Radiosynthesis of Fluorine-18 labeled Albumin: Methodology Towards Developing Peanut Protein Tracer

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Disclosures

- There are no known conflicts of interest.

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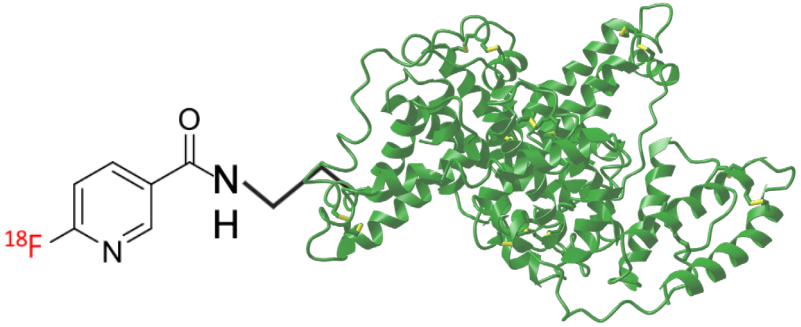
Background

- There is no accurate method to assess in vivo the pharmacokinetics of food allergens after ingestion [1].
- We are working to develop a clinically relevant reproducible method for radiolabeling Ara h 2, an allergenic peanut protein, responsible for anaphylactic reactions.

Downloaded from NCIB. 3OB4:MBP-fusion protein of the major peanut allergen Ara h 2.

- There are currently several methods for radiolabeling peptides and proteins with fluorine-18 for use as a tracer for PET imaging in animals and humans [2].
- In this work, we present a fast and simple semi-automated *optimized* procedure for the ^{18}F-labeling of **bovine serum albumin (BSA)** via [^{18}F]fluoro-pyridine-tetrafluorophenol ([^{18}F]F-Py-TFP) as a proof-of-concept.



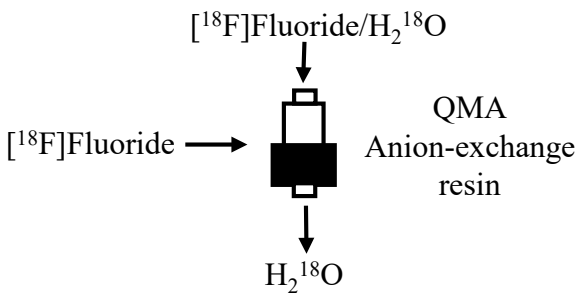
Adapted from NCIB. 4F5S: Crystal Structure of Bovine Serum Albumin.

- A similar methodological approach was adapted for the ^{18}F-labeling of the *expensive* Ara h 2 peanut protein.

