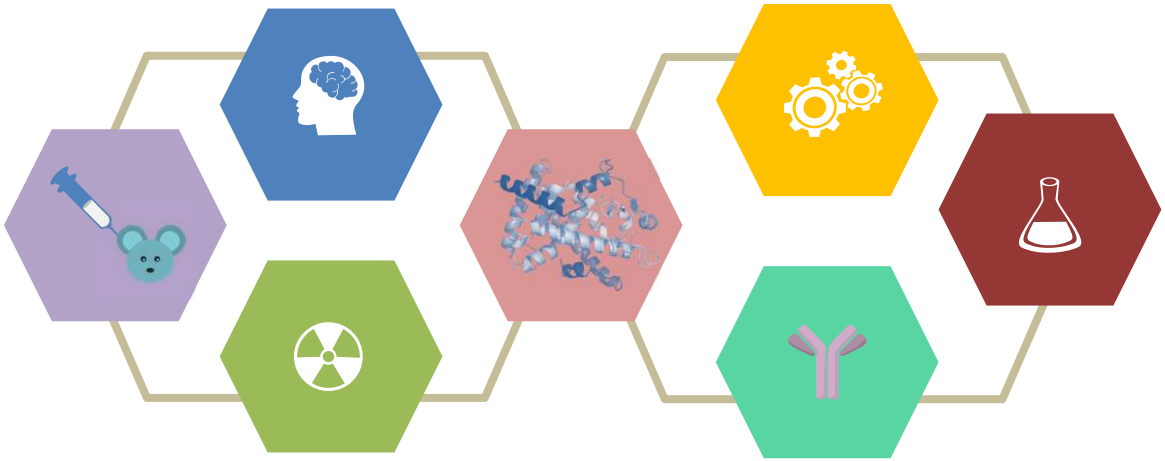
LIÈGE université
GIGA
CRC In vivo Imaging



^{18}F PET chemistry

Dr.Dammicco S.

aps American Physiological Society | Physiological Reviews®

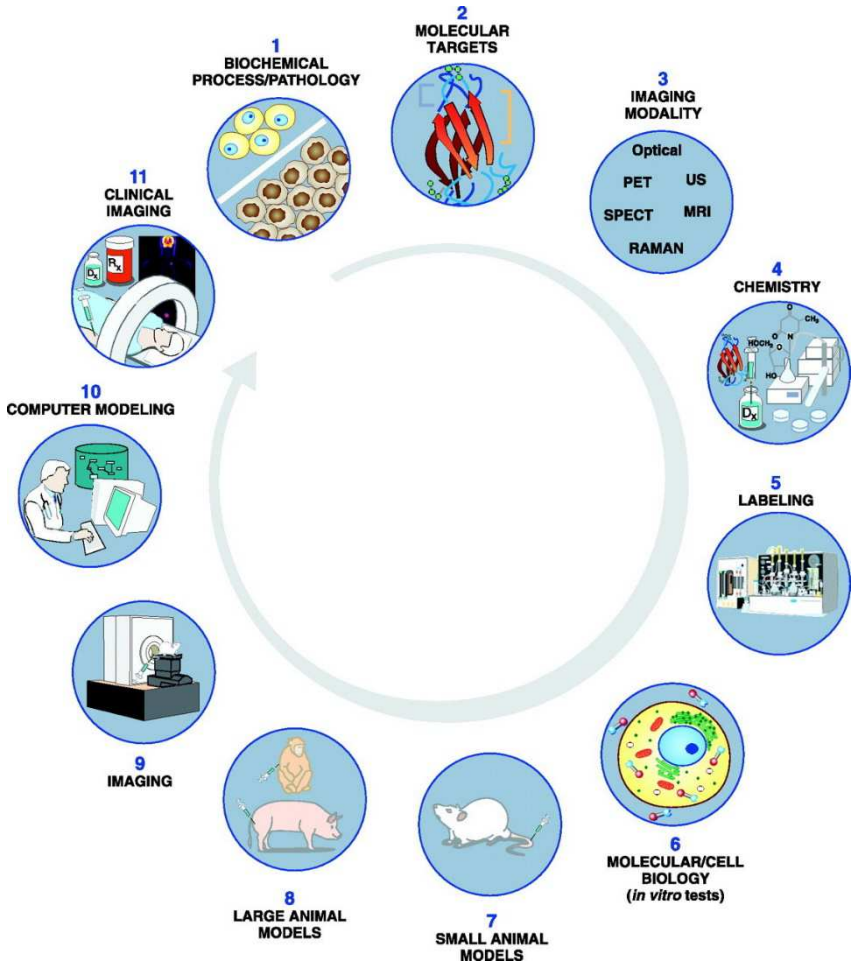
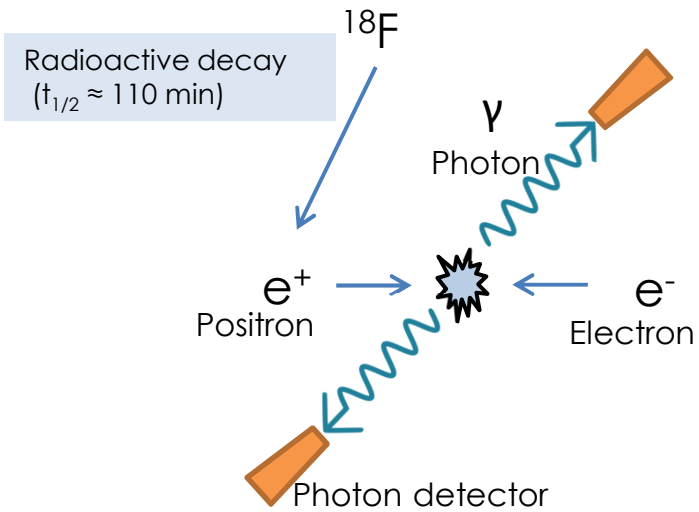
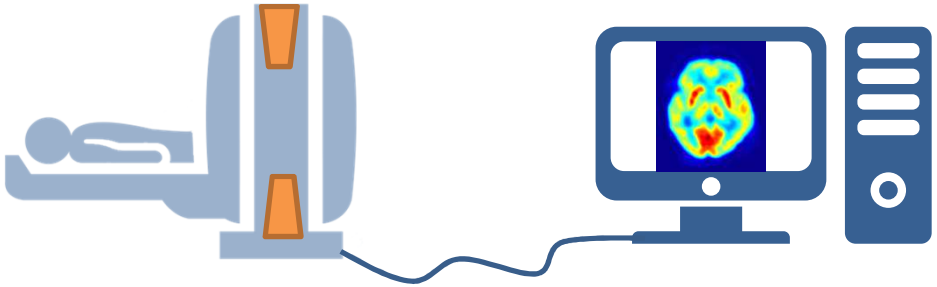


Figure 1. Schematic of some key steps involved in a molecular imaging study. The first step is to identify a biochemical process or pathology of interest, and to assess the significance of visualizing this process/pathology noninvasively via the tools of molecular imaging. The second step is to decide on a molecular target that will enable direct or indirect visualization of the phenomena of interest. This is usually followed by selection of an appropriate imaging modality (like those discussed in sect. II) and, if necessary, an imaging agent (discussed in sect. III). Typically, some chemistry and labeling are required to synthesize the imaging agent (so that it contains both a targeting and signaling component). A number of in vitro (molecular/cell biology-based) and in vivo (animal model-based) tests are required to evaluate the specificity and selectivity of the imaging method for visualizing the phenomena of interest. If clinical studies are the end goal, FDA approval is required, and certain mathematical models/algorithms might need to be developed so that meaningful data can be obtained from images. Please refer to section V for a step-by-step guide to performing a molecular imaging study. PET, positron emission tomography; US, ultrasound; SPECT, single photon emission computed tomography; MRI, magnetic resonance imaging; RAMAN, surface enhanced Raman spectroscopy.

Published in: Michelle L. James; Sanjiv S. Gambhir; *Physiological Reviews* **2012,** 92, 897-965.
DOI: 10.1152/physrev.00049.2010

PET scan





PET Camera

3D image

- A Positron is an anti-matter electron, it is identical in mass but has an apposite charge of +1.

- Positron can come from different number of sources, but for PET they are produced by nuclear decay.

- Nuclear decay is basically when unstable nuclei are produced in a cyclotron by bombarding the target material with protons, and as a result a neutron is released.

$$^{18}O + p \rightarrow {}^{18}F + n$$

- In PET the target material is chosen so that the product of the bombardment decays to a more stable state isotope by emitting a positron, for instance ^{18}F has too many protons, so one of these protons decays into a neutron emitting in the process a positron an a neutrino.

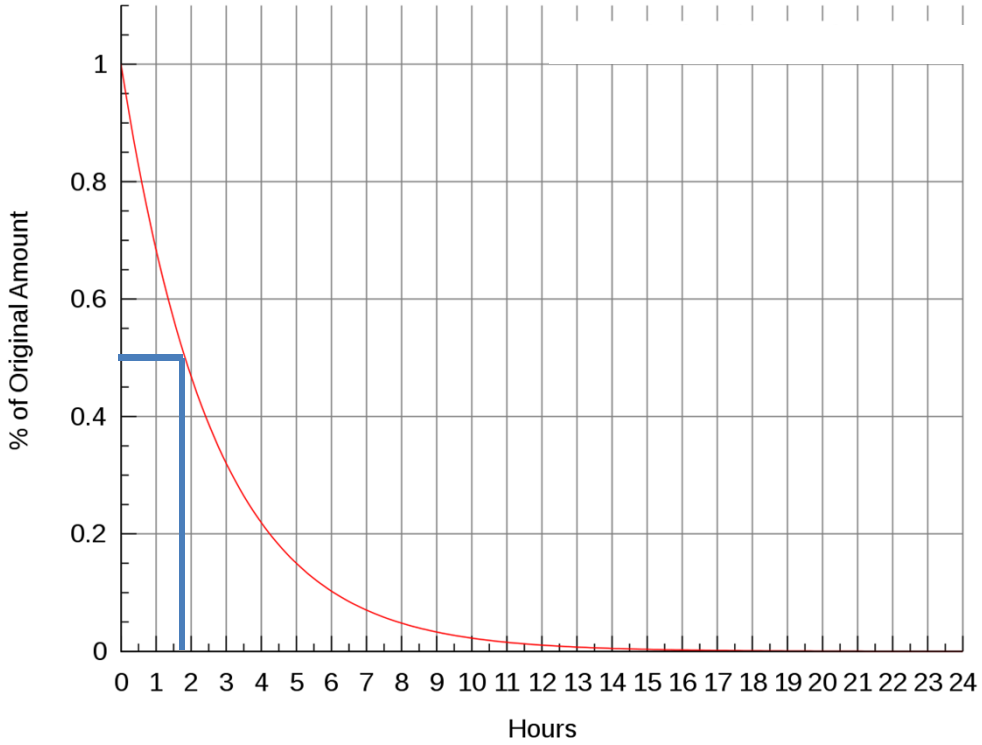
$$^{18}F \rightarrow {}^{18}O + \beta^{+} + \nu^{0}$$

PET radionuclides

PET nuclide	Half life	β^+ energy (keV)	β^+ yield (%)	Tissue range (mm)	Production
^{11}C	20.4 min	980	99.8	3.9	cyclotron
^{13}N	9.97 min	1198	99.8	5.1	cyclotron
^{15}O	2.04 min	1732	99.9	8.0	cyclotron
^{18}F	109.7 min	633	97	2.3	cyclotron
^{68}Ga	67.7 min	1899	89	8.9	generator
^{124}I	4.18 d	2100	26	No data	cyclotron

Among them, fluorine-18 is certainly the radionuclide with the most benefits

The half-life



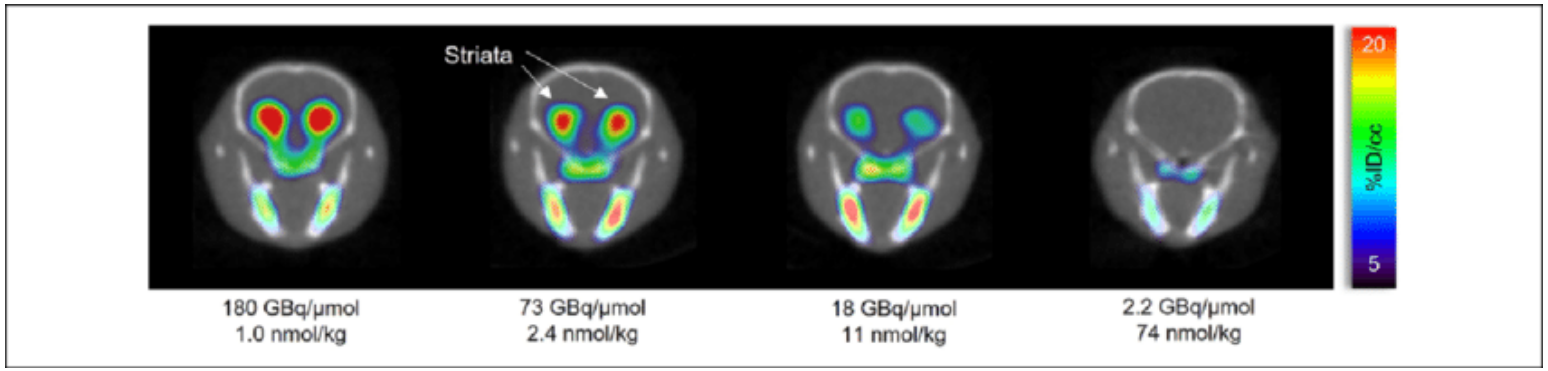
t ½= 109.7 min

The molar activity

$$A_m \text{ max (Bq / mol)} = \frac{\ln 2 \; x \; N_A}{t_{1/2}}$$

Directly correlated to the half-life, the molar activity (A_m) of a radioelement corresponds to its measured activity per mol of compound. It is expressed in Becquerel (Bq) or curies (Ci) per mole.

