^{18}F-radiolabelling

Where are we now?

Inês F. Antunes
31-01-2025

umcg

Direct radiolabelling

- ^{18}F-Nucleophilic
- Metal-mediated ^{18}F-Radiofluorination
- Light-mediated ^{18}F-Radiofluorination
- Metal-complexation ^{18}F-Radiofluorination

Indirect radiolabelling

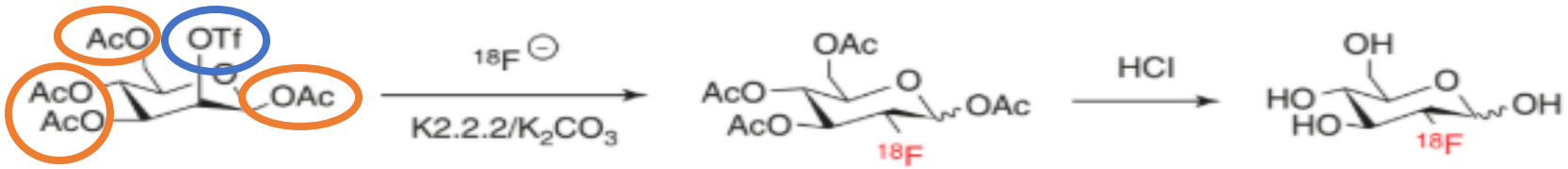
- Prosthetic groups (Synthons)
- Click chemistry

^{18}F-Nucleophilic Substitution

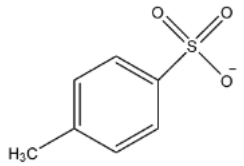
- ^{18}F-fluoride must be dehydrated
- Higher temperatures (~80–100 °C)
- Presence of poorly nucleophilic bases (carbonate; bicarbonate or oxalate ions)
- Polar aprotic solvents: ACN, DMF or DMSO.
- phase transfer catalyst (PTC) (Kryptofix 222) or tetrabutylammonium cation.

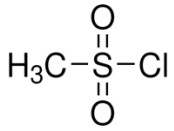
- **Reactivity of leaving group and its sensitivity to basic conditions should be considered**

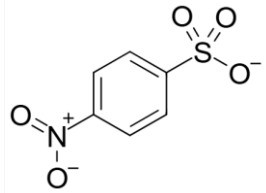
^{18}F-Nucleophilic Aliphatic Substitution

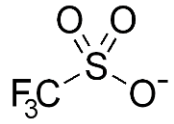


1. A good **leaving group**: Halides and sulfonates
Cl < Br < I < tosylate ~ mesylate < nosylate < triflate


Ts

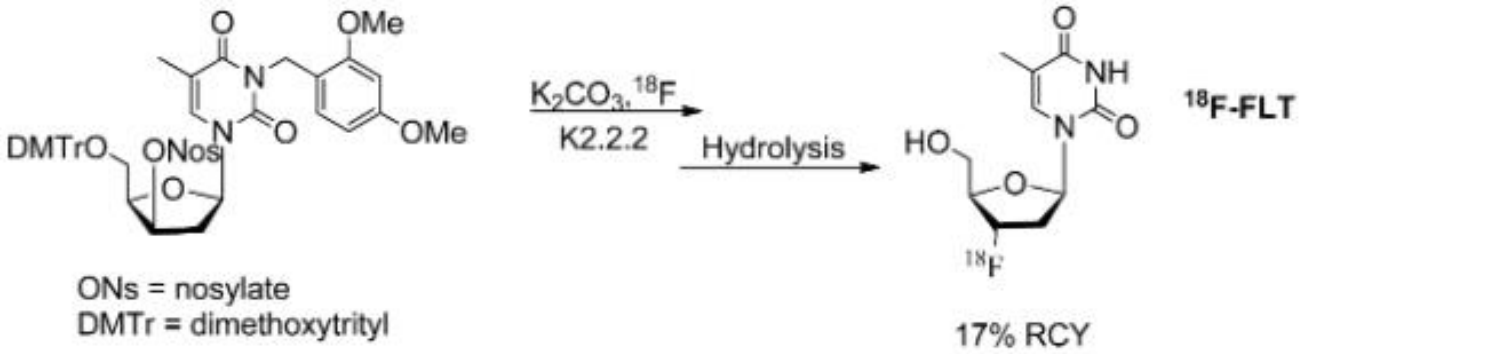

Ms


Nos

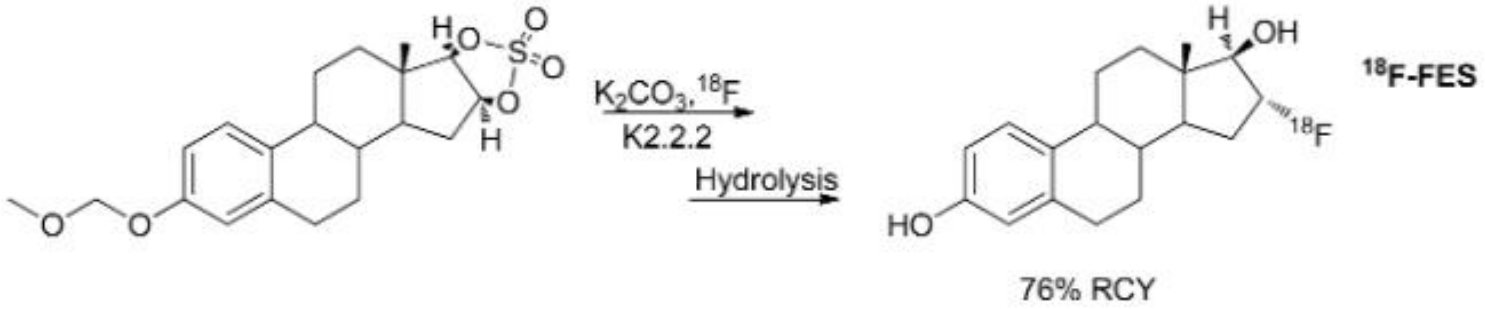

Tf

2. Protecting groups: Precursors that contain functional groups that might hamper nucleophilic 18F-substitution reactions either due to their acidity or steric hindrance

^{18}F-Nucleophilic Aliphatic Substitution



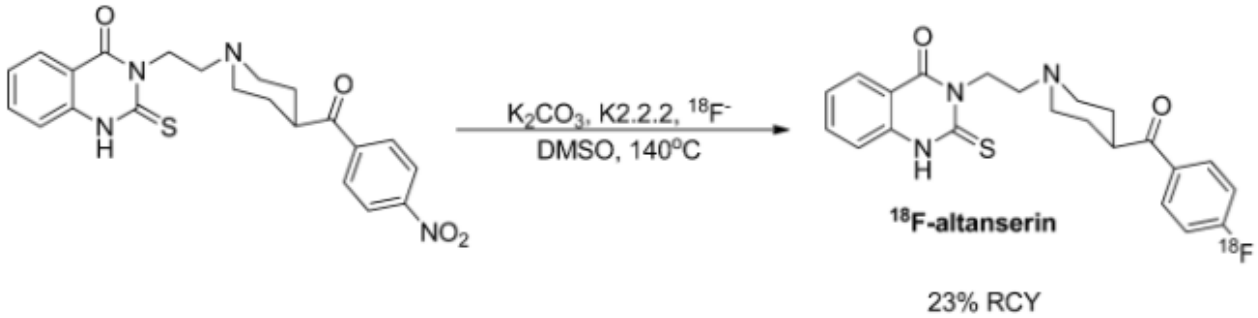
ONs = nosylate
DMTr = dimethoxytrityl



dx.doi.org/10.1021/bc500475e | Bioconjugate Chem. 2015, 26, 1−183

^{18}F-Nucleophilic Aromatic Substitution

Issue: It requires sufficient activation or the phenyl ring→ electron-withdrawing groups (-NO_2, –CN, –CF_3, or carbonyl groups) in the ***ortho*** or ***para*** position to the leaving group



1. A good leaving group: F, NO_2 and trimethylammonium salt

LG	F	NO_2	NMe_3^+
Temperature (°C)	>100	120-180	100-110
Solvent	DMSO	DMF/DMSO	ACN
Molar activity	Low	Low	High