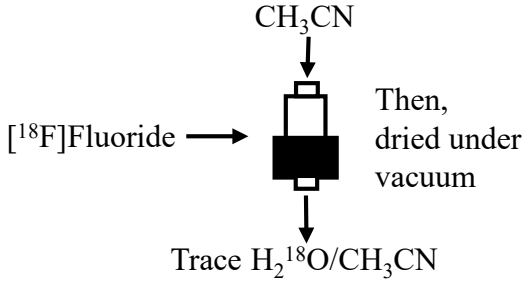
(Aqueous) Method

Radiosynthesis of [^{18}F]F-Py-TFP

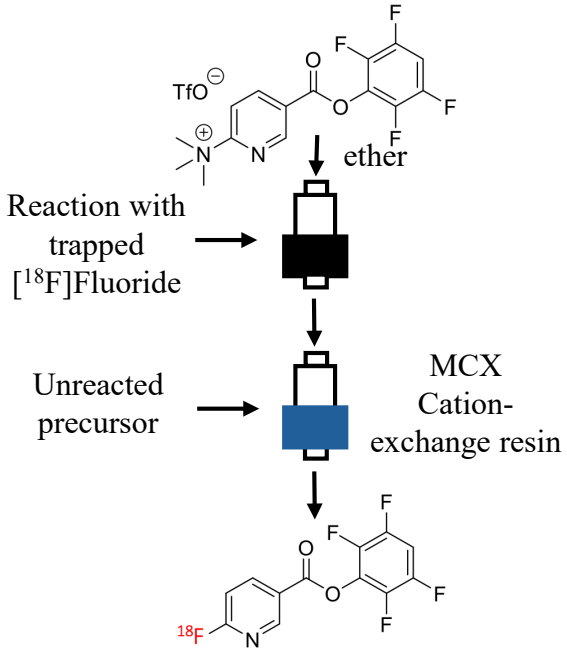
1. [^{18}F]Fluoride Capture

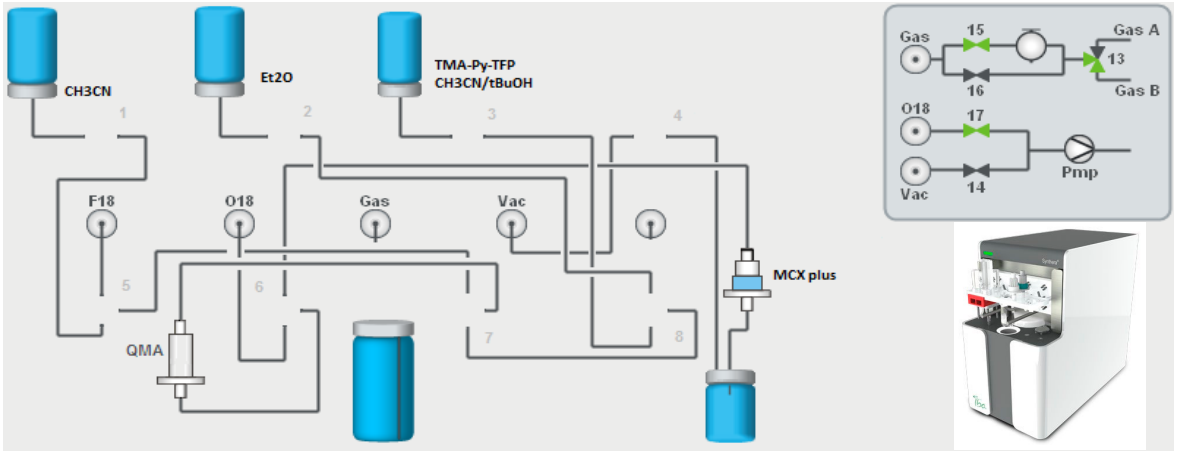


2. Rinsing & Drying

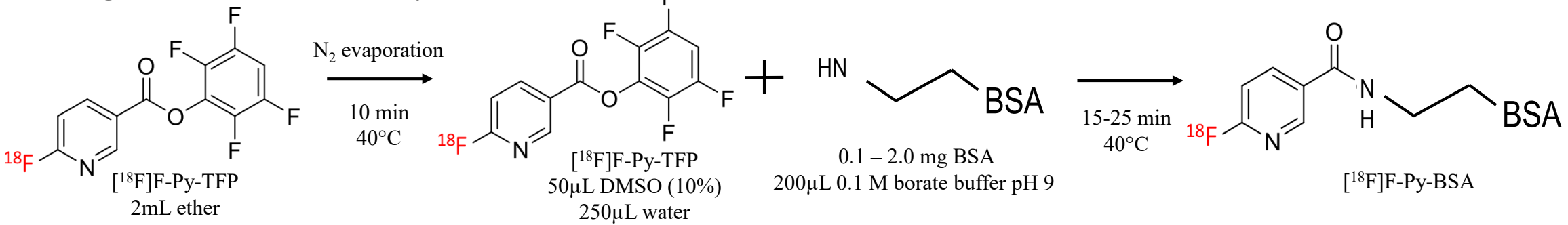


3. Radiolabeling, purification, & elution with ether





Radiolabeling of BSA with [^{18}F]F-Py-TFP



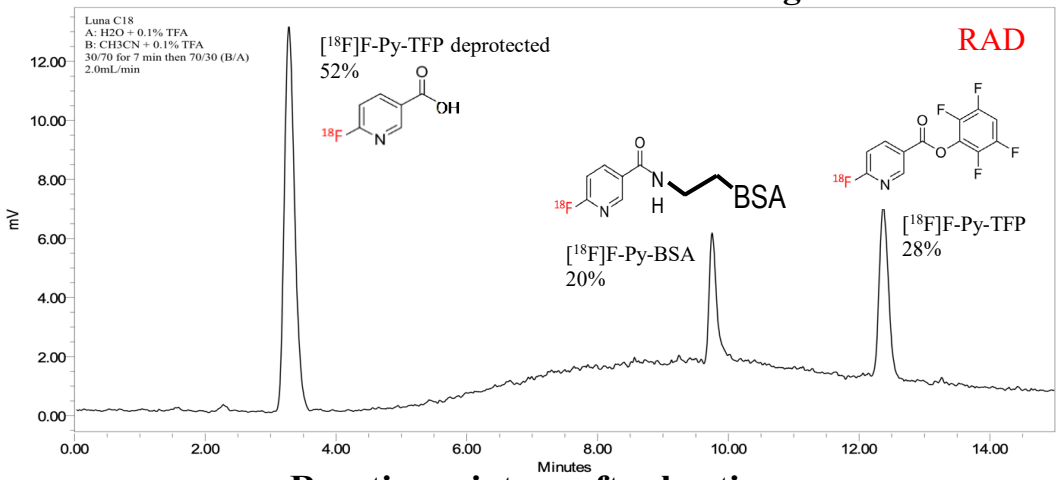
Results – BSA & Ara h 2

Table 1. Optimization of the ^{18}F-labeling of BSA

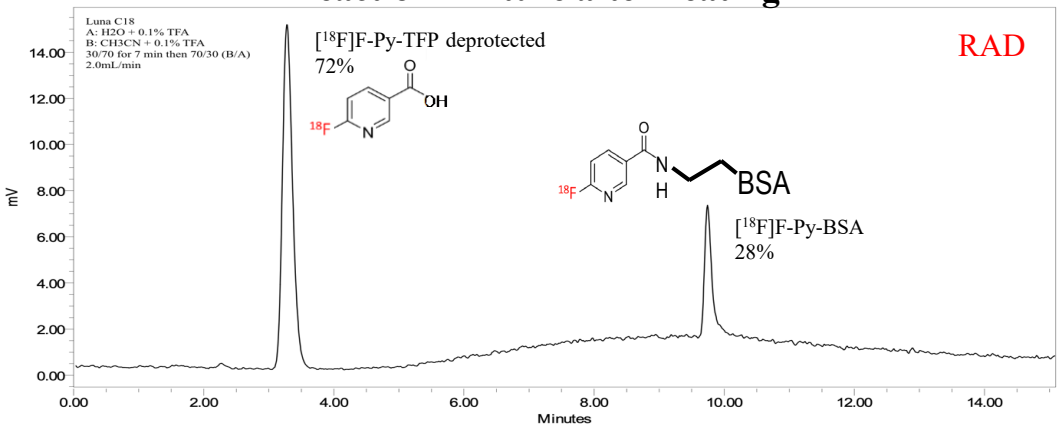
Conditions		Radiochemical Purity (%)*	Radiochemical Yield (%)**
Buffer (1µg/µL BSA)	Carbonate-bicarbonate buffer, 0.1M pH 10 (n = 3)	17.3 ± 7.7	6.7 ± 3.2
	Carbonate-bicarbonate buffer, 0.1M pH 9 (n = 2)	12	7
	Borate buffer, 0.1M pH 9 (n = 4)	**30.7 ± 9.6**	**17.1 ± 7.4**
Protein Concentration (in borate buffer)	0.1 µg/µL (n = 1)	No labeling	-
	0.3 µg/µL (n = 1)	No labeling	-
	0.5 µg/µL (n = 1)	18	13
	1.0 µg/µL (n = 4)	**23 ± 5.7**	**8.62 ± 3.1**
	2.0 µg/µL (n = 2)	42.5	26.6
Heating time (1µg/µL BSA in borate buffer)	**15 minutes (n = 9)**	**23.7 ± 10.2**	**12.3 ± 7.1**
	25 minutes (n = 2)	24	15.5

*Proportions of the peaks from the analytical HPLC
**Decay-corrected to the end of prosthetic group [^{18}F]F-Py-TFP synthesis, mean ± SD