The theoretical A_m of fluorine-18 is 6.33 10^4 GBq/µmol or 1710 Ci/µmol. However, in practice, this molar activity is always lower due to contamination by fluorine-19 present in trace amount in the glassware, plastics, reagents or even in the water. The fluorine-18 produced by the cyclotron is therefore always diluted by fluorine-19 significantly decreasing the molar activity.



Effect of molar activity on [18F]fallypride PET/CT imaging in mice. Sergeev et al. 44 with permission from Springer Nature, 2018

The energy of the emitted positrons

Positrons with high energy can travel deeper in the tissue before annihilation with an electron resulting to a lower resolution PET imaging. With a 2.3 mm positron range, fluorine-18 is one of the less energetic β^+ emitter.

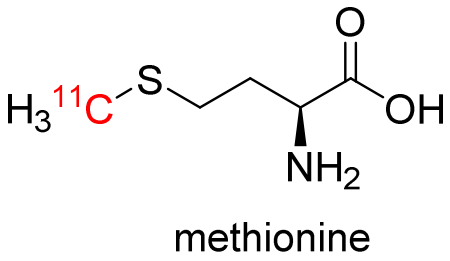
β^+ ratio

A high ratio of the β^+ emission over the global decay emission is also important to increase the sensitivity of PET detection by reducing the noise level. ^{18}F presents a high β^+ yield of 97%.

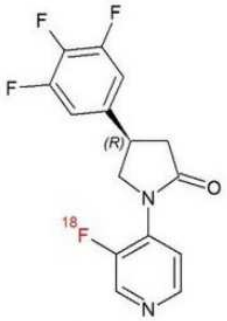
Other possible decay:
- Alpha: He^{2+}
- β^-: electron
- gamma

The chemistry

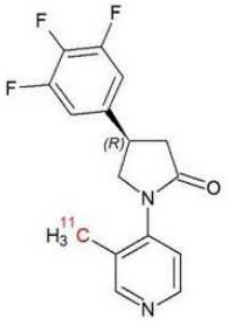
^{11}C: identical biological properties than ^{12}C
BUT short half-life not adapted for PET examination.
The fluorine atom is not present in natural products but more and more frequently found in drugs.
Fluorine substitution of a hydrogen atom or a hydroxyl group is considered as bioisosteric, and can even create better pharmacophores.



methionine



[^{18}F]UCB-H



[^{11}C]UCB-J

t ½ = 20.4 min

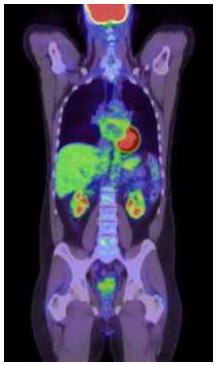
Why a nucleophilic approach?

	Electrophilic route	Nucleophilic route
Nuclear reaction	$^{18}O_2(p, n)^{18}F$ $Ne(d, \alpha)^{18}F$	$H_2{}^{18}O(p,n)^{18}F$
Target material	Gaz	Water
Typical batch of production	250-3000 mCi	1- >10 Ci
Fluorinating agent	$[^{18}F]F_2$/ $CH_3COO[^{18}F]F$	$^{18}F^-$
Theoretical maximum radiochemical yield	50 %	100 %
Amount involved	50-200 µmol	nca
Specific activity	2 mCi/µmol	> 1 Ci/µmol
Amount (mg)	2-10 mg	nca
Radiochemical Yield (d.c., %)	33	20-30

^{18}F-labeled radiotracer production

→ 

→ Incorporation in a molecule of interest via an automated synthesis



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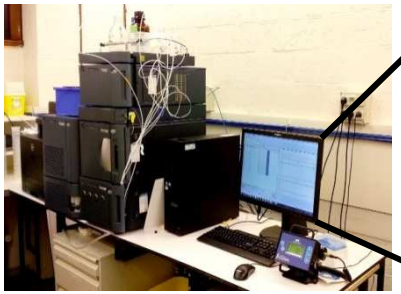
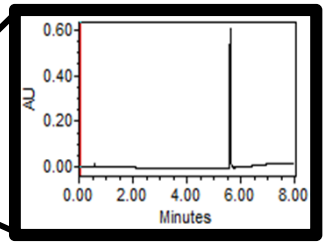
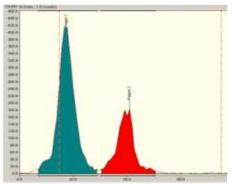
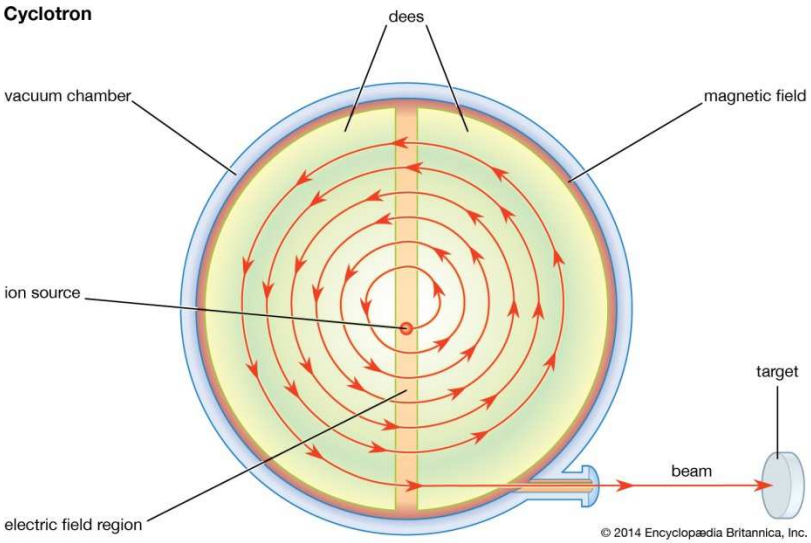






^{18}F production



^{18}O(p,n)^{18}F reaction produces [^{18}F]fluoride ion

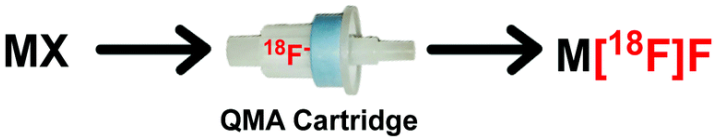
Only small fraction of protons undergo reaction

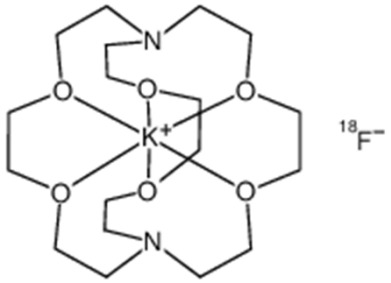
Transfer solution of [^{18}F]fluoride ion/[^{18}O]water from target to chemistry module

^{18}F production

Pass through ion exchange cartridge – Recover [^{18}O]water – “Trap and release”
[^{18}F]fluoride ion

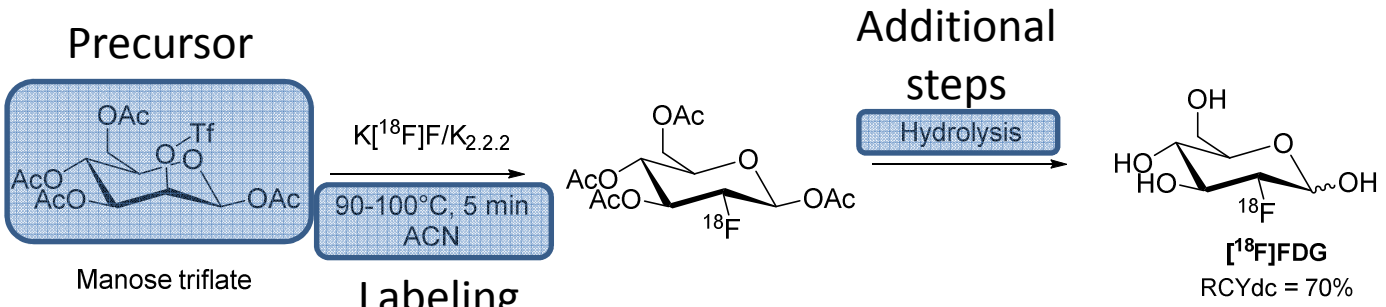
Solid phase process based on resins
Relies on electrical charge
Anion exchange resins positively charged
Cation exchange resins negatively charged
[^{18}F]Fluoride ion is displaced from resin with potassium carbonate
Therefore, the counterion is potassium that is disolved in acetonitrile/water with Kryptofix®