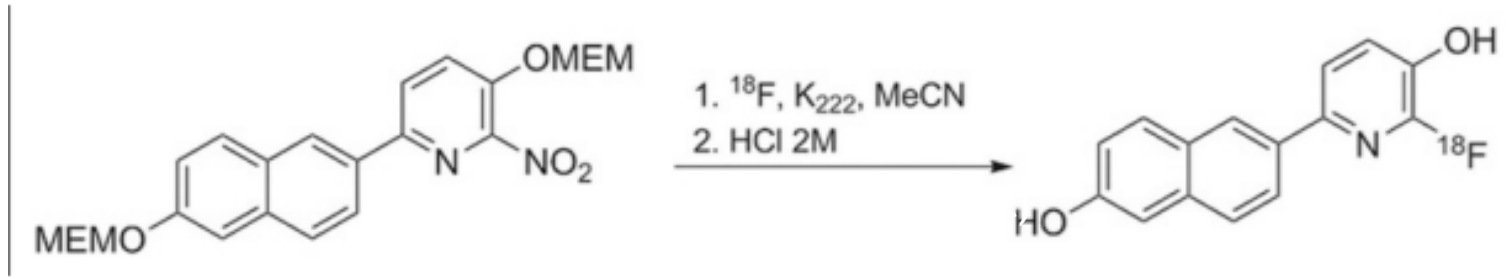
dx.doi.org/10.1021/bc500475e | Bioconjugate Chem. 2015, 26, 1–184

^{18}F-Nucleophilic Aromatic Substitution

Solution:

Heteroarene (pyridine)→ no need of electron-withdrawing groups

- substituition occurs preferentially in the *ortho* position of the pyridine
- good leaving group :F, Cl, Br, I, NO_2 and trimethylammonium salt
- Temperatures: 120-180 ^{0}C
- Solvents: DMF, DMSO
- Protecting groups



Antunes et.al. J Nucl Med. 2017 Apr;58(4):554-559

^{18}F-Nucleophilic Aromatic Substitution

Issue: most suitable fluorinated drugs contain a phenyl group instead of an heteroarene

Substitution of the phenyl group with pyridine

Enable the synthesis of the ^{18}F analog

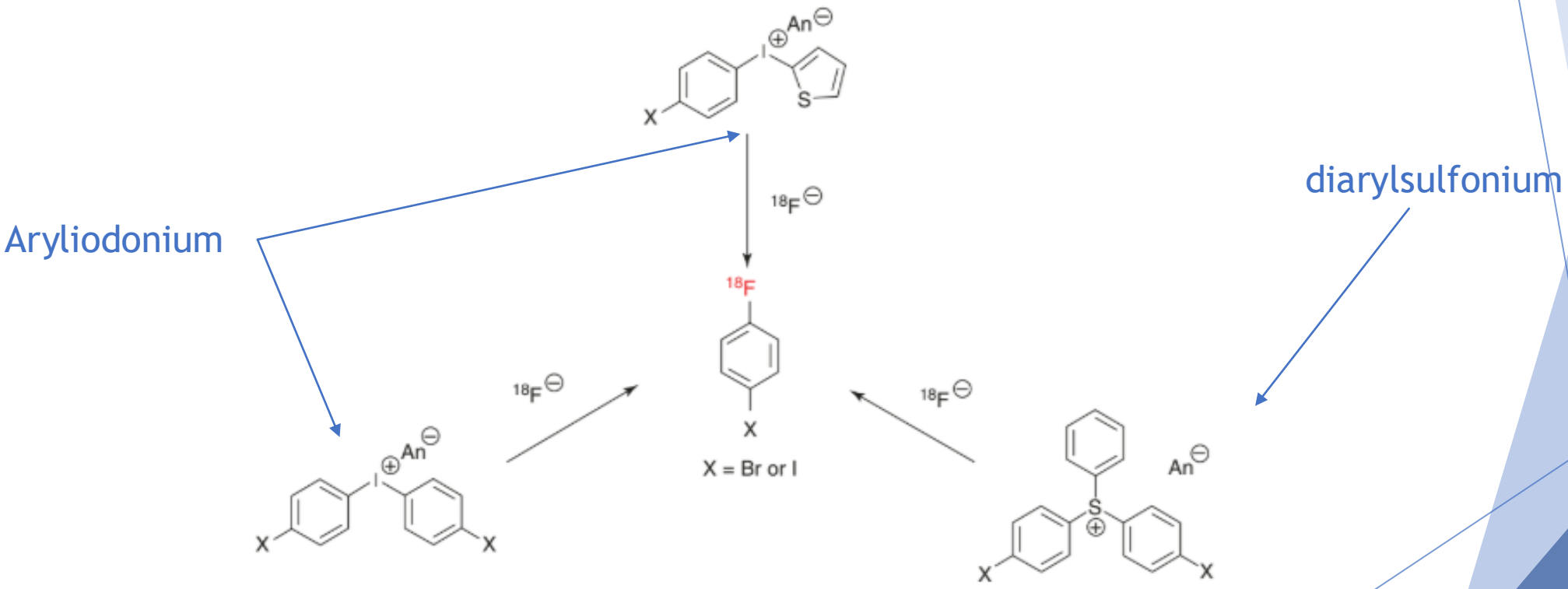
Might alter the structure→affinity for target

Need better radiofluorination strategies to add ^{18}F to a phenyl group

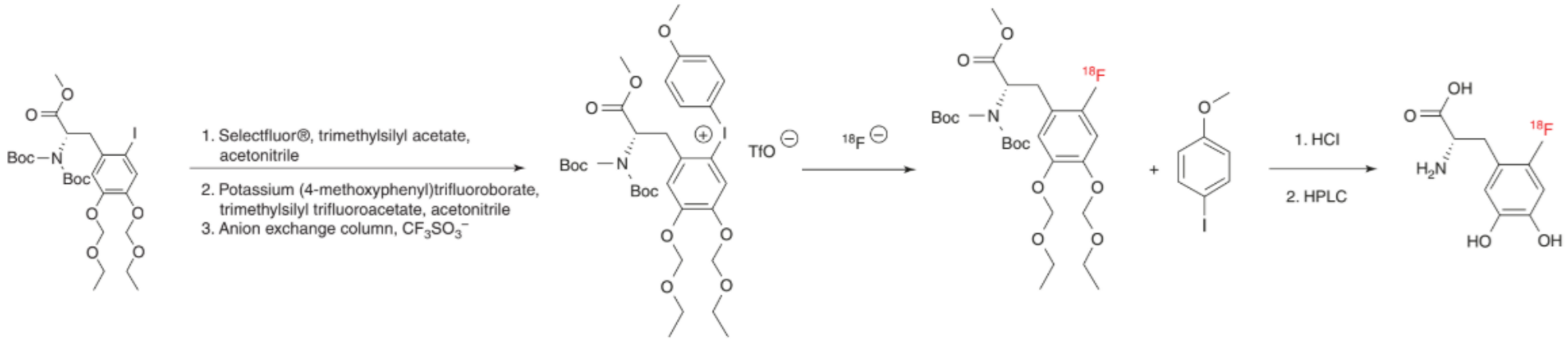
^{18}F-Nucleophilic Aromatic Substitution

Possible Solution: Use of "Onium"salts

Onium leaving groups →labeling of electron-neutral and moderately electron-rich aromatics.



^{18}F-Nucleophilic Aromatic Substitution



RCY= 14% (d.c) **Vs**
RCY(ES)=33%
M_A=35GBq/μmol **Vs** M_A(ES)=0.011GBq/ μmol

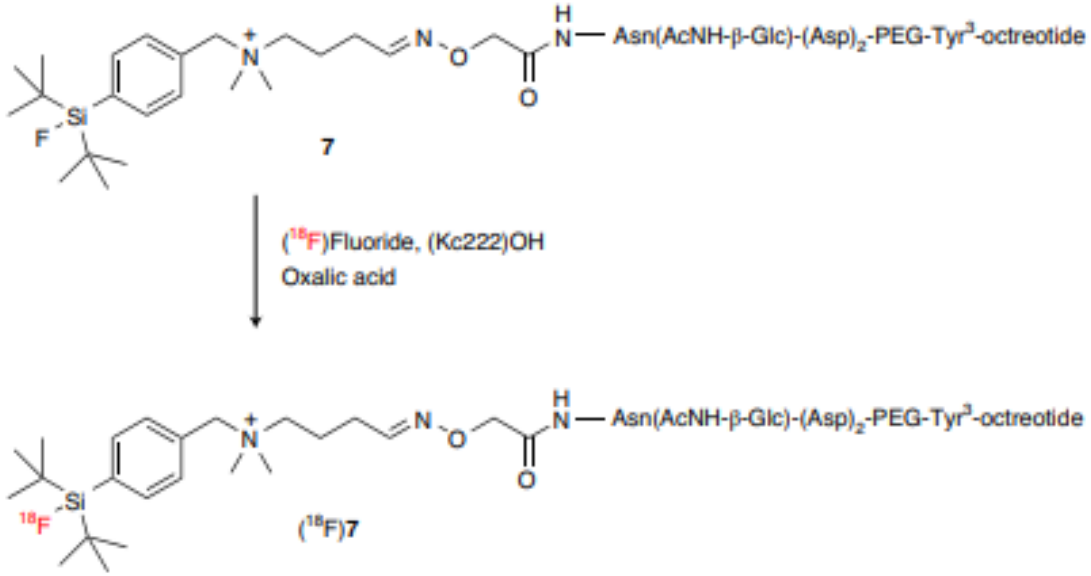
Difficult to synthesize (Harsh conditions)
Not stable
Difficult to make if molecules are more complex

Kuik et al., *J Nuc Med* 2015, 56 (1) 106-112

^{18}F-Nucleophilic Aromatic Substitution

SiFA chemistry (Silicon-Fluoride-Acceptor)

Isotopic exchange reaction



No azeotropic drying

Synthesis time ~20–30 min (No HPLC)

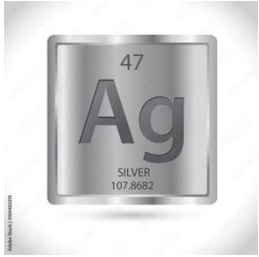
Yield=42±3% (n = 6) d.c.c.