Reaction mixture before heating



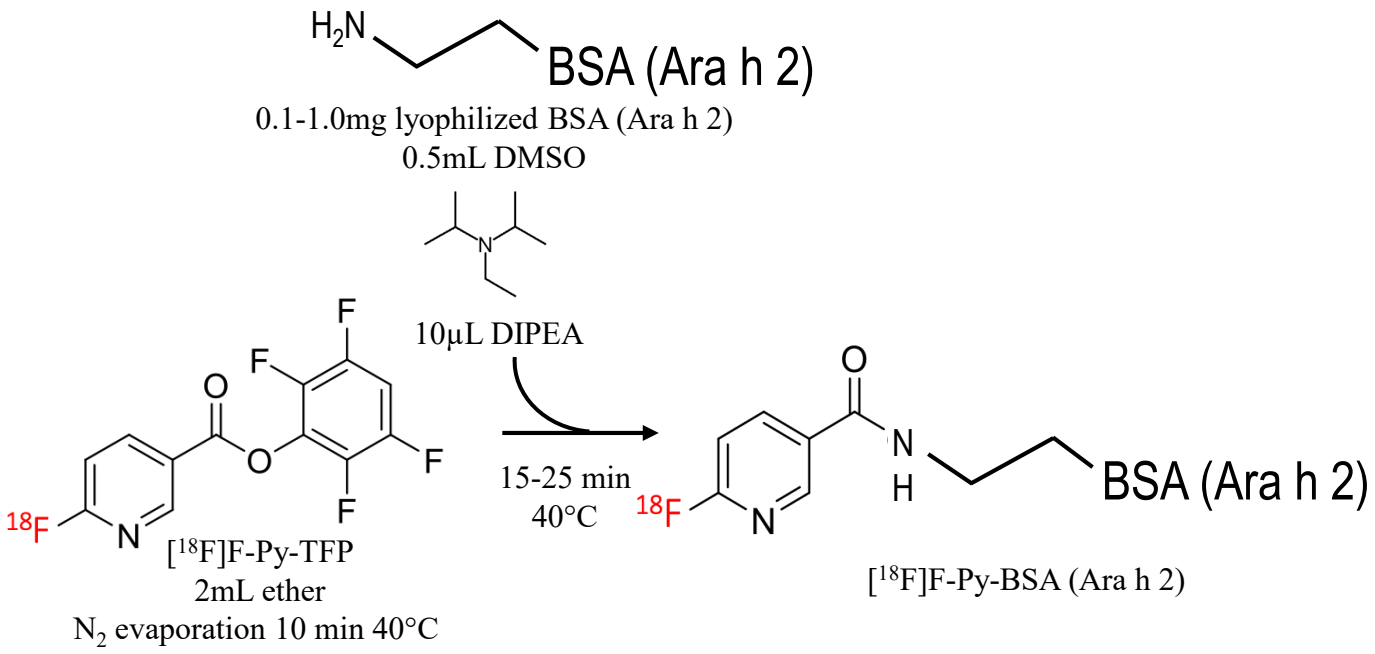
Reaction mixture after heating



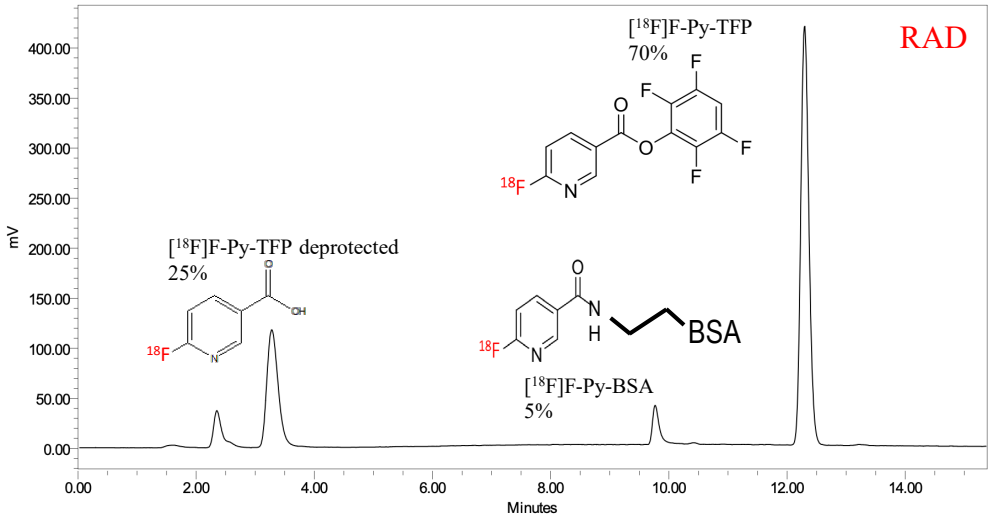
No successful Ara h 2 radiolabeling was obtained with aqueous method regardless of various conditions attempted (buffer, reaction time, concentration, heating time).