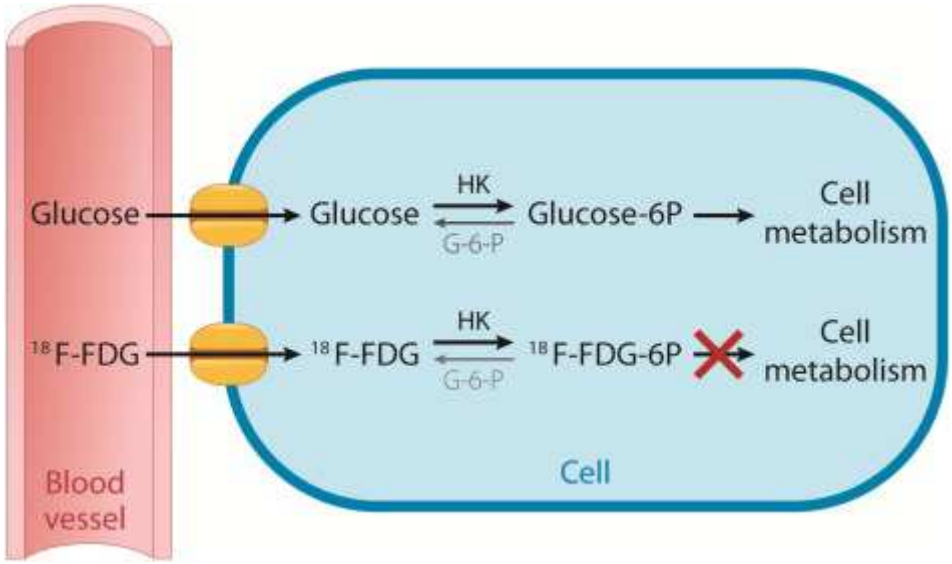


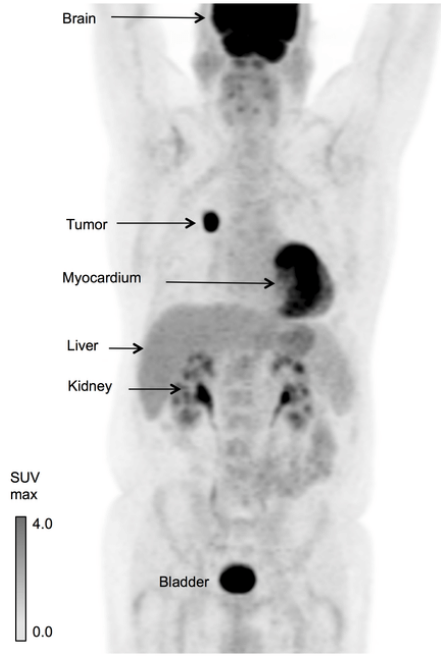


^{18}F Radiotracers

[^{18}F]FDG

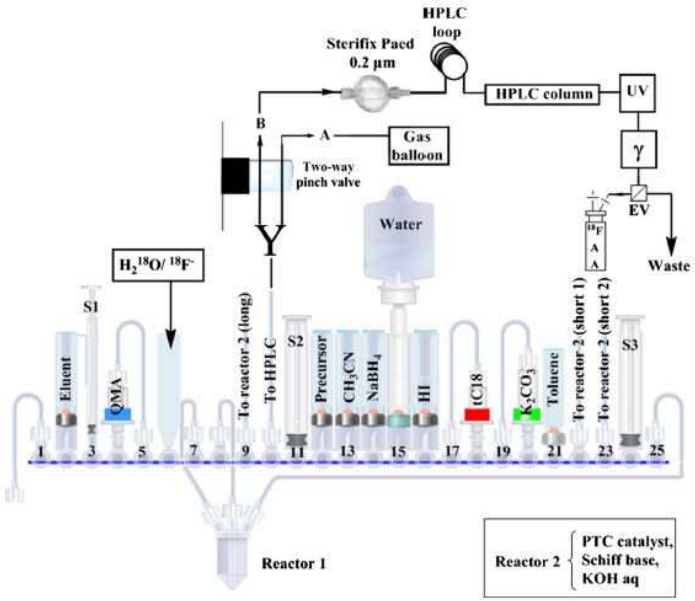


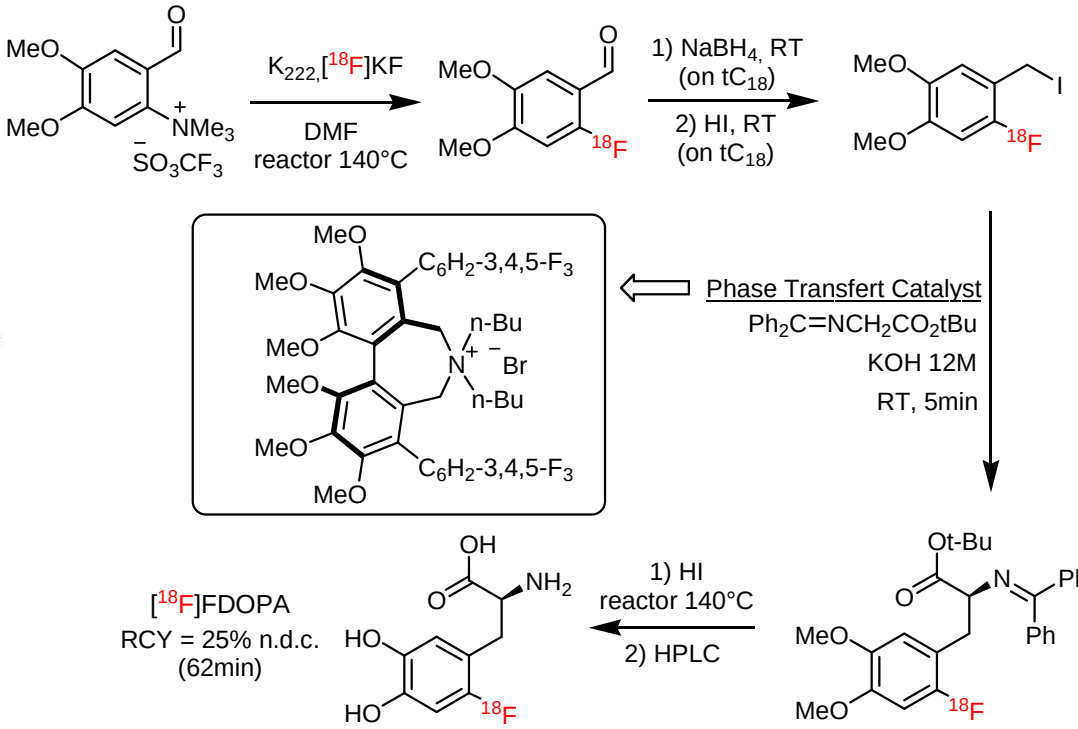




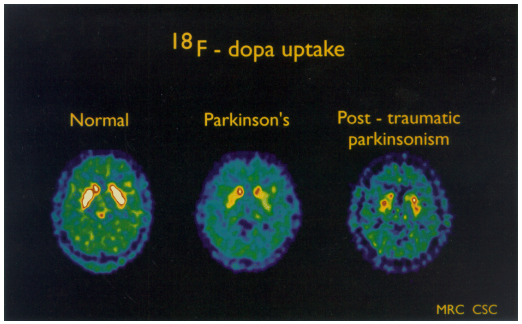
^{18}F Radiotracers

[^{18}F]DOPA



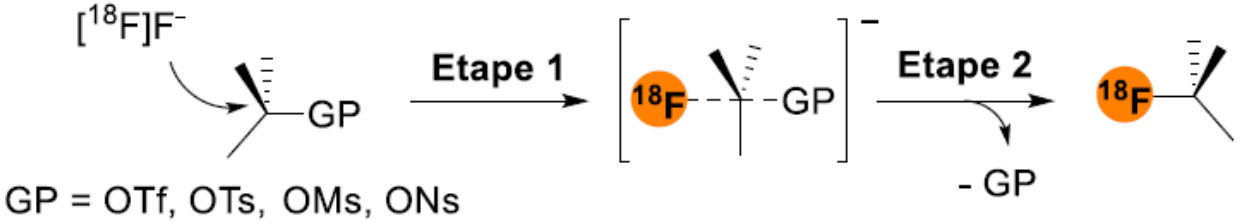


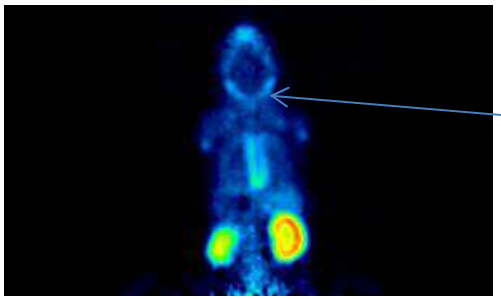
J. Label Compd, Radiopharm, vol. 58, pp. 281–290, 2015



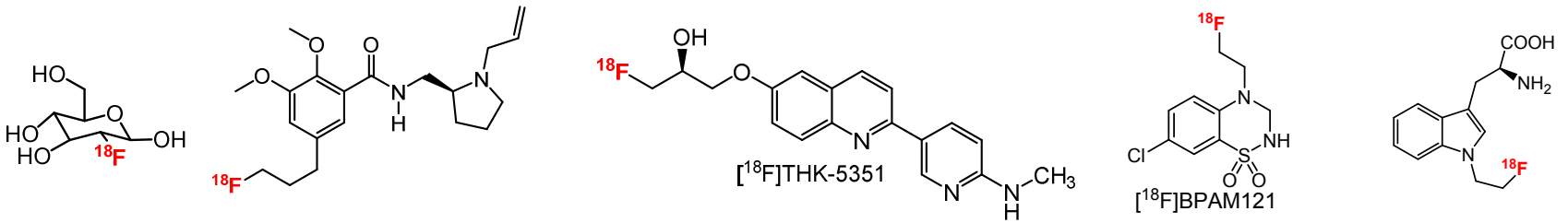
^{18}F Chemistry

Aliphatic labeling



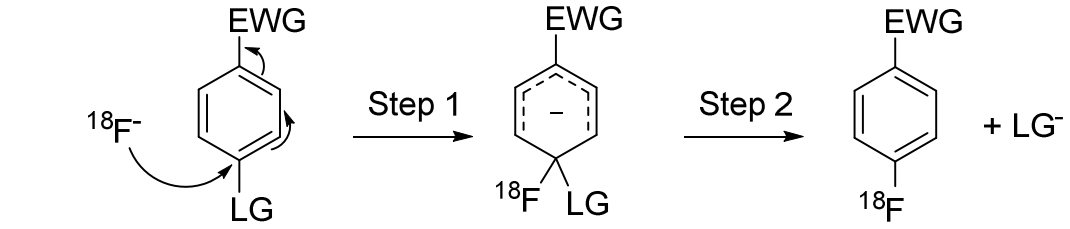


Defluorination issue



^{18}F Chemistry

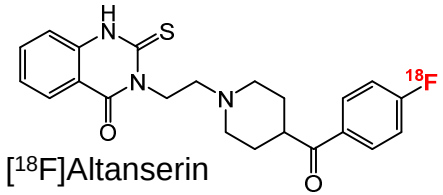
Aromatic labeling



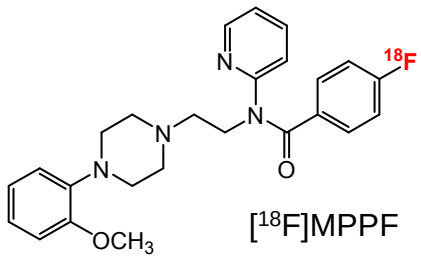
LG: NMe_3^+, NO_2, halogenes
EWG: CN, CF3, Carbonyl

Meisenheimer complex

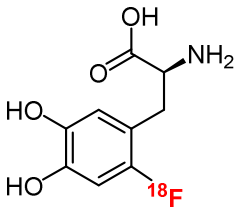
Less defluorination
EWG in para or ortho position is required