RCP >97%

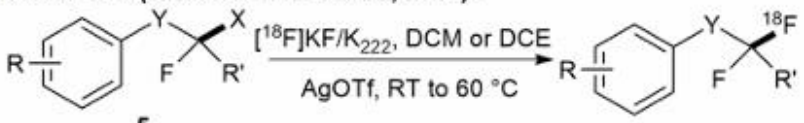
M_A= 60 ± 7 GBq/µmol (n = 6)

Lindner et al., *Nature Protocols* volume 15, pages3827–3843 (2020)

Metal mediated ^{18}F-Radiofluorination



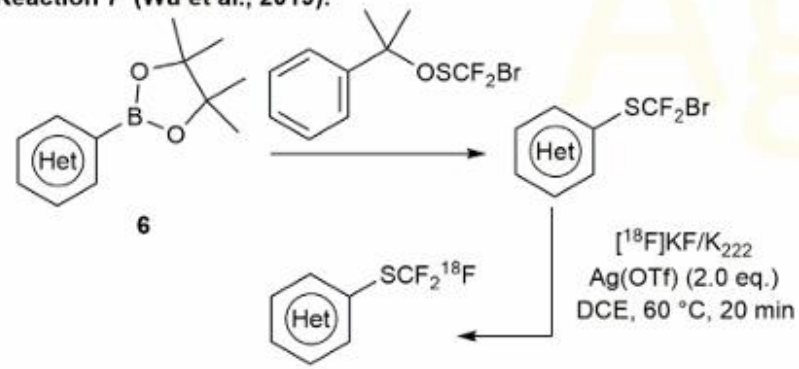
Reaction 6 (Khotavivattana et al., 2015):



5

Y = S, X = Br, R' = F 60 °C, AgOTf (2 eq.) 9 examples RCYs = 34-92 %
Y = O, X = Br, R' = F 60 °C, AgOTf (2 eq.) 8 examples RCYs = 10-72 %
Y = O, X = Cl, R' = H RT, AgOTf (1 eq.) 9 examples RCYs = 66-79 %

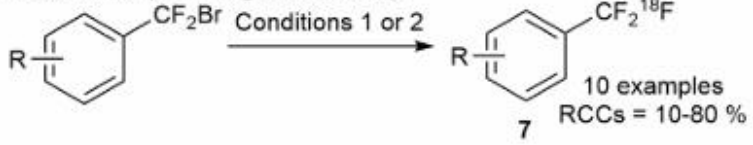
Reaction 7 (Wu et al., 2019):



6

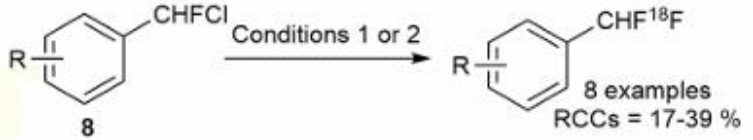
11 examples
RCCs = 2-86 %

Reaction 8 (Verhoog et al., 2016):



7

10 examples
RCCs = 10-80 %



8

8 examples
RCCs = 17-39 %

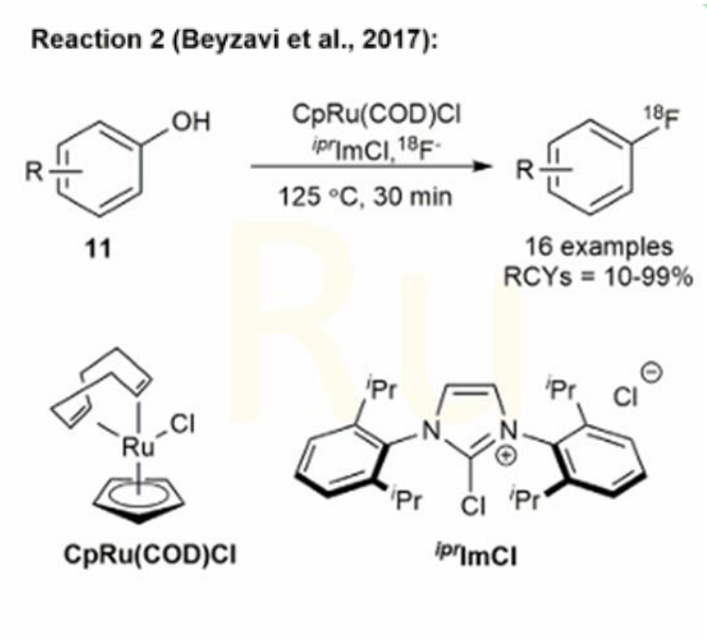
Conditions 1: [^{18}F]KF/K_{222}, AgOTf (1.0 eq.)
CH_2Cl_2, r.t., 20 min

Conditions 2: [^{18}F]KF/K_{222}, AgOTf (2.0 eq.)
DCE, 60 °C, 20 min

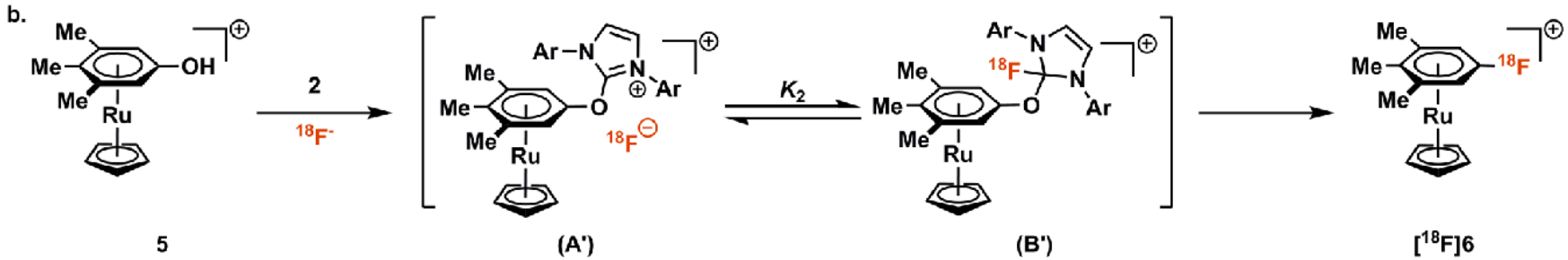
Reactivity towards ^{18}F: ArOCHFCl >$ArCF_2Br$ ≈ ArCHFCl > $ArSCF_2Br$ > $ArOCF_2Br$.

