{18}F Chemistry: Challenges & Opportunities

- ^{18}F exhibits attractive nuclear qualities for PET imaging, including a high positron abundance (97%) and low positron energy (E_{max}=635 keV), which allows for the acquisition of high-resolution molecular images.
- However, the radiolabeling of functionally complex molecules such as peptides and proteins with ^{18}F is challenging, owing to a number of factors, including:
  - the short half-life of ^{18}F (110 min)
  - the perceived need for 'dry' $[^{18}F]F^-$, which is obtained through extraction from cyclotron target $[^{18}O]H_2O$ and multiple azeotropic distillation steps from MeCN (**Fig. 1a**)
  - the incompatibility of many sensitive biological targeting vectors with the high temperatures and basic conditions usually required to incorporate $[^{18}F]F^-$.
- Often a smaller prosthetic group is radiolabeled first, and then attached to the vector in a subsequent step– a complex, *indirect* labeling strategy that often requires HPLC purification. This is contrast with radio-metalation, in which a chelator-modified biomolecule is simply incubated in an aqueous solution of radioisotope.
- Further radiosynthetic challenge arises from the need to prepare radiotracers remotely, using automated synthesizers, if they are intended for clinical use.

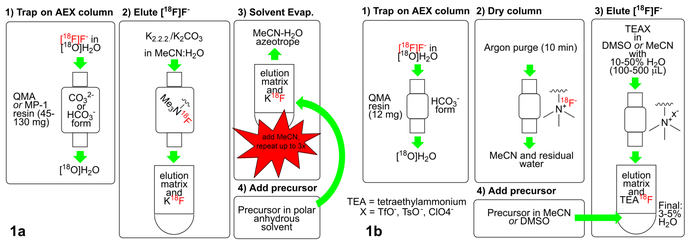

Fig. 1. a) Canonical method for radiolabeling with $[^{18}F]F^-$. A basic anion (usually carbonate) is used to elute $[^{18}F]F^-$ from QMA anion exchange resin, then water is removed *via* successive MeCN:H_2O azeotropic distillation ('drydown') steps. **b)** The "non-anhydrous, minimally basic" (NAMB) method involves the trapping of $[^{18}F]F^-$ on small QMA columns, elution with non-basic anions, and subsequent nucleophilic ^{18}F-fluorinations in 'damp' reaction mixtures. No 'drydown' steps are required.

Merging New Technologies

- A 'next generation' direct (*i.e.* 1-step) labeling strategy called "Silicon-Fluoride Acceptor" (aka SiFA) employs highly efficient ^{19}F-for-^{18}F isotopic exchange reactions to ^{18}F-label di-*tert*-butyl-fluorosilane-modified peptide targeting vectors at room temp.[1] Only nmol of ^{19}F-peptide precursor is required to achieve high radiochemical yields, and thus high molar activity radiopharmaceutical can be obtained without HPLC purification.
- SiFA has led to the discovery of promising ^{18}F-labeled octreotide derivative **$[^{18}F]$Si-TATE** (**Fig. 2**). When compared with [^{68}Ga]Ga-DOTA-TOC in clinical NET imaging scans, **$[^{18}F]$Si-TATE** showed higher tumor uptake in most metastatic sites and comparable biodistribution in healthy tissues.[2]

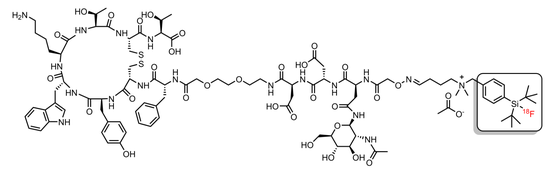

Fig. 2. Structure of **$[^{18}F]$Si-TATE**.

- Translation to automation has hamstrung clinical progress, owing to a radiosynthetic protocol that requires highly basic (hydroxide anion) $[^{18}F]F^-$ eluates that must be carefully titrated with small volumes (15 µL) of oxalic acid.[3] As the ^{19}F-peptide is sensitive to both the base and the acid alone, it must be quantitatively added to the reaction vessel *via* cannula after titration, a step that is difficult to automate.
- We introduced an alternative means to prepare reactive $[^{18}F]F^-$ called "Non-Anhydrous, Minimally Basic" (NAMB) ^{18}F-fluorination chemistry, in which $[^{18}F]F^-$ is eluted from smaller-than-usual anion exchange (AEX) quaternary methylammonium (QMA) columns with *non-basic* tetraalkylammonium salts, then used for nucleophilic $^{18}F^-$fluorination reactions directly, despite reaction mixtures containing 3-5% water (**Fig. 1b**).[4]