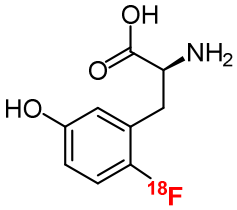
[^{18}F]Altanserin



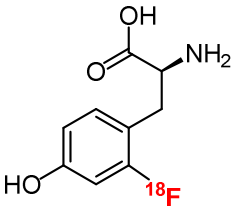
[^{18}F]MPPF



[^{18}F]FDOPA



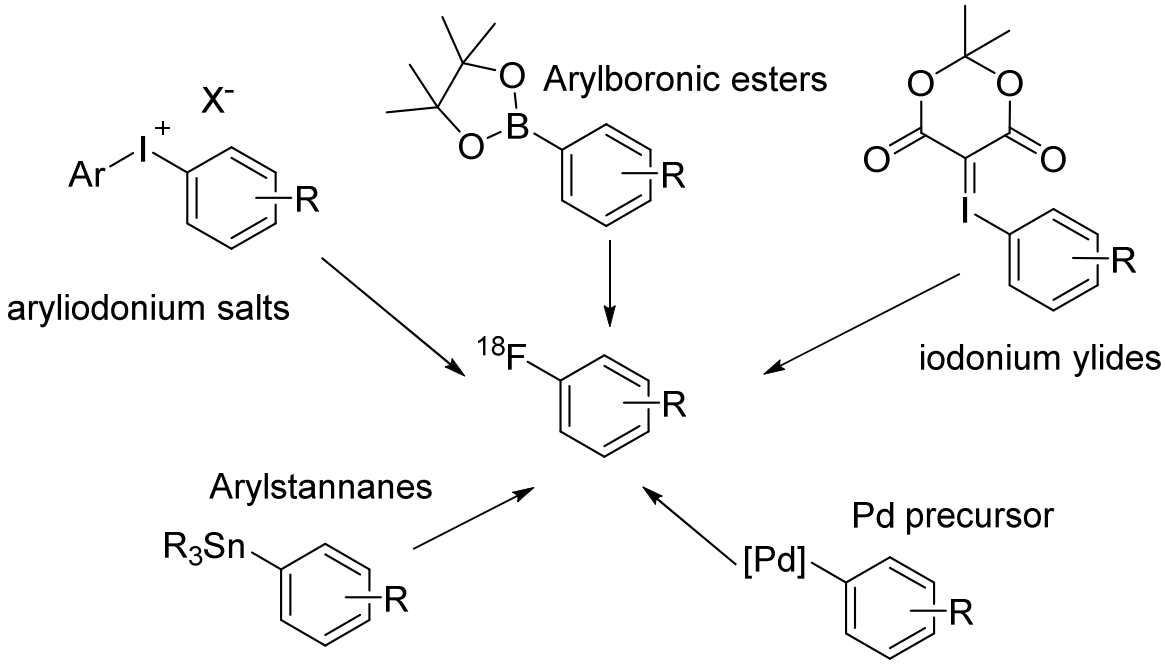
[^{18}F]FMT



[^{18}F]TYR

^{18}F Chemistry

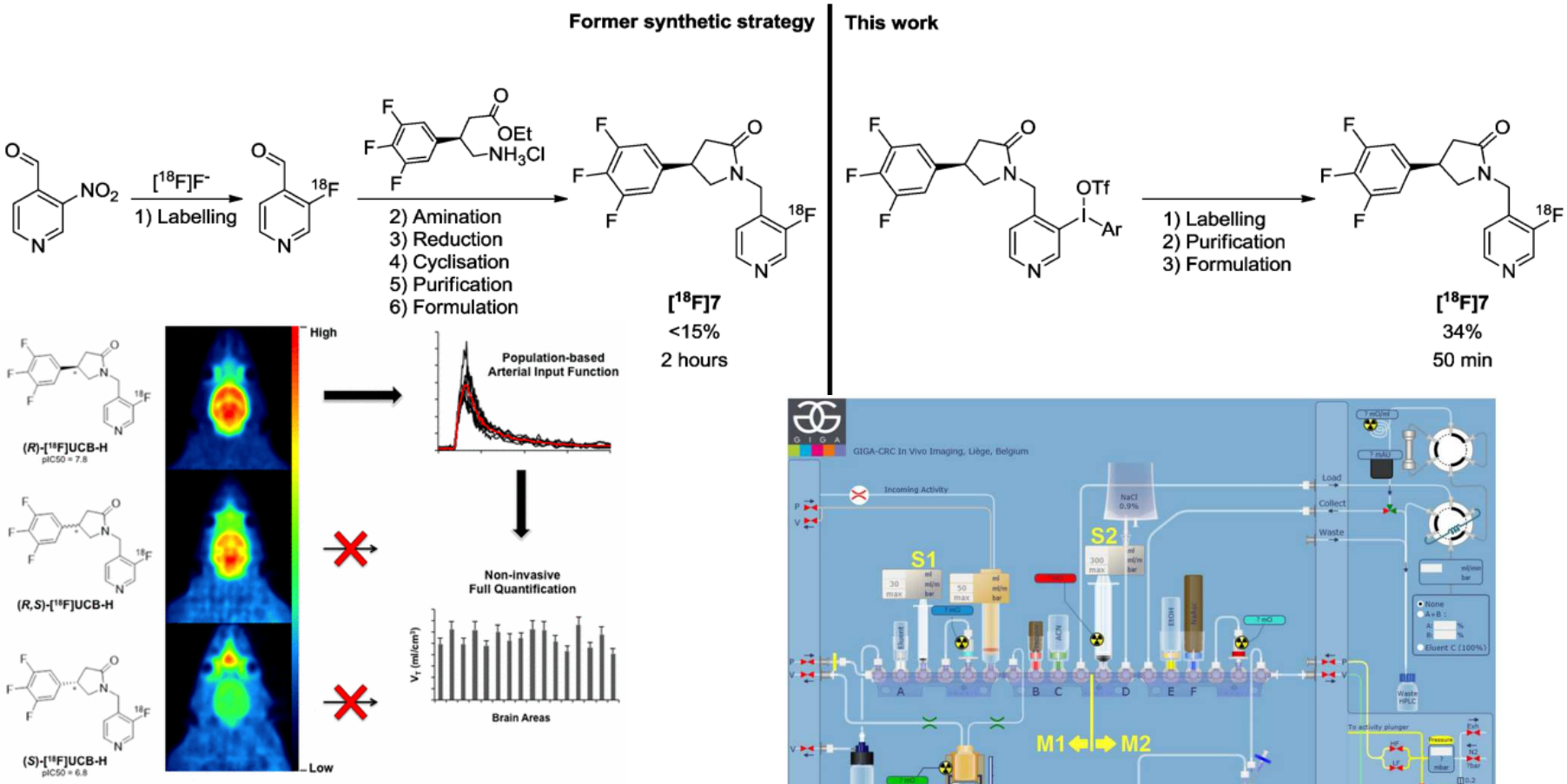
Aromatic labeling



No EWG required for these new methods

^{18}F Chemistry

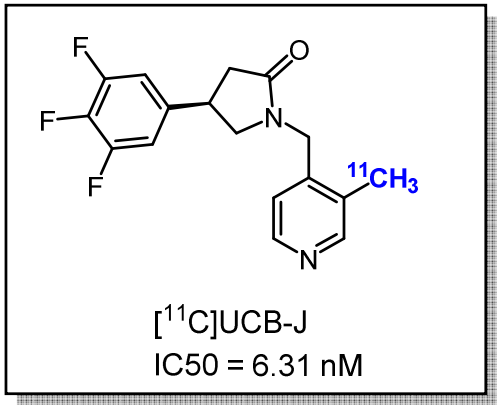
Aromatic labeling UCB-H example



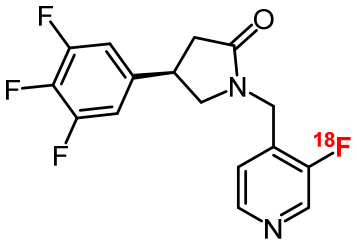
Warnier C., J. Med. Chem. 2016, 59, 8955−8966

Becker, G. Mol. Pharmaceutics 2017, 14, 8, 2719-2725

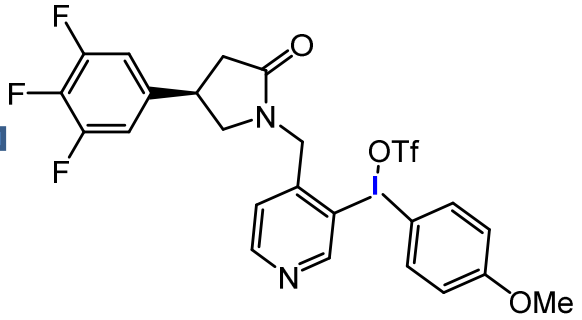
Synthesis of a PET tracer for imaging of synaptic vesicle glycoprotein 2A (SV2A)

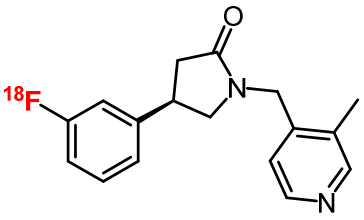


[^{11}C]UCB-J
IC50 = 6.31 nM

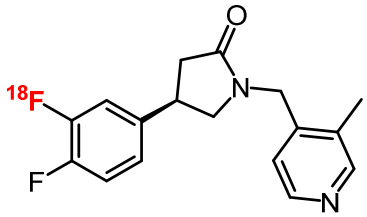


[^{18}F]UCB-H

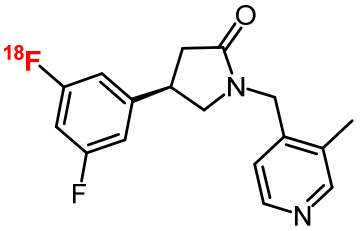




^{18}F-SDM-2
IC50 = 26.9 nM



^{18}F-SDM-7
IC50= 12.6 nM



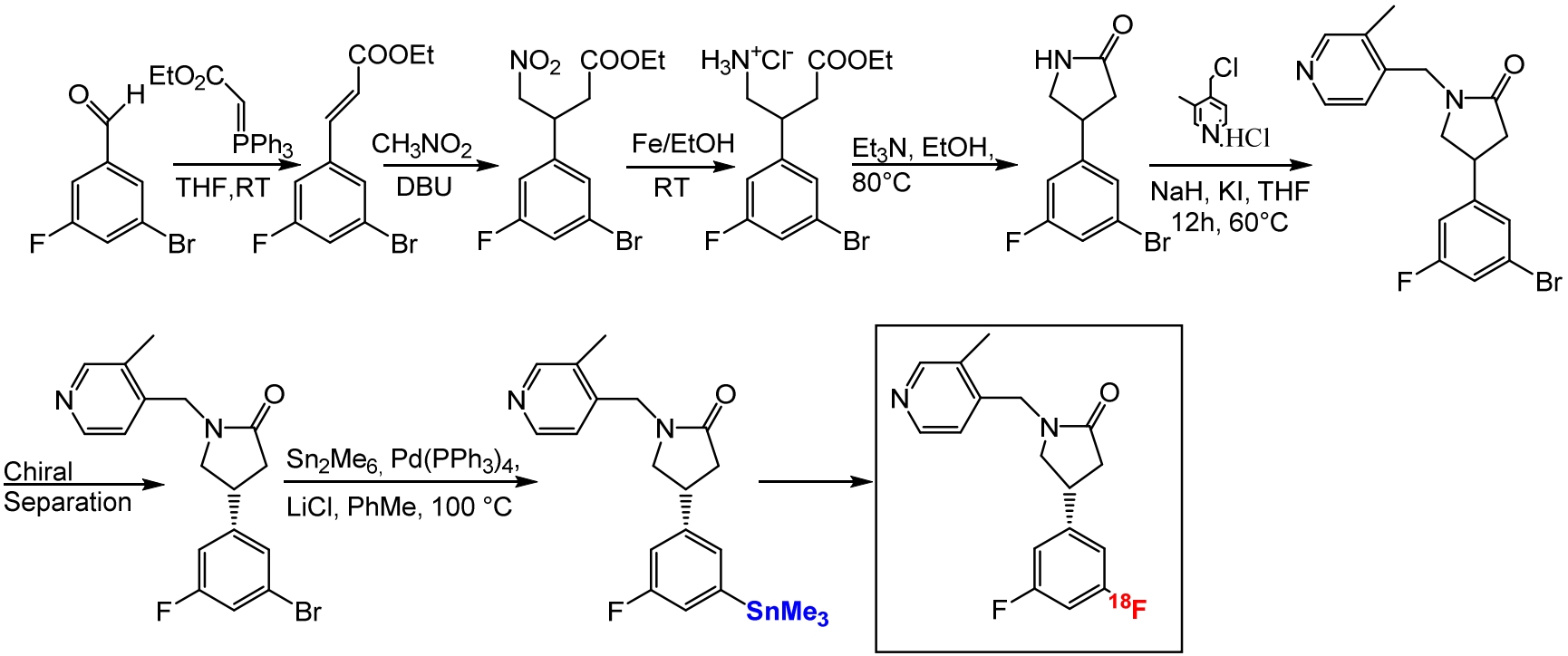
^{18}F-SDM-8
IC50 = 3.52 nM

[^{11}C]UCB-J and [^{18}F]SDM-8 display the same attractive image properties for SV2A:

- high brain uptake,
- appropriate tissue kinetics,
- high level of specific binding.