(Non-Aqueous) Method & Preliminary Results

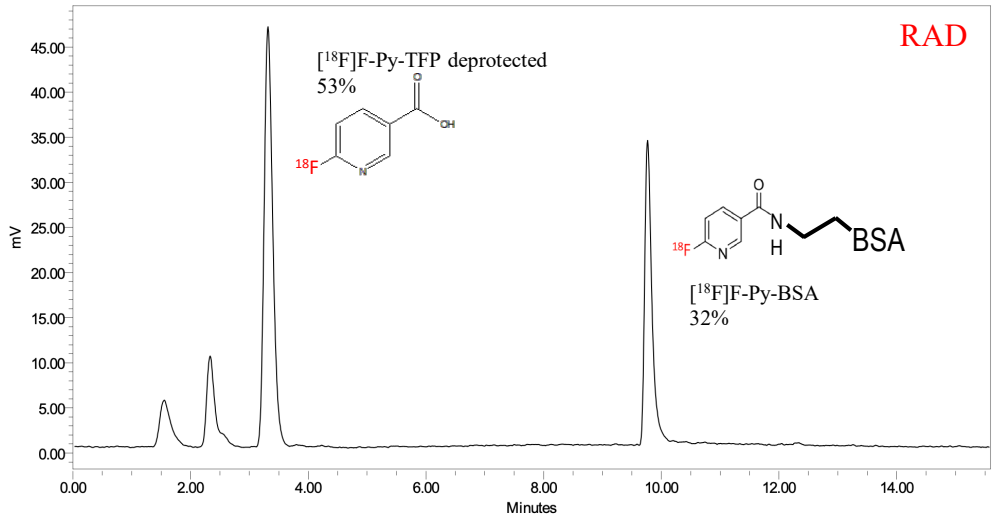
- New non-aqueous method, modified from Davis et al. [3]
- Using lyophilized BSA as a proof-of-concept.
  - Achieved higher RCY (up to 32%, decay-corrected to the end of prosthetic group [^{18}F]F-Py-TFP synthesis) then aqueous method.
- Optimization of non-aqueous method will be adapted for lyophilized Ara h 2.



Reaction mixture before heating

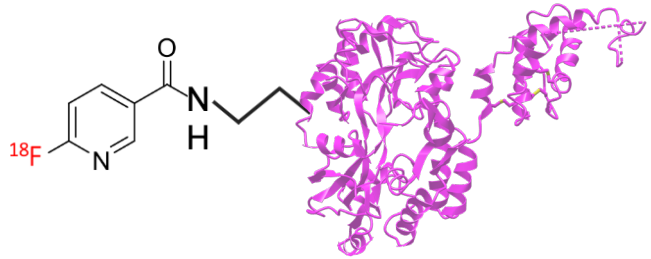


Reaction mixture after heating



Conclusions

- Pure [^{18}F]F-Py-BSA was successfully produced and optimized using an aqueous reaction.
  - No labeling was observed below 0.5µg/µL.
  - Most effective labeling buffer was borate buffer.
  - Higher RCY (up to 27%, decay-corrected to the end of prosthetic group [^{18}F]F-Py-TFP synthesis) were obtained with increasing concentrations of BSA.
  - No successful Ara h 2 radiolabeling with aqueous method regardless of various conditions attempted.
- A non-aqueous method for producing [^{18}F]F-Py-Ara h 2 is currently in progress using DMSO and DIPEA with lyophilized Ara h 2.
  - Successful radiolabeling of lyophilized BSA with high RCY (up to 32%, decay-corrected to the end of prosthetic group [^{18}F]F-Py-TFP synthesis).
- Future Directions
  - This translational work chemistry-human has the potential for *in vivo* tracking of peanut proteins via PET imaging and contribute to the understanding peanut allergies.



Adapted from NCIB. 3OB4:MBP-fusion protein of the major peanut allergen Ara h 2.

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