Low Molar Activity

Metal mediated ^{18}F-Radiofluorination

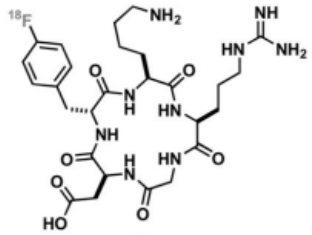


- Aryl systems
- Heterocyclic compounds
- Wide variety of functional groups
- Some basic amines

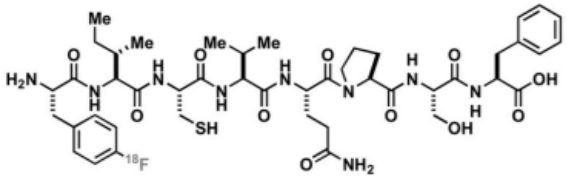


Beyzavi et al., ACS Central Science 2017 3 (9), 944-948

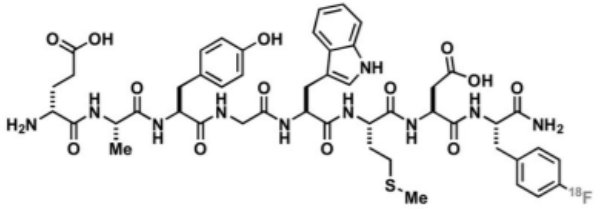
Metal mediated ^{18}F-Radiofluorination



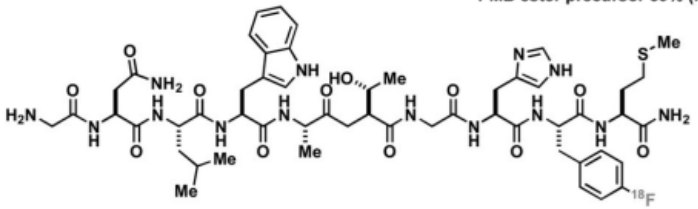
c(Asp-*D*-Phe(4-[^{18}F]F)-Lys-Arg-Gly) (7)
c(RGDfk) analog
25% (n=2)



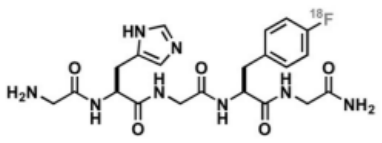
H-Phe(4-[^{18}F]F)-Ile-Cys-Val-Gln-Pro-Ser-Phe-OH (8)
free acid precursor 11% (n=2)
PMB ester precursor 39% (n=2)



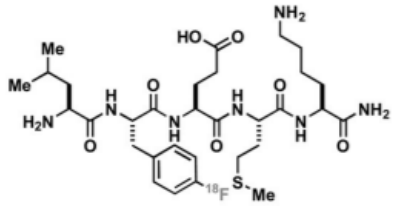
H-*D*-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe(4-[^{18}F]F)-NH$_2$ (9)
MG11 analog
24% (n=3)



H-Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe(4-[^{18}F]F)-Met-NH$_2$ (10)
Neuromedin B analog
29% (n=2)



H-Gly-His-Gly-Phe(4-[^{18}F]F)-Gly-NH$_2$ (11)
39% (n=2)

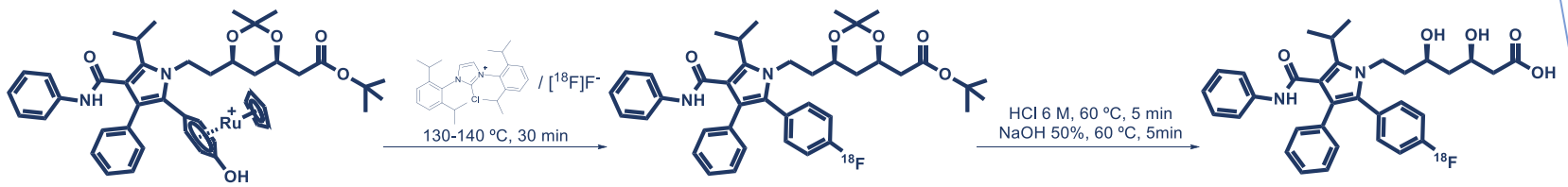


H-Leu-Phe(4-[^{18}F]F)-Glu-Met-Lys-NH$_2$ (12)
20% (n=2)

Only suitable for tyrosine containing peptides

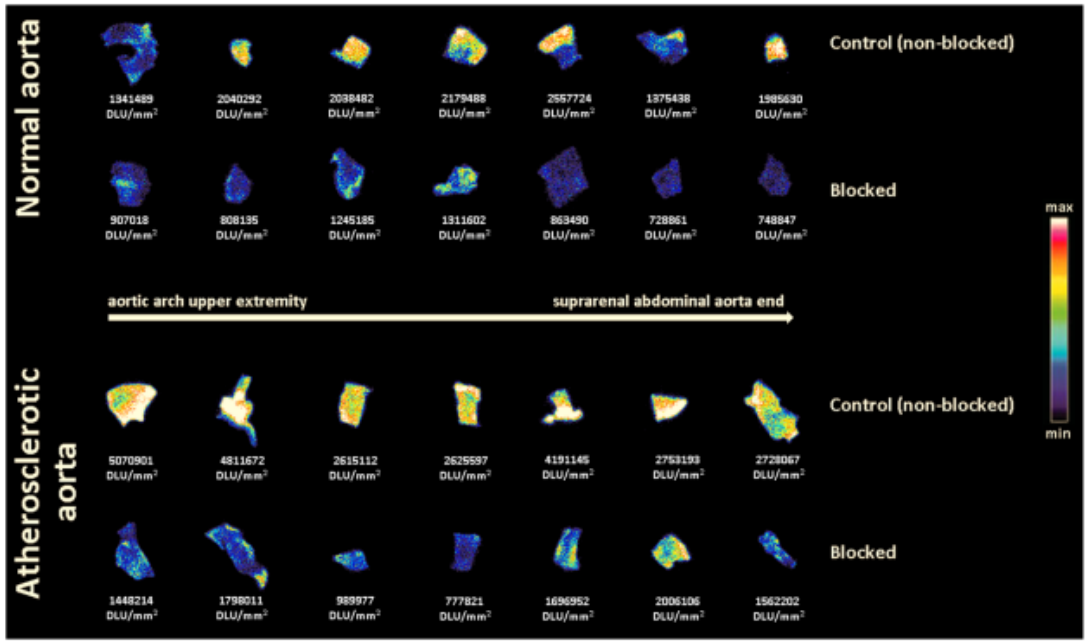
Angew. Chem. Int. Ed. 2018, 57, 14207 –14211

Metal mediated ^{18}F-Radiofluorination



10%

RCY=19% (d.c)
M_A=65GBq/μmol

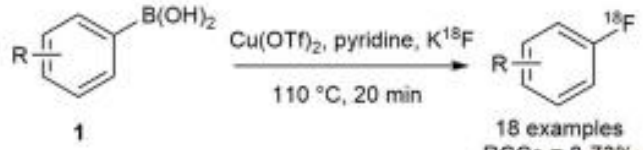


Not all ligands can handle the Ru complex

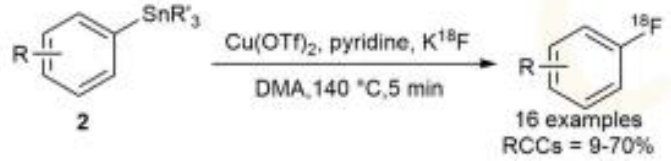
Clemente et al. EJNMMI Research 2020, 10,

Metal mediated ^{18}F-Radiofluorination

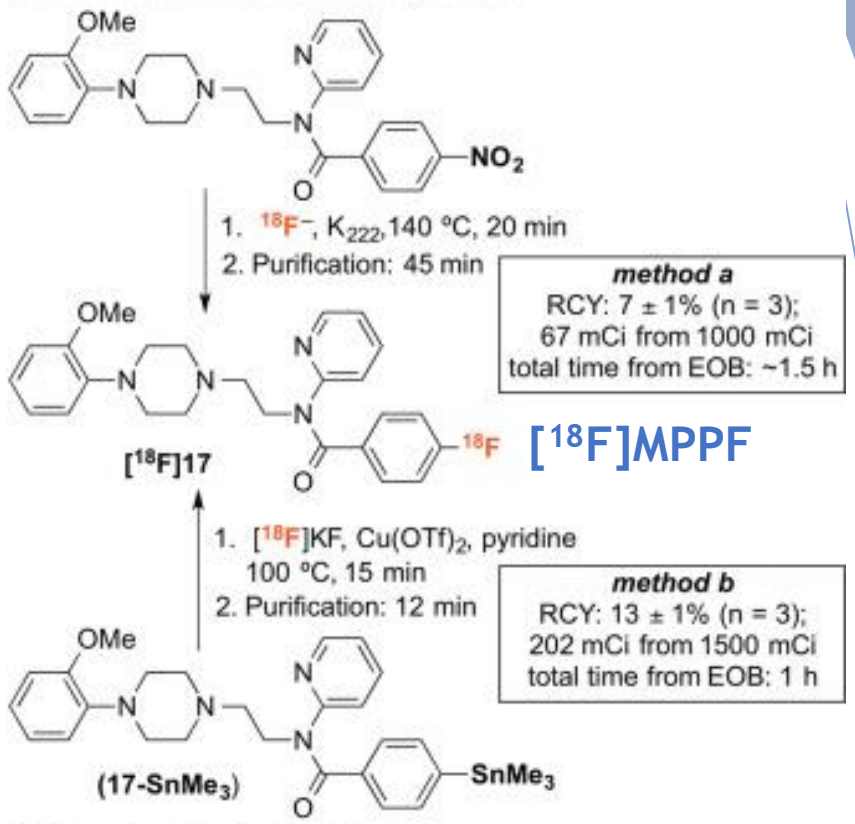
Reaction 1 (Mossine et al., 2015):