Warnier et al, 2016 J. Med. Chem.
Becker G et al. 2017 Mol Pharm
Li et al. ACS Chemical Neuroscience 2018, accepted for publication
Constantinescu et al. Mol Imaging Biol 2018

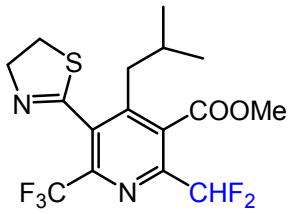
In progress



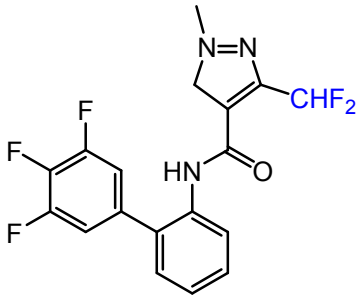
Li S. (2018) ACS Chem. Neurosci. In press

Synthesized the precursor and the ^{19}F-reference (chiral separation required)
Automated the synthesis and the purification
Developed a QC
Stability studies
Pre clinical studies
GMP production

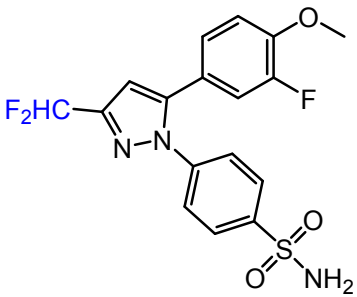
^{18}F-Difluoromethylation



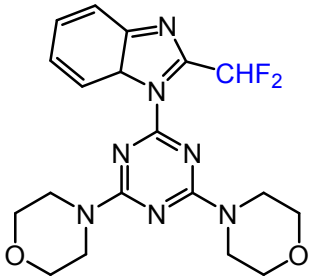
Thiazopyr



Fluxapyroxad

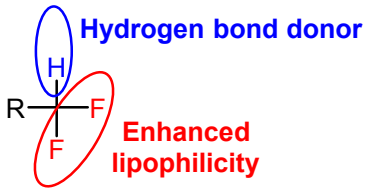


Deracoxib



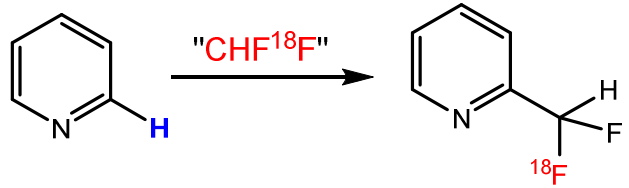
ZSTK474

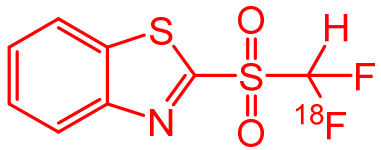
Zafrani Y et al. (2017) J Med Chem



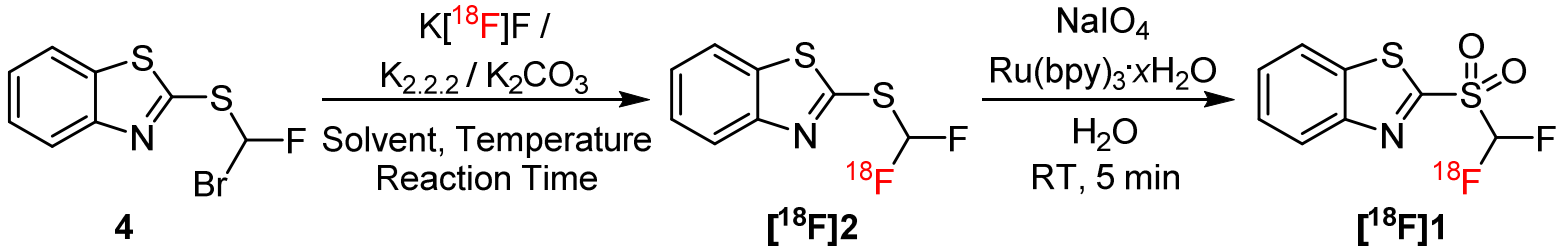
Difluoromethylgroup is considered as a lipophilic hydrogen bond donor that may act a bioisostere of hydroxyl (-OH), thiol (-SH), or amine group (-NH_2)

Lemos A et al. Advanced Synthesis & Catalysis (2018)





- C-H activation
- No prefunctionalization required
- Late stage [^{18}F]fluoride insertion
- Radical pathway to introduce « $CH^{18}F_2$ »
- Mild conditions

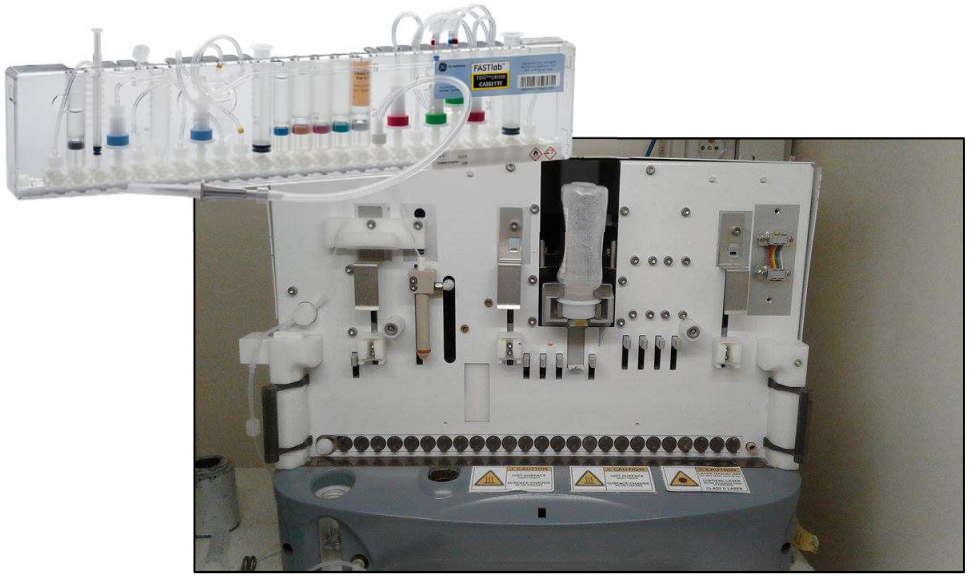


4 **[^{18}F]2** **[^{18}F]1**

RCY: 15,2 ± 0,3 %

RCY: 13,4 ± 0,4 %

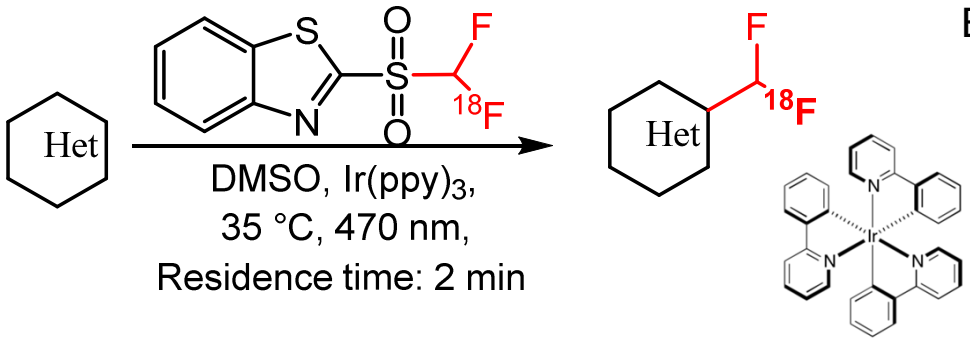
Molar activity = 81 GBq/μmol (dc)



GE FASTlab™ synthesizer

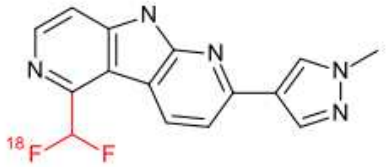
Trasis AllInOne synthesizer

Photoredox reaction

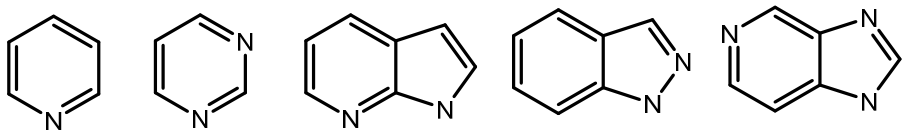


Experimental:

- 37 MBq (1 mCi) of sulfone
- Substrate (20 µmol)
- Ir(ppy)3 (0,01 µmol)
- DMSO
- Blue LED (470 nm, 2W)

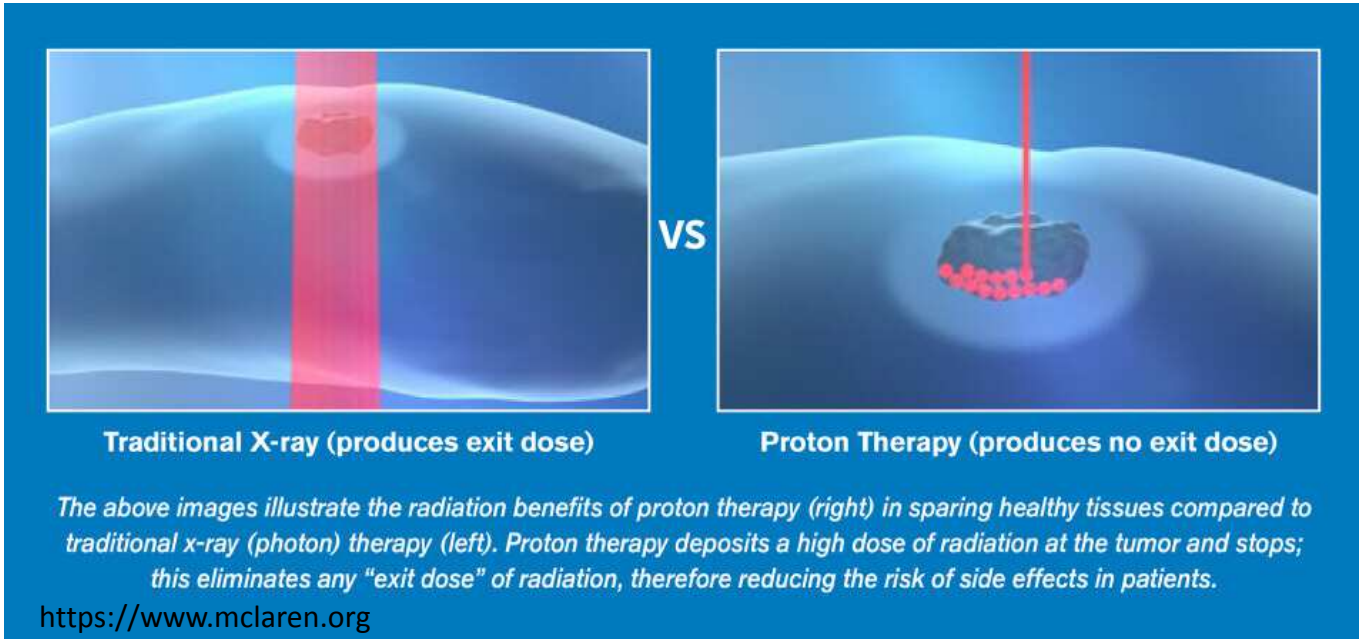


22 examples
(18-75 %)