SiFA×NAMB-based syntheses of $[^{18}F]$Si-TATE

- We hypothesize that SiFA isotopic exchange reactions can be coupled with 'NAMB' techniques, allowing for the highly simplified and fully automated preparation of **$[^{18}F]$Si-TATE** and other peptide-based ^{18}F-PET radiopharmaceuticals.
- The feasibility of labeling [^{19}F]Si-TATE (50 nmol) under NAMB conditions has been investigated (**Fig. 3**). Apart from the parameters in **Table 1**, we also tracked the efficiency of $[^{18}F]F^-$ trap-and-release from our small (12 mg) QMA columns, which we 3D-print ourselves and also sell commercially.[5]

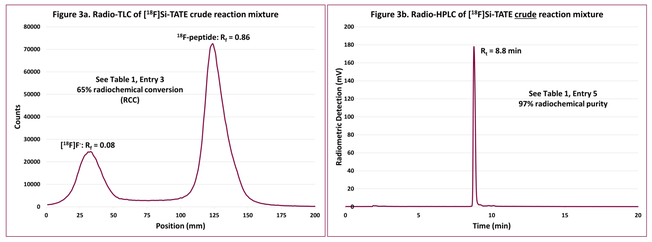

Fig. 3 Example radio-traces of the manual syntheses of **$[^{18}F]$Si-TATE**.

Entry	Eluate MeCN:H_2O	% Water	% DMSO	React. (min)	% RCC*	NDC-RCY†
1	7:3 (0.1 mL)	3	2.5	10	31	30
2	7:3 (0.1 mL)	3	2.5	20	37	25
3	7:3 (0.1 mL)	3	20	20	60-84‡	37-52‡
4	9:1 (0.3 mL)	3	20	20	87	26
5	8:2 (0.3 mL)	6	20	20	70	49
6§	8:2 (0.3 mL)	6	20	30	–	10

Table 1. Summary of **$[^{18}F]$Si-TATE** syntheses. All elution efficiencies from QMA resin were >99%, except Entry 4 (66%). All reactions at room temp. Total syntheses times = 41-59 min. *Radiochemical conversion assayed by radio-TLC. †Non-decay-corrected radiochemical yield. ‡*n*=3 experiments. §Automated synthesis.

- In contrast with many other novel radiochemical approaches, efforts were made to develop a general protocol that could be *easily translatable to automated synthesis* (e.g. the avoidance of steps that require micro-pipetting).
- This strategy proved worthwhile as our first attempt to synthesize **$[^{18}F]$Si-TATE** on a GE FASTlab™ (**Fig. 4**) was successful (**Table 1, Entry 6**). Final radiochemical purity was 98%.

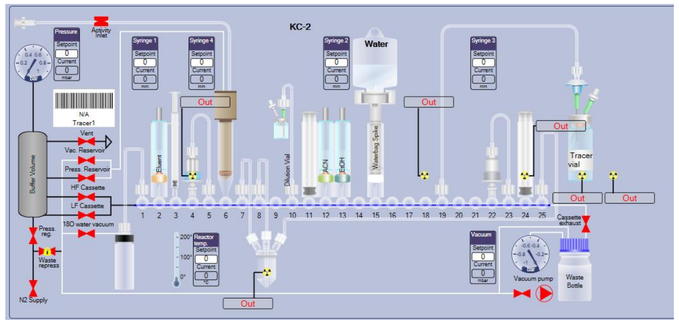

Fig. 4. Summary diagram of the automated synthesis of **$[^{18}F]$Si-TATE.**

New PET agents for pancreatic cancer

- We intend to apply the "SiFA×NAMB" radiolabeling strategy towards the design of new SiFA-bearing peptide receptor ligands which target certain pancreatic cancers (insulinomas).
- The overexpression of glucagon-like peptide 1 receptor (GLP-1r) in most insulinomas has led to nuclear imaging with radiolabeled exendin-4 analogues prior to surgery becoming common practice.[6]
- Most GLP-1r-targeting radioprobes invented thus far employ metallic radioisotopes and tend to distribute into the kidneys, which can complicate pancreas imaging due to the proximity of the two organs.
- However, many ^{18}F radio-peptides that contain lipophilic prosthetic groups clear quickly from the kidneys, making SiFA-bearing exendin-4 derivatives a worthy class of NET imaging agents for design and preclinical assessment.

References

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