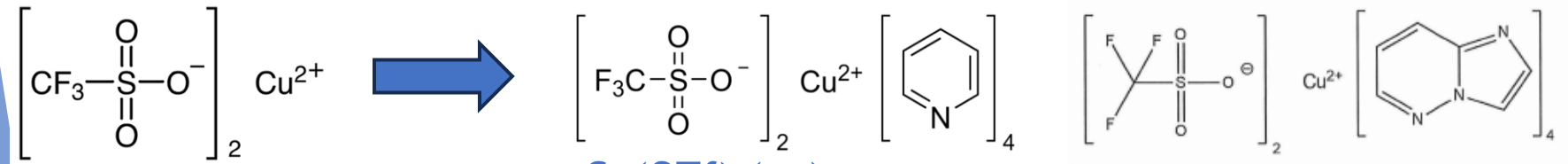
Reaction 2 (Makaravage et al., 2016):



a. Current automated clinical synthesis



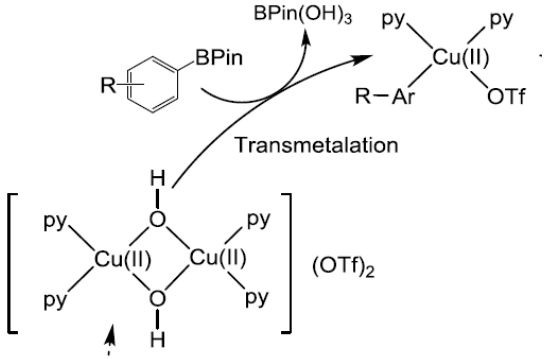
b. Our automated synthesis



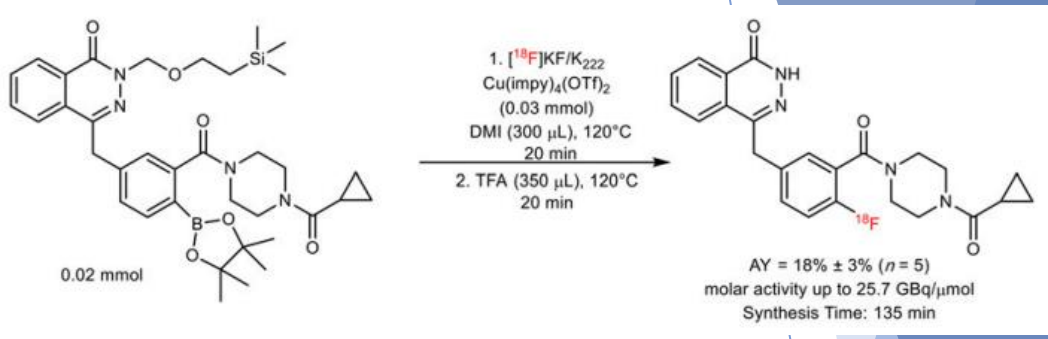
$Cu(OTf)_2$ $Cu(OTf)_2(py)_4$ $Cu(OTf)_2(impy)_4$

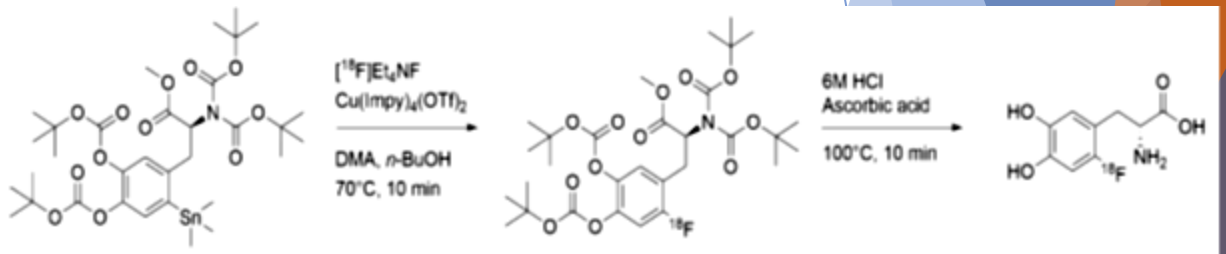
Scott et al., *Org. Lett.* 2016, 18, 20, 5440–5443

Metal mediated ^{18}F-Radiofluorination



Wilson et al. 2019 and Kwizera C et.al.2024):

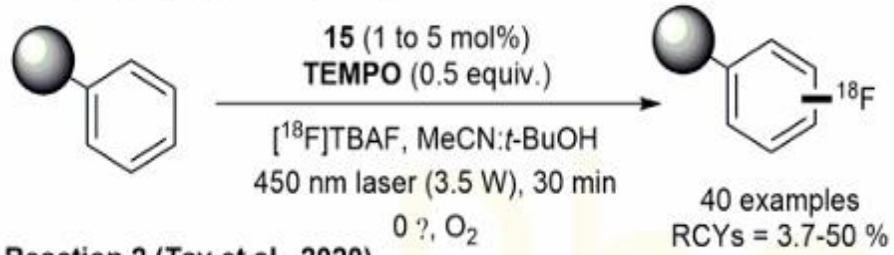




RCY=20% (n.d.c) M_A>25GBq/µmol

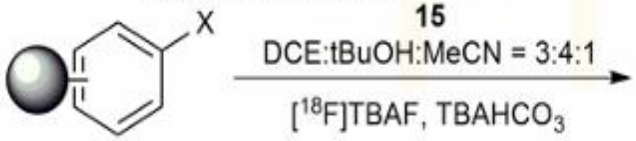
Light mediated ^{18}F-Radiofluorination

Reaction 1 (Chen et al., 2019):



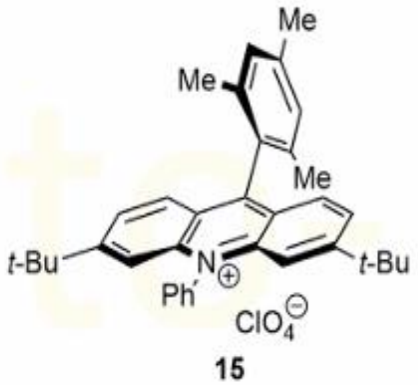
40 examples
RCYs = 3.7-50 %

Reaction 2 (Tay et al., 2020)
(Chen et al., 2021):