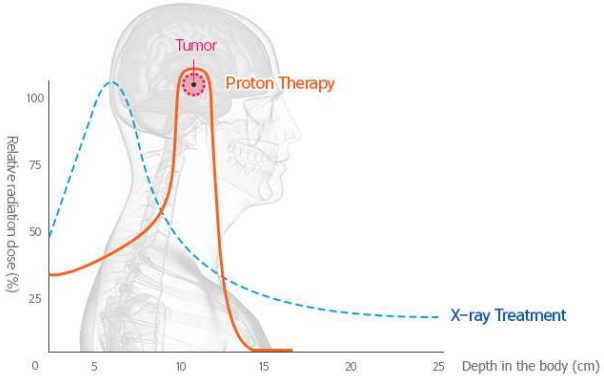


Pyrimidine

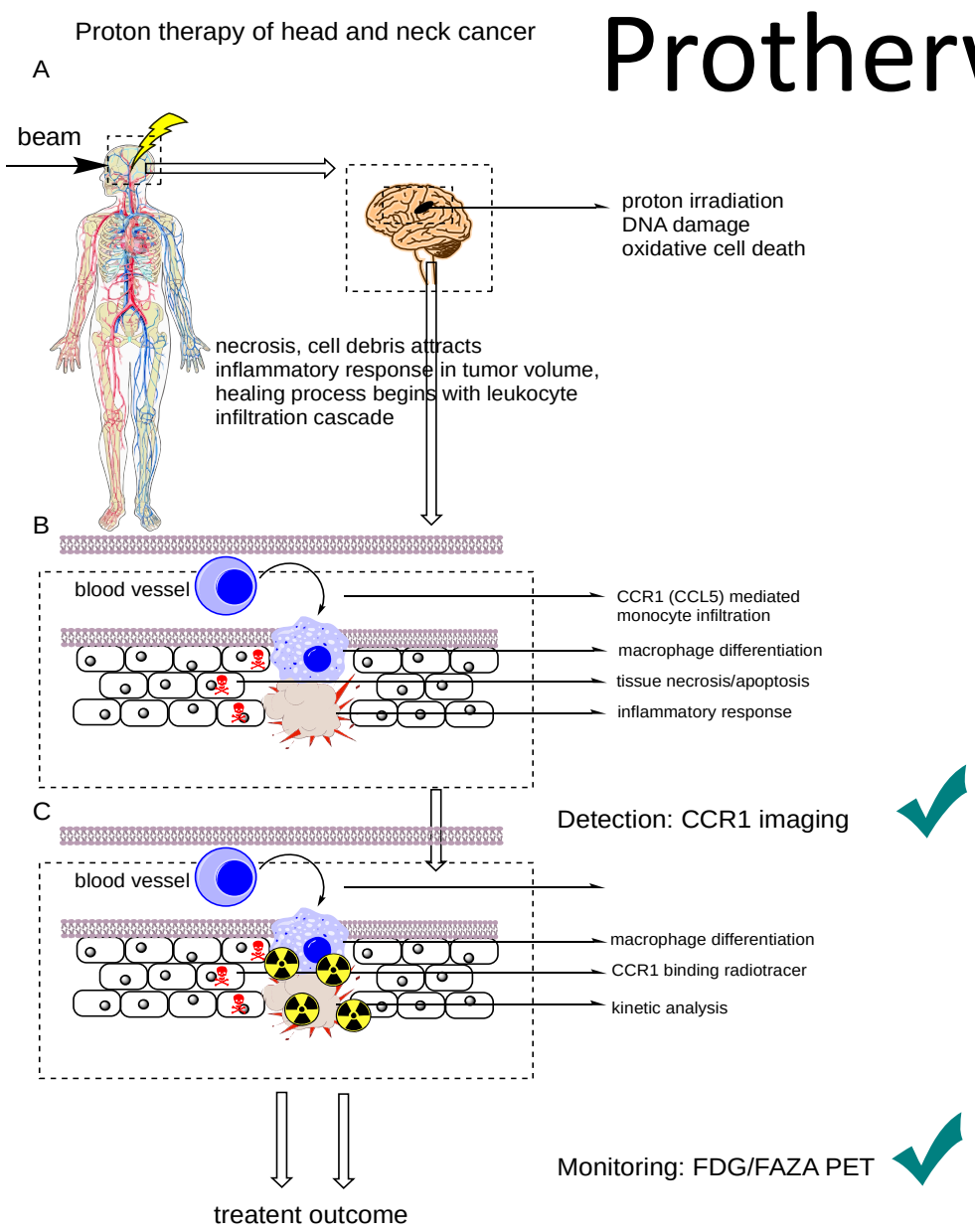
Protherwal



https://www.mclaren.org

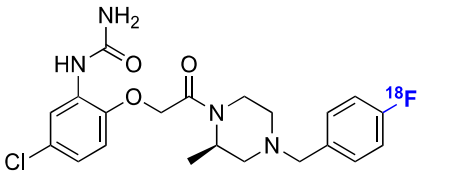


Protherwal



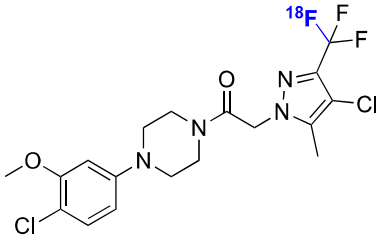
The overall objective of this project is to introduce a new method for diagnosis and detection of inflammation by the translational development of CC chemokine receptor 1 (CCR1) antagonist PET radiotracers. Moreover, a better knowledge of inflammation and treatment response allows for advanced decision making for adjustment of the treatment tailored to individual patients.

Two selected CCR1 ^{18}F-tracers



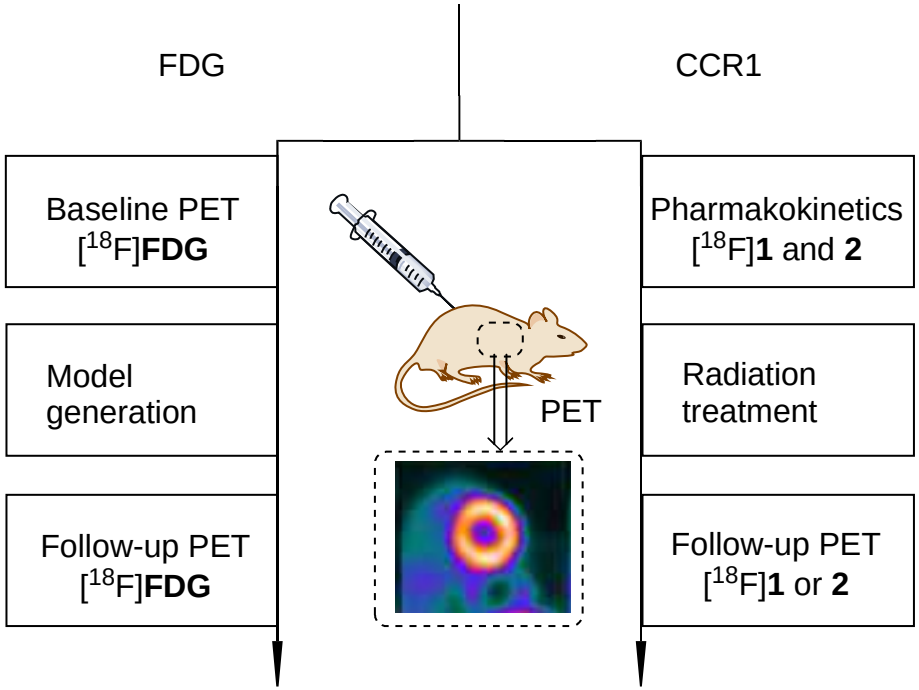
N-[5-chloro-2-[2-[(2R)-4-[(4-fluorophenyl)methyl]-2-methyl-1-piperazinyl]-2-oxoethoxy]phenyl]-urea,

1 (ZK-811752)



2-(4-chloro-3-(trifluoromethyl)-5-methyl-1H-pyrazol-1-yl)-1-(4-(4-chloro-3-methoxyphenyl)piperazin-1-yl)ethan-1-one

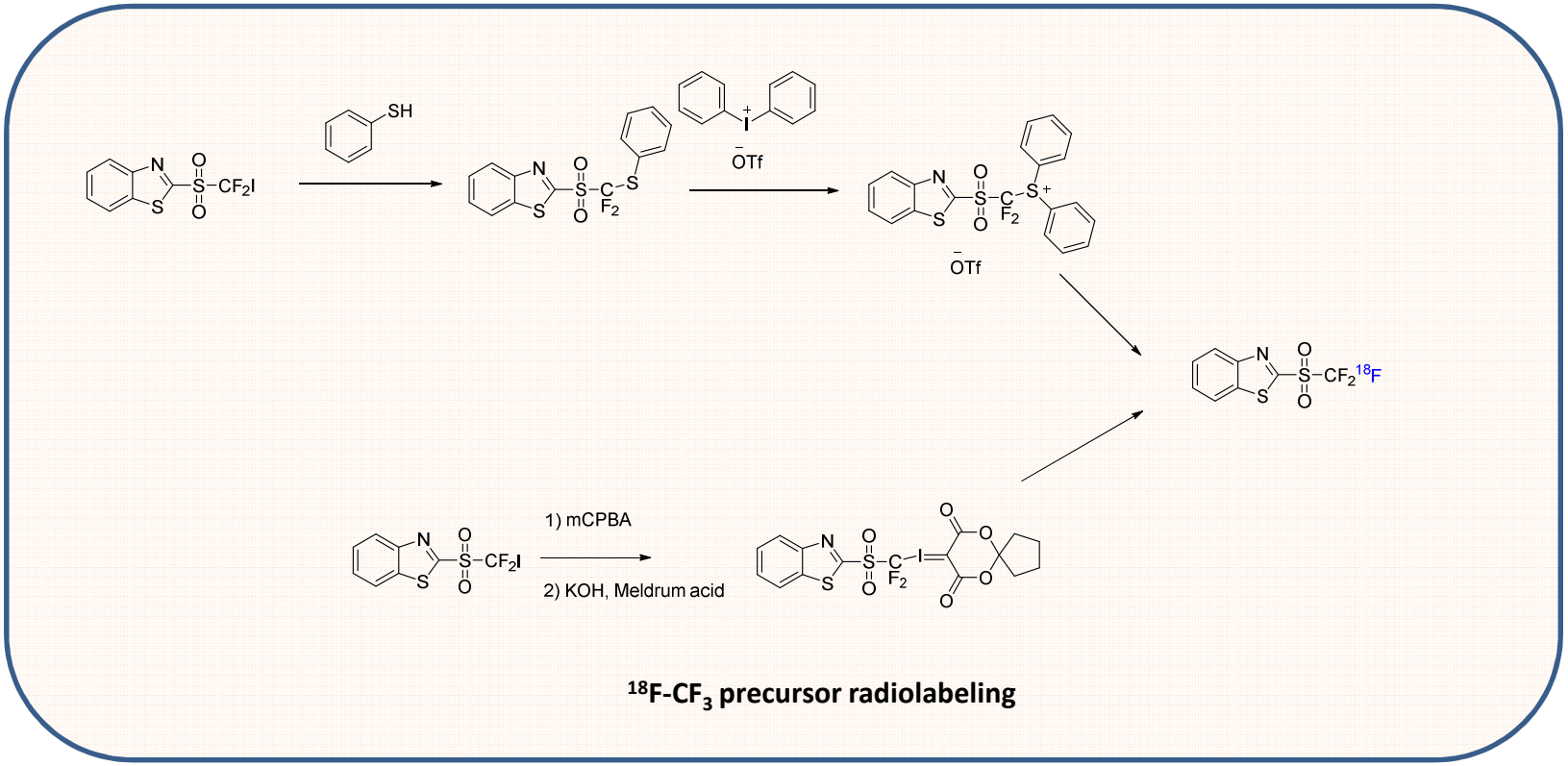
2 (CCX354)



1 has a high affinity c-type chemokine receptor 1 (CCR1) ligand with a binding affinity (IC_{50}) of 1.5 nM and functional antagonism.

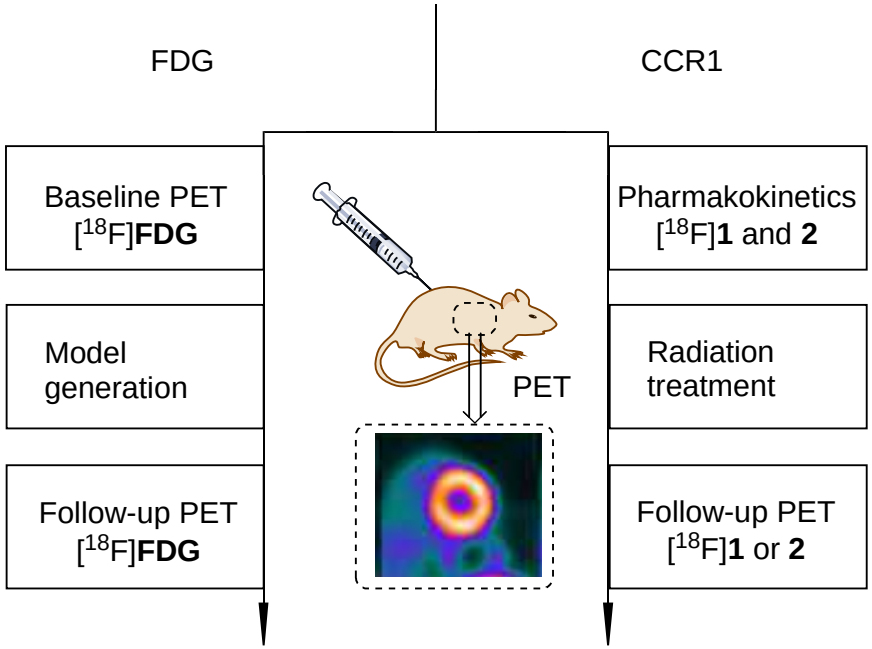
Candidate **2**, is a selective CCR1 antagonist with a nanomolar potency of inhibition of CCL5-induced chemotaxis and a nanomolar binding affinity. In a safety screen against a panel of >50 ion channels, transporters, and GPCRs no significant inhibitory activity was observed at a concentration of 1 µM.