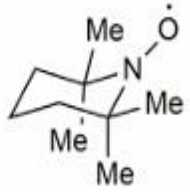


X = F, Cl, Br, I,
OTf, NO_2, OR

RCYs =
non-detected to 79.7%



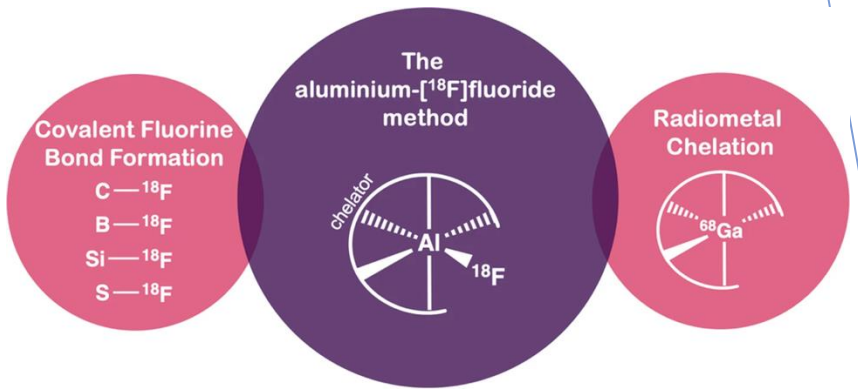
15

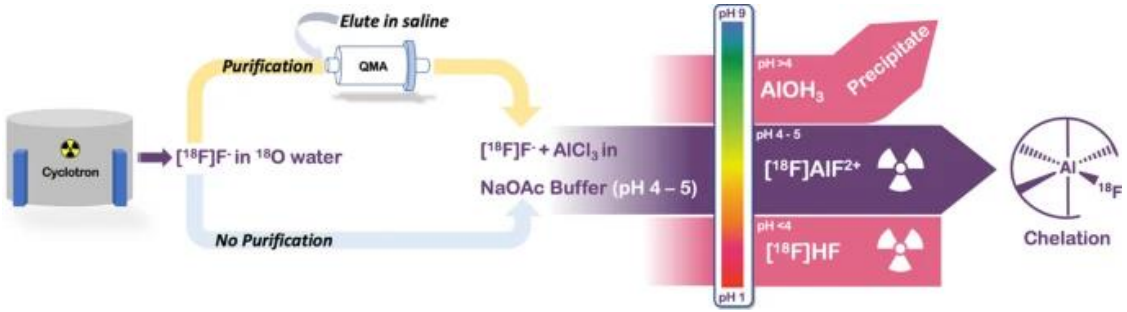


TEMPO

Metal complexation ^{18}F-Radiofluorination

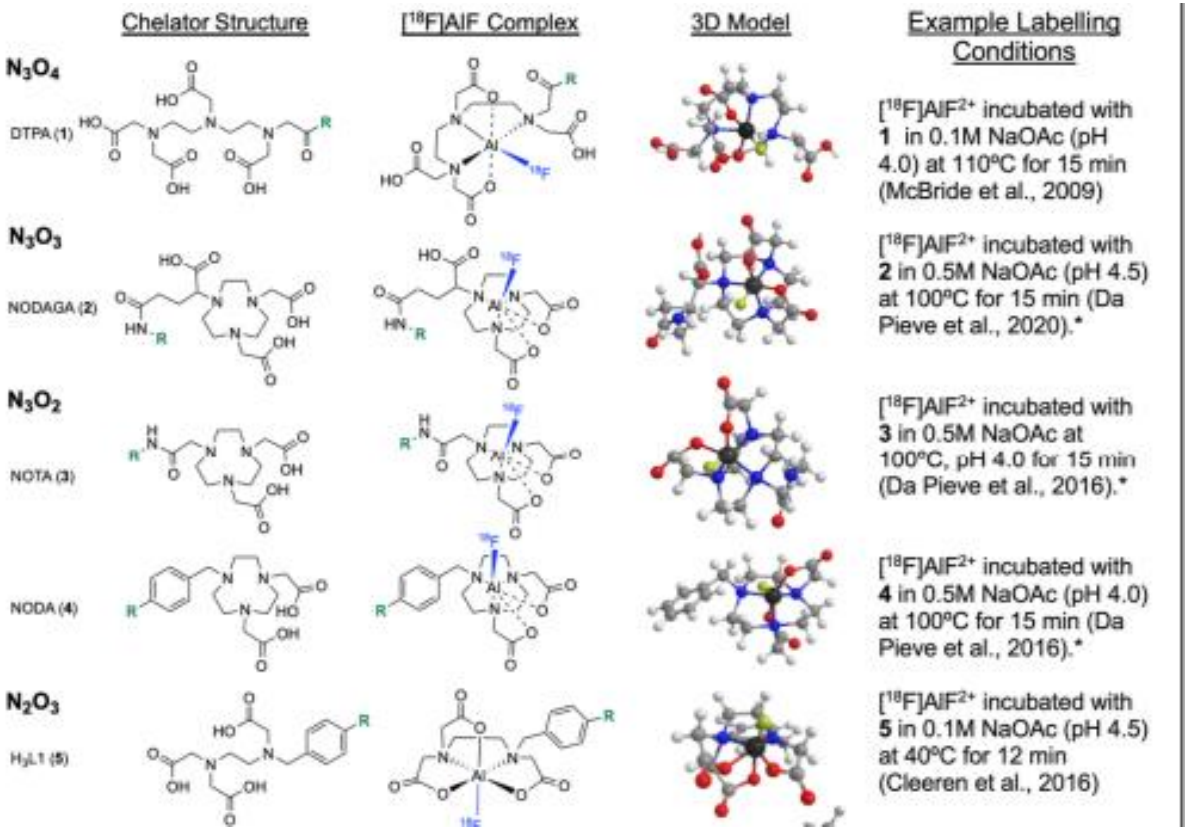
- Metal-like coordination
- Short synthesis time
- Fluorination may be in an aqueous solution
- pH dependent
- Organic co-solvent ↑ RCY
- Elution efficiency per volume depends on the base

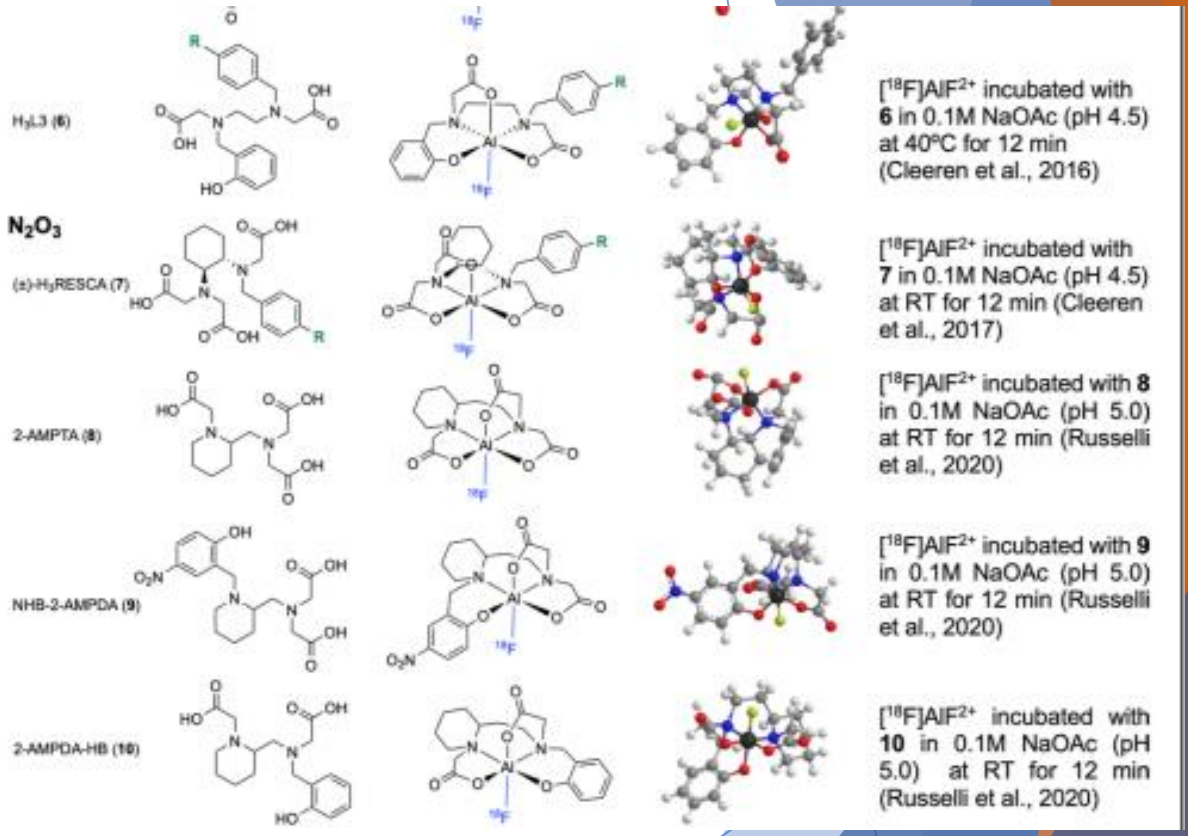




Allott et al., EJNMMI radiopharm. chem. 6, 30 (2021).

Metal complexation ^{18}F-Radiofluorination





S.J. Archibald et al, EJNMMI Radiopharmacy and Chemistry volume 6, 30 (2021)

Metal complexation ^{18}F-Radiofluorination

Examples in clinic

PubMed®

18F-AlF and patients

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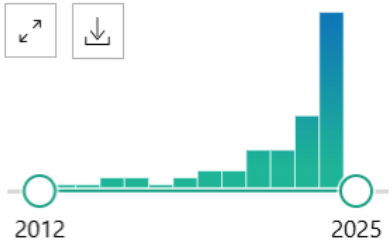
RESULTS BY YEAR



PUBLICATION DATE

11 Cite Share

Comparative assessment of the diagnostic efficacy of [(18)F]AlF-NOTA-FAPI-04 and [(18)F]FDG PET/CT imaging for detecting postoperative recurrence in gastric cancer **patients**: a pilot study.

Yang J, Wu Y, Zhang Y, Peng X, Jiang C, Zhou W, Dai J, Xie A, Ye H, Zheng K.

Front Oncol. 2024 Sep 11;14:1427649. doi: 10.3389/fonc.2024.1427649. eCollection 2024.

PMID: 39323998 **Free PMC article.**

PURPOSE: This study aimed to compare the efficacy of [(18)F]AlF-NOTA-FAPI-04 PET/CT with that of [(18)F]FDG PET/CT for detecting postoperative recurrence in **patients** with gastric cancer. METHODS: This single-center retrospective clinical study was performed at Hunan Cancer ...

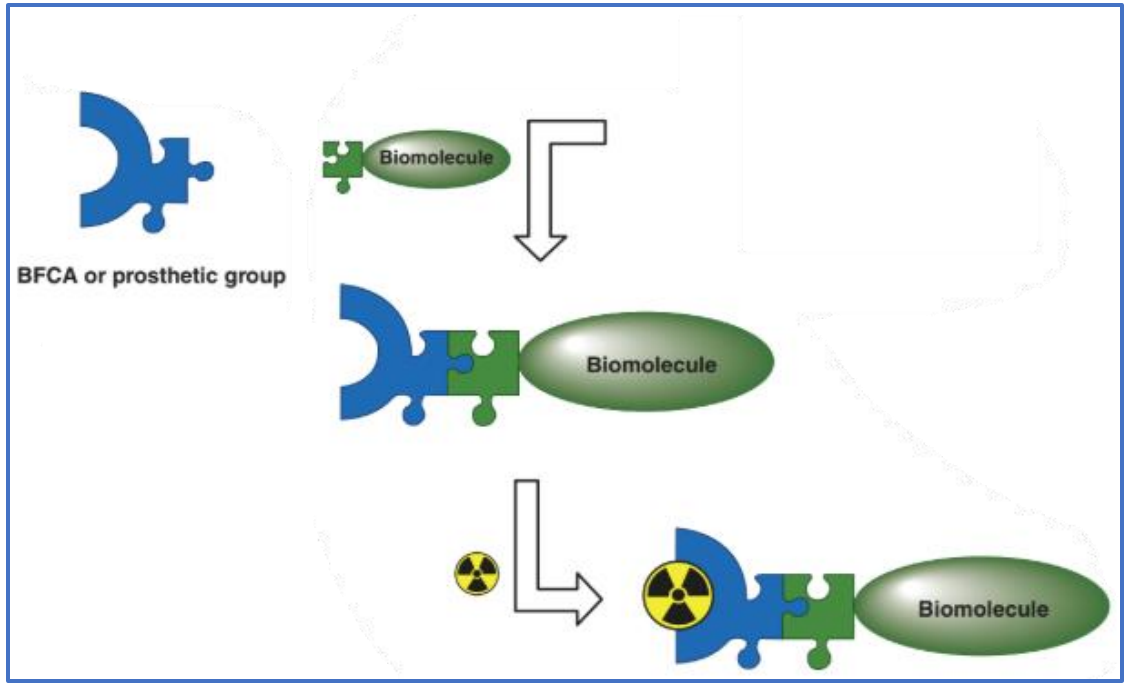
Metal complexation ^{18}F-Radiofluorination Examples in clinic

PET tracer	Target	Malignancy	Reference
[^{18}F]AlF-NOTA-Octreotide [^{18}F]AlF-NOTA-LM3	SSTR	NET	Hou G, et al. Theranostics. 2024. PMID: 38855183 Liu M, et al. Eur J Nucl Med Mol Imaging. 2024. PMID: 38878175
[^{18}F]AlF-NOTA-FAPI-04 [^{18}F]AlF-NOTA-FAPI-42	FAP	Pancreatic ductal adenocarcinoma Gastric cancer Kidney tubular injury Liver fibrosis Multyple Myeloma Rheumatoid arthritis Ischemic stroke	Zhang Y, et al. Eur J Nucl Med Mol Imaging. 2025. PMID: 39820598 Lv J, et al. Eur Radiol. 2024. PMID: 39604653 Yang J, et al. Front Oncol. 2024. PMID: 39323998 Wang H, et al. Clin Kidney J. 2024. PMID: 38803395 Rao W, et al. Jpn J Radiol. 2024. PMID: 38316724 Wang H, et al. Clin Nucl Med. 2023. PMID: 37682606 Ge L, et al. Eur J Nucl Med Mol Imaging. 2022. PMID: 35715613 Tang P, et al. Transl Stroke Res. 2024. PMID: 38940873
[18F]AlF-NYM005	CAIX	clear cell renal cell carcinoma	Yang L, et al. Eur J Nucl Med Mol Imaging. 2024. PMID: 39676103
[^{18}F]AlF-NOTA-Pentixather	CXCR4	primary hyperaldosteronism	He L, et al. Theranostics. 2024. PMID: 39659571
[^{18}F]AlF-NOTA-RGD2 [^{18}F]AlF-NOTA-PRGD	integrin αvβ3	Liver fibrosis Melanoma Myocardial infraction	Liu W, et al. Heliyon. 2024. PMID: 39170113 Wang L, et al. EJNMMI Res. 2024. PMID: 38967722 Gao H, et al. Eur J Nucl Med Mol Imaging. 2012. PMID: 22274731
[18F]AlF-PSMA-11 [^{18}F]AlF-thretide [18F]AlF-P16-093	PSMA	Prostate cancer	Li X, et al. EJNMMI Rep. 2024. PMID: 39245688 Zang J, et al. J Nucl Med. 2024. PMID: 38724276 Zhao R, et al. Eur J Nucl Med Mol Imaging. 2024. PMID: 38285206
[^{18}F]AlF-NOTA/RESCA-HER2-BCH [^{18}F]AlF-RESCA-MIRC213	HER2	Breast cancer	Liu J, et al. Eur J Nucl Med Mol Imaging. 2023. PMID: 37093312 Qin X, et al. Eur J Nucl Med Mol Imaging. 2023. PMID: 36129493
[^{18}F]AlF-NOTA-FAPI-RGD	integrin αvβ3+FAP	rheumatoid arthritis	Wang H, et al. Theranostics. 2024. PMID: 39629127

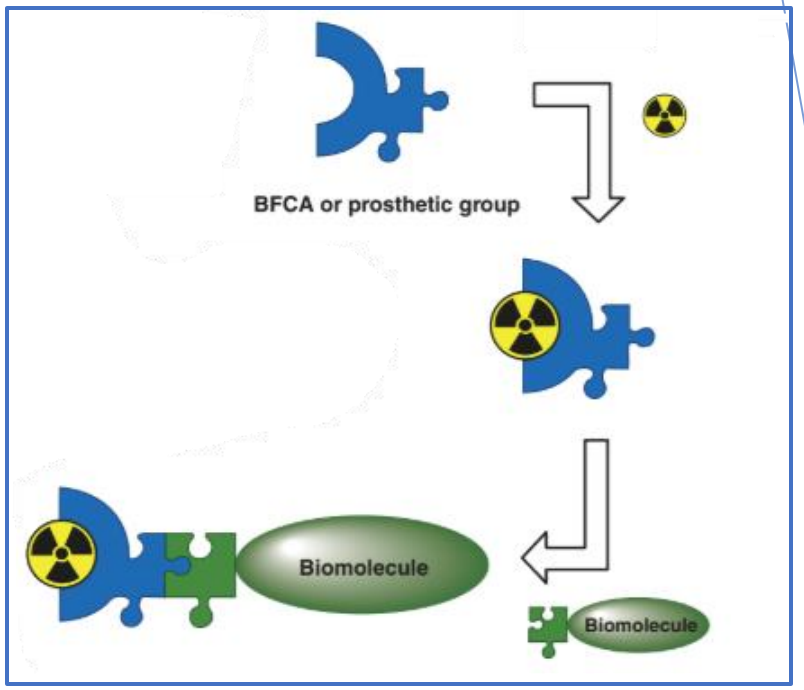
Indirect radiolabelling
(Mostly applies to biomolecules)