- **WP1**: Synthesis and radiosynthesis: **2**(CCX354)



- **WP2**: Preclinical studies

- A CTI Focus 120 small animal PET scanner at the CRC will be used for imaging.
- Blood sampling will be conducted to evaluate the metabolic stability of the radiotracers.
- Animals will be sacrificed and tissue samples will be harvested for further investigation using microscopy, immunohistochemistry and autoradiography to co-localize radiotracer binding with the inflammatory lesions.
- For comparison, FDG scans will be repeated in groups of animals which will undergo scans with [^{18}F]**1**+**2** to evaluate the pharmacokinetics of the radioligands and validate the inflammation models. The data obtained hitherto will be used for evaluation of the study and validation of the tracers.



Small radiotracers vs biomolecules

Biomolecules (peptide, protein, antibody…) allow more specific targeting with higher affinity compare to small molecules

➔ use these biomolecules as radiotracers

➔ radiolabel it with ^{18}F

Fluorine-18 labeling conditions are too harsh (organic solvent, high temperature, high pH) to add the fluorine directly on the native biomolecule

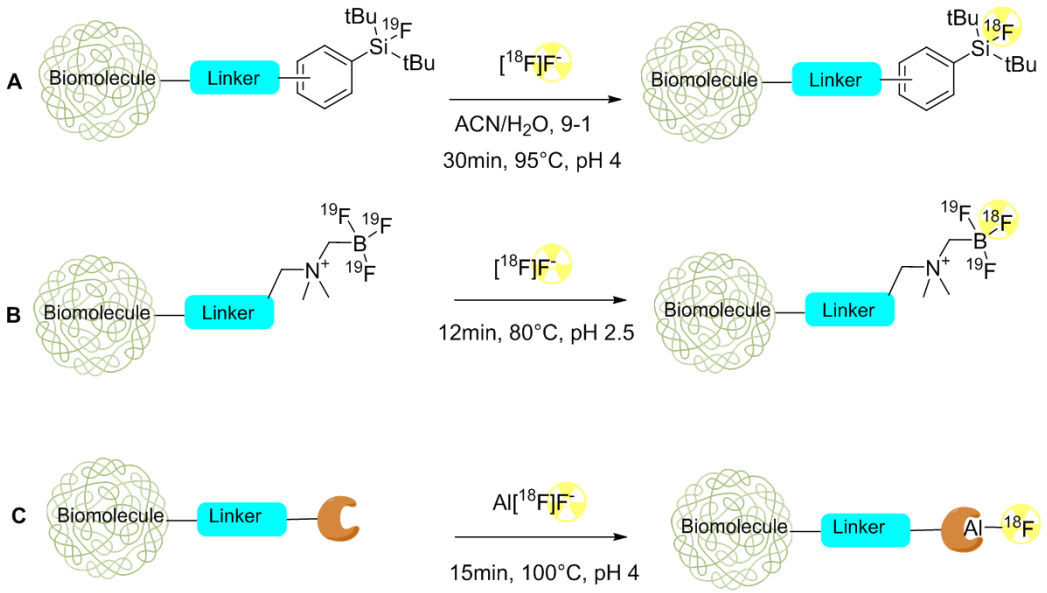
➔ Degradation

➔ Diffferent methods to radiolabel a biomolecule with fluorine-18:

Direct labeling
or « late stage labeling »

Prosthetic approach

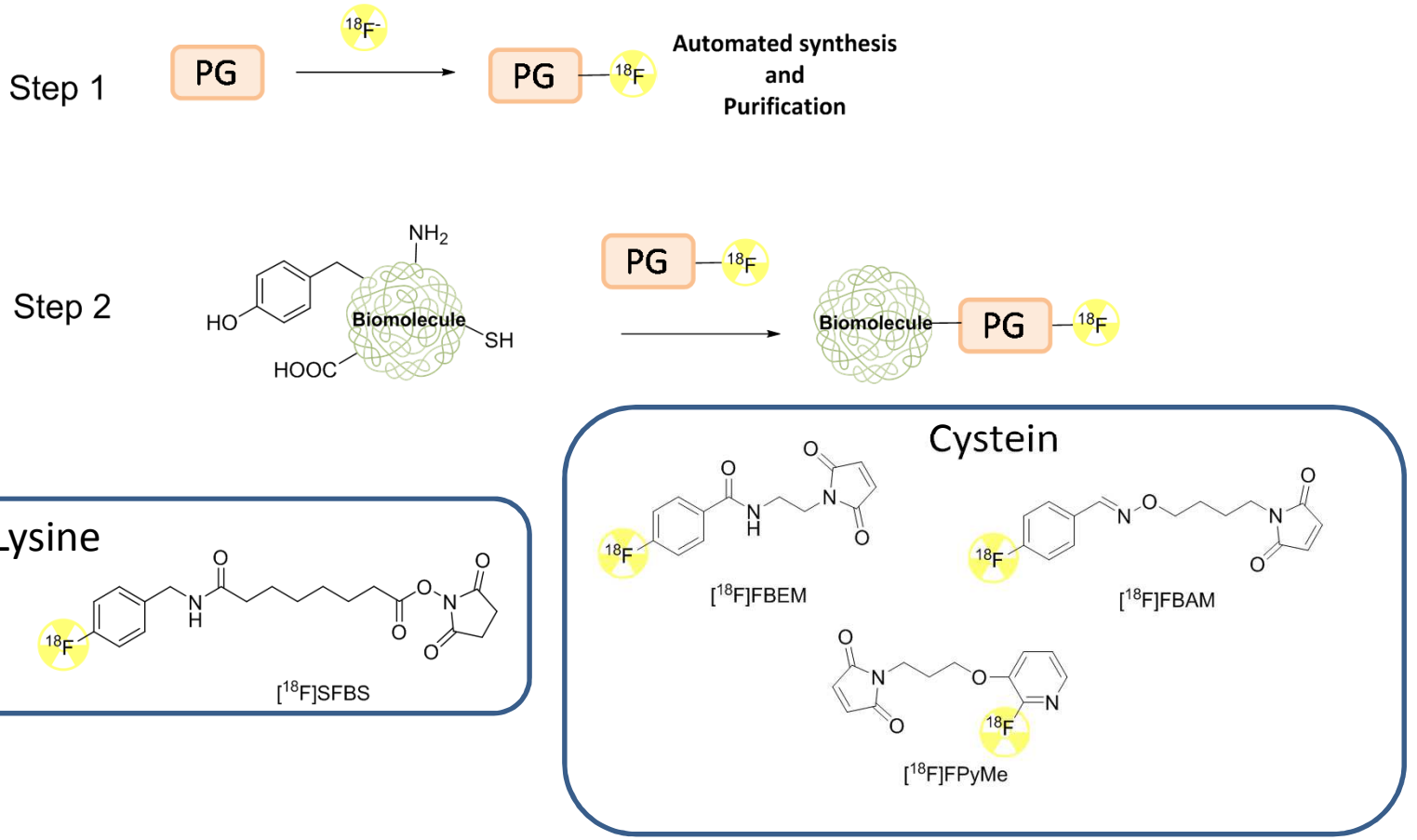
Direct labeling



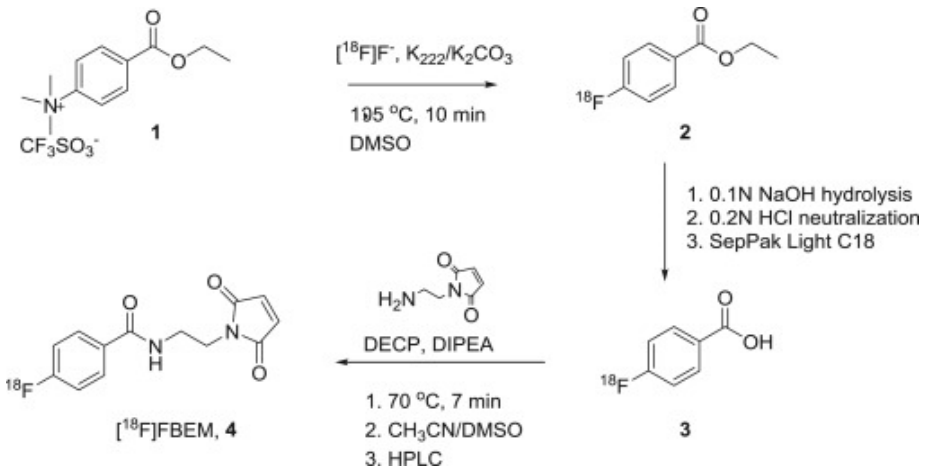
Many issue:

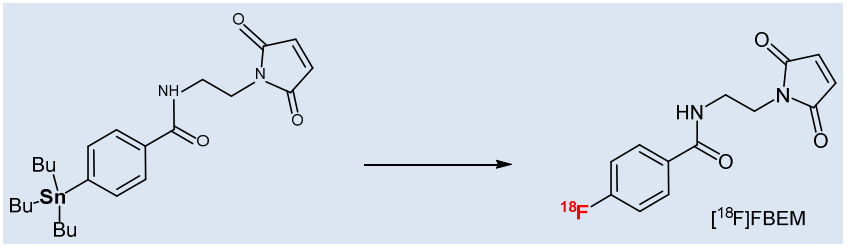
- Low specific acitivity due to the presence of fluorine-19
- The addition of these chemical entities may alter the biological activity of the biomolecule.
- Labeling condition relatively harsh (pH, temperature…) leading to biomolecule degradation.