Indirect radiolabelling

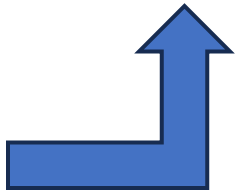
Direct Radiolabelling



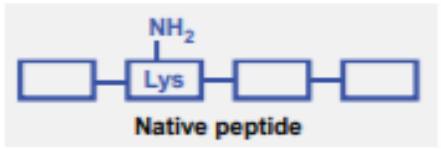
Indirect Radiolabelling

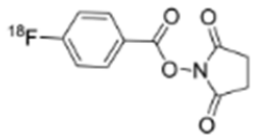


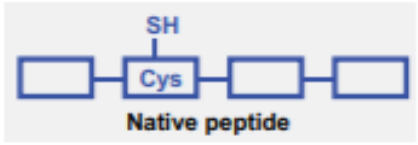
Non physological pH
High temperatures

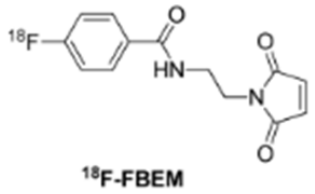


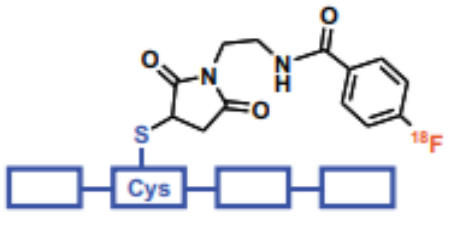
Indirect radiolabelling

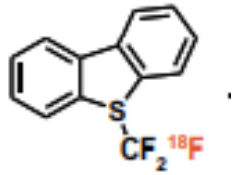


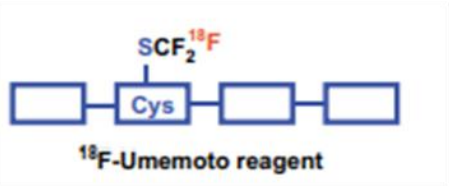




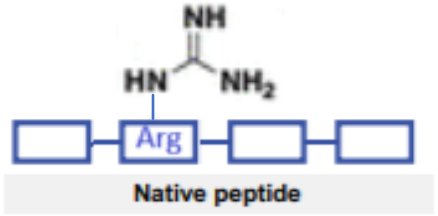


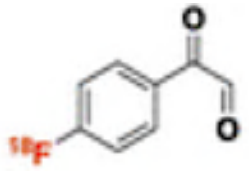






Gouveneur 2018

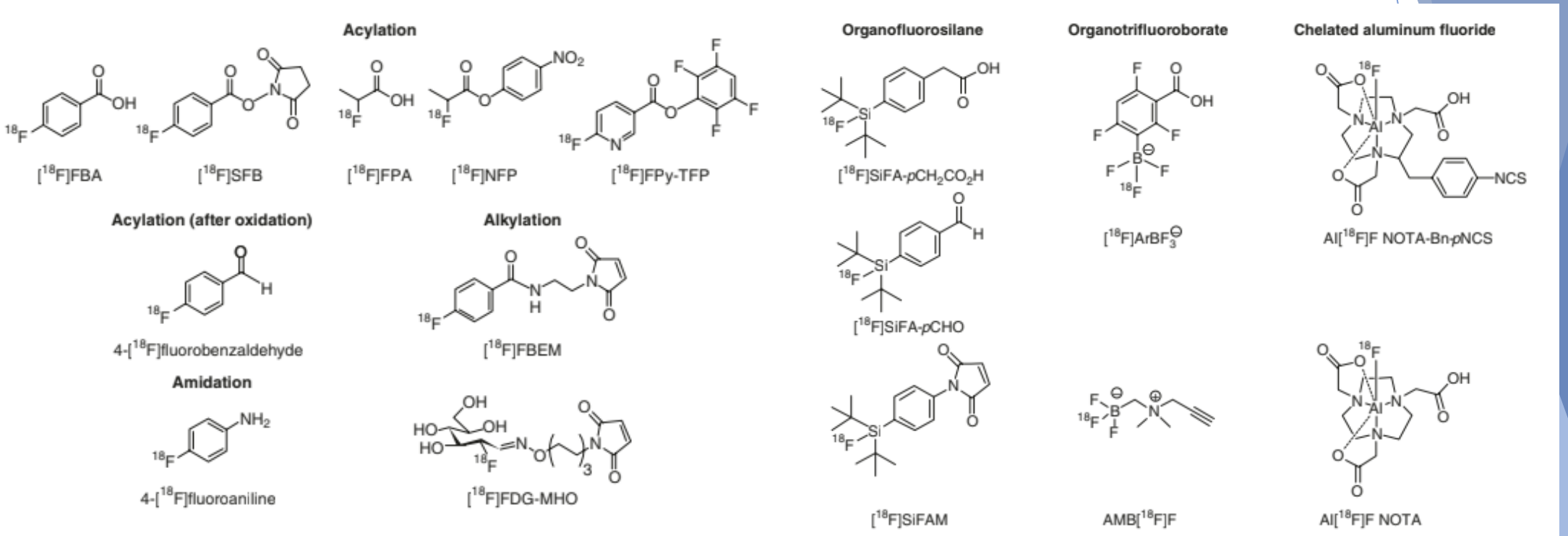




Sadasivam 2024

Rickmeier, J. et al. Nat Protoc 18, 3614–3651 (2023), Verhoog et al., Synlett. 2016;27:25,
Sadasivam et al., J. Med. Chem. 2024, 67, 5064−5074

Indirect radiolabelling

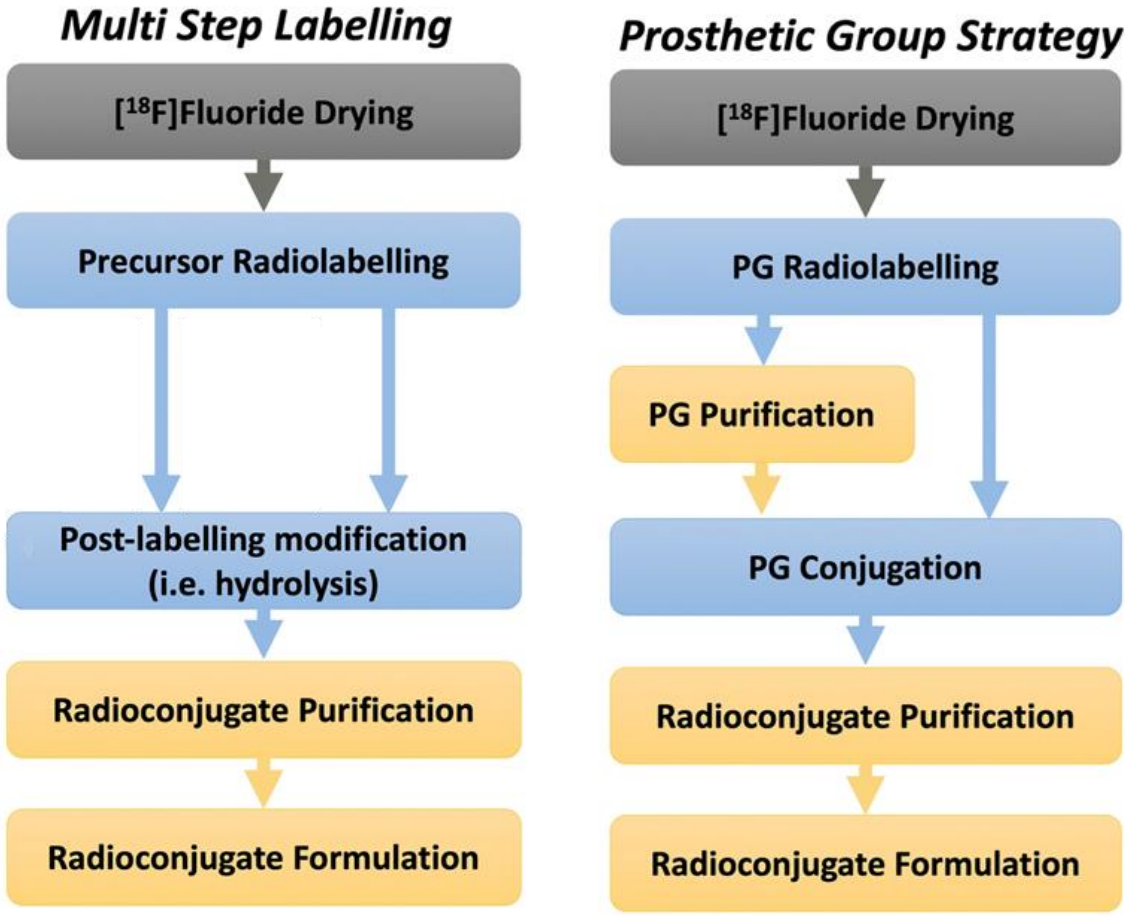


Versatility

Rickmeier, J. et al. Nat Protoc 18, 3614–3651 (2023), Verhoog et al., Synlett. 2016;27:25, Sadasivam et al., J. Med. Chem. 2024, 67, 5064−5074

Indirect radiolabelling

Laborious and time-consuming



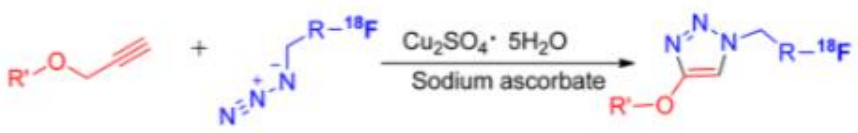
Other disadvantages?