Prosthetic approach

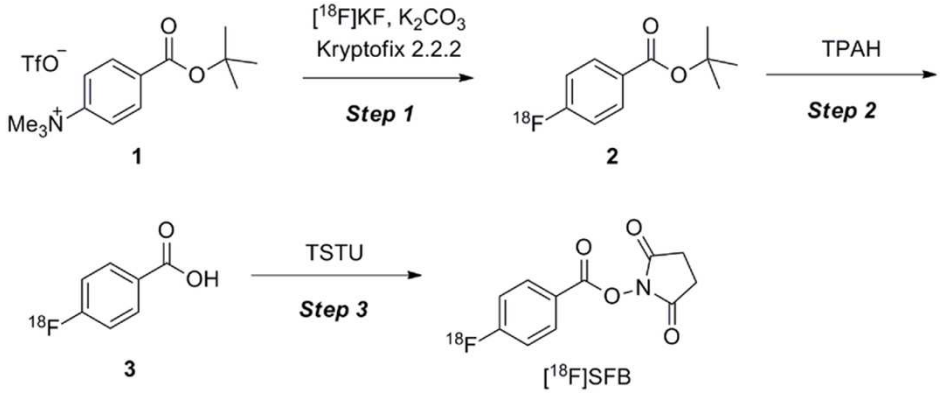


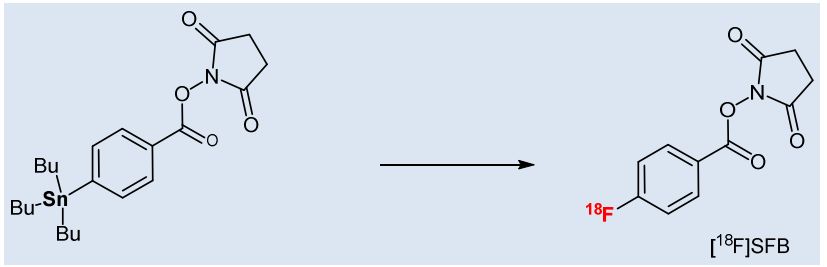
In progress





K. Lim, et al. Applied Radiation and Isotopes, Vol. 140, 2018, 294-299,



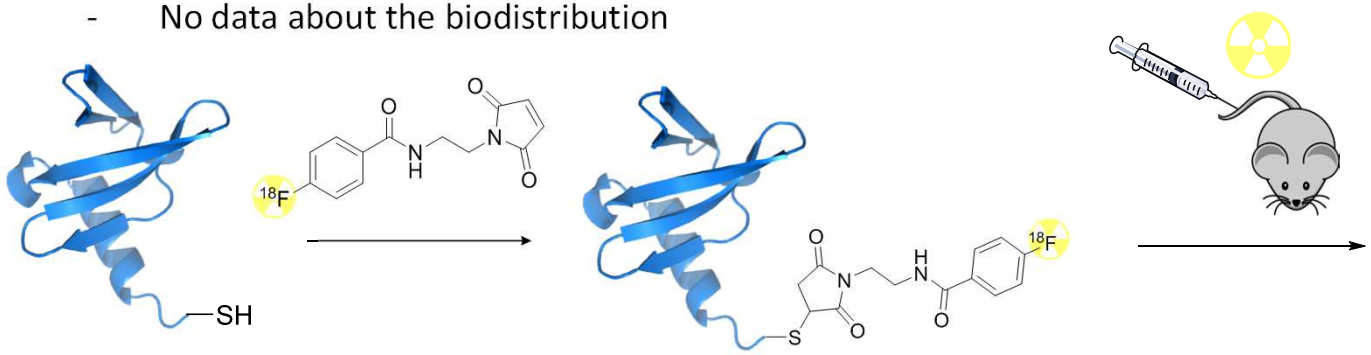


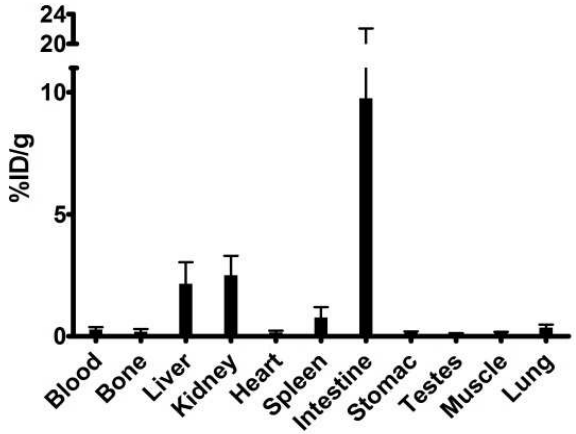
H.Kimura et al. PLoS ONE, 2016, 11(7):e0159303

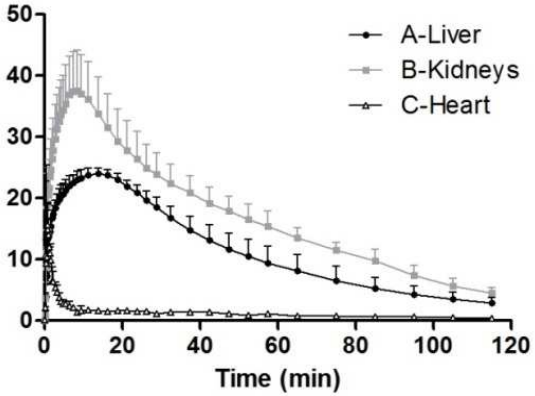
Radiolabeling of Nanofitin with [^{18}F]FBEM

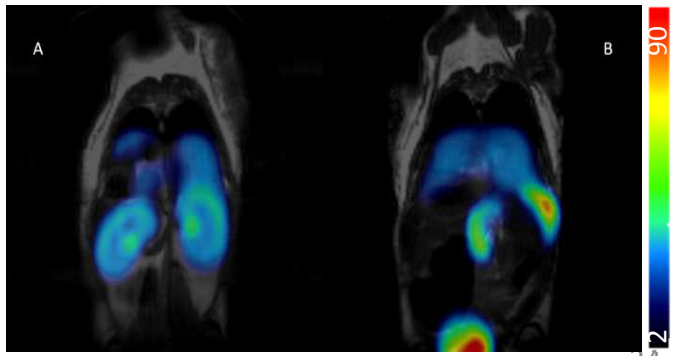
Nanofitin

- Alternative to antibody
- Produced by bacterial fermentation
- No data about the biodistribution









Pharmacokinetic of macrobiomolecule

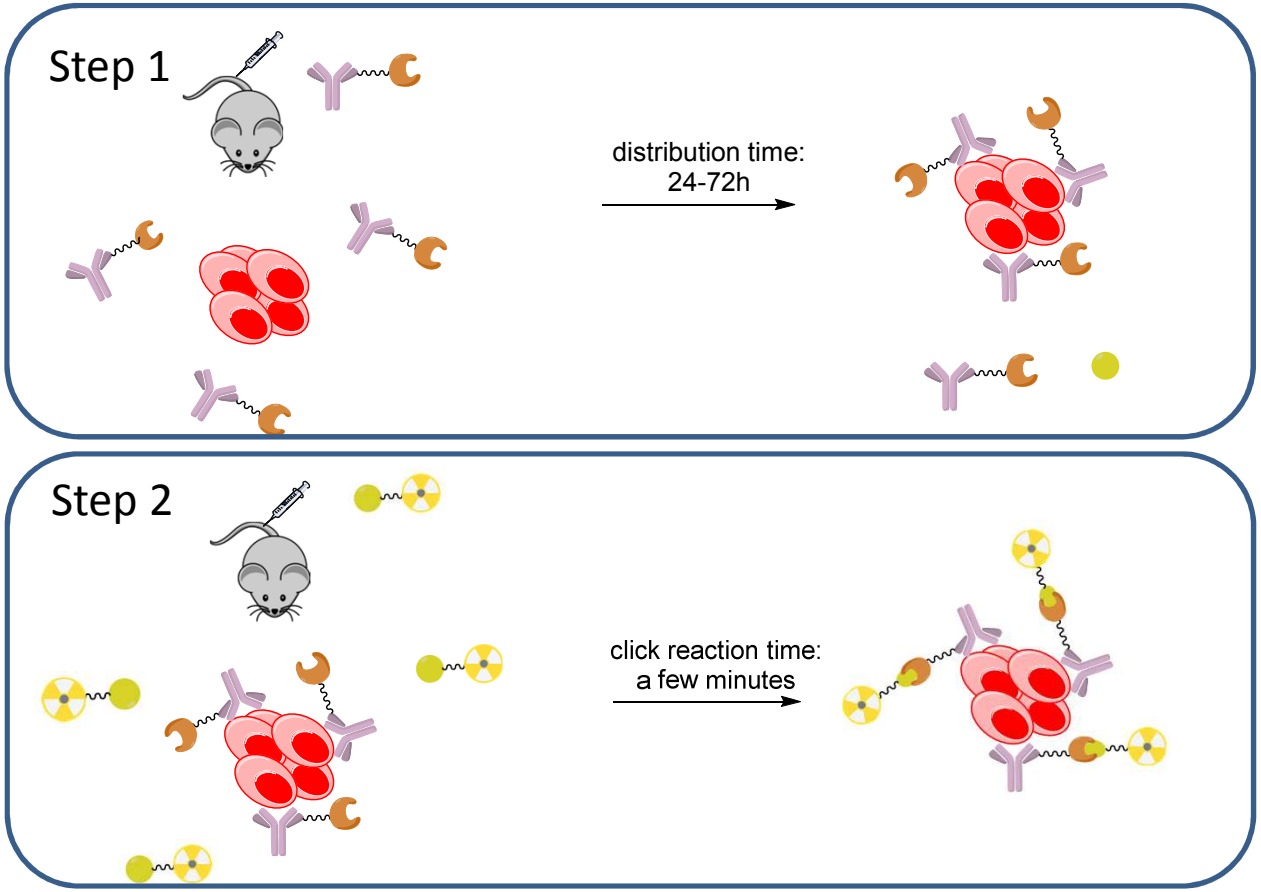
Macrobiomolecules as antibody have low pharmacokinetic (few days)
fluorine-18 isotope (110 min)
→ low signal-to-noise ratio is exploitable only a few days after injection because of slow elimination of free-circulating antibodies that did not reached their target.

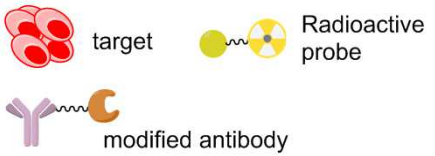
Two solutions:

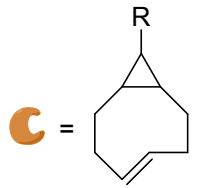
- Working with longer half-life radionuclides (^{89}Zr $t_{1/2}$= 3.2 days, ^{124}I $t_{1/2}$ = 4.1 days)
BUT the effective dose received by the patient due to long exposure

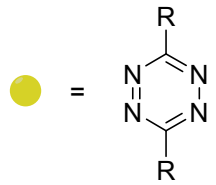
- Using the pretargeting technique

IEDDA for pretargeting experiment



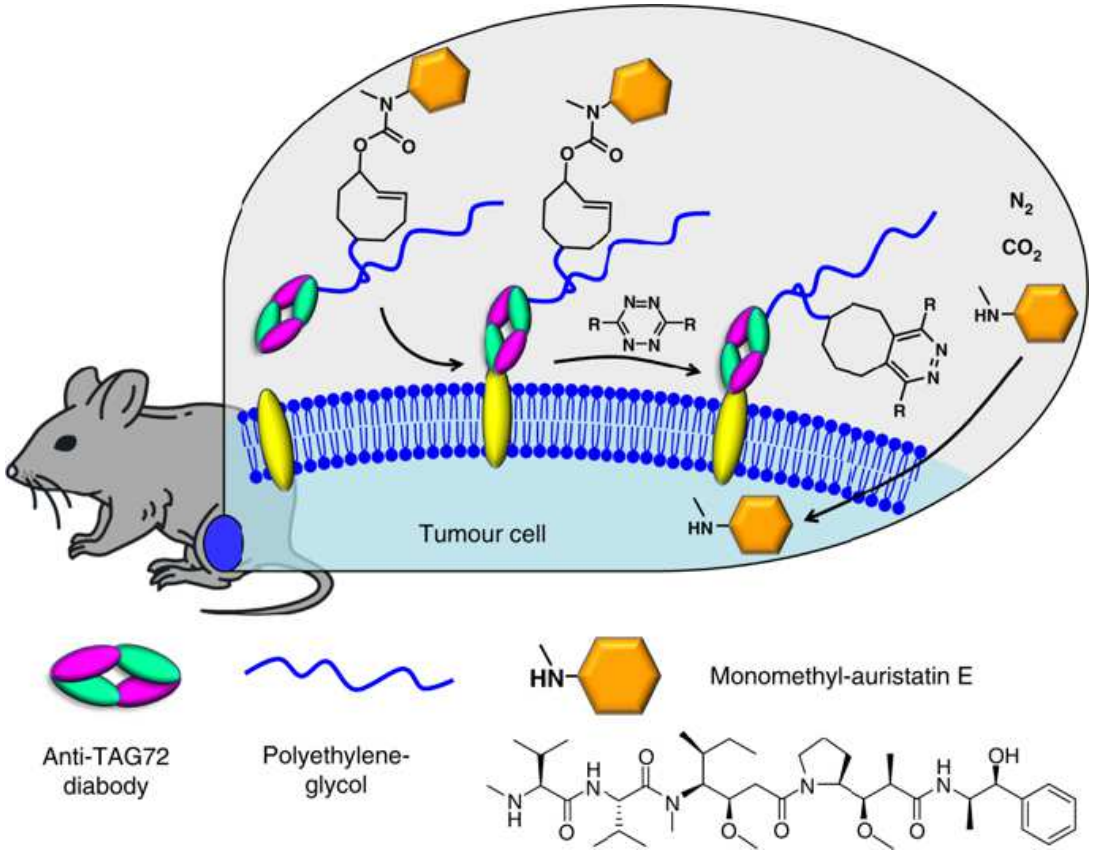






Addition of a clearing agent between the two steps to clear the Ab circulating in the blood

Click and release



Nat Commun **9,** 1484 (2018) doi:10.1038/s41467-018-03880-y

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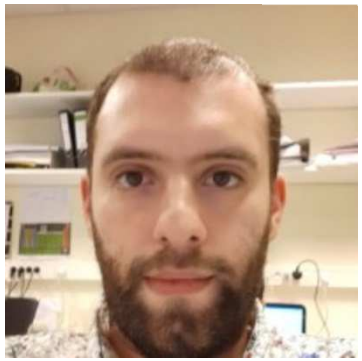
LIÈGE université
GIGA
CRC In vivo Imaging

Pr. Riss
Head of the radiochemistry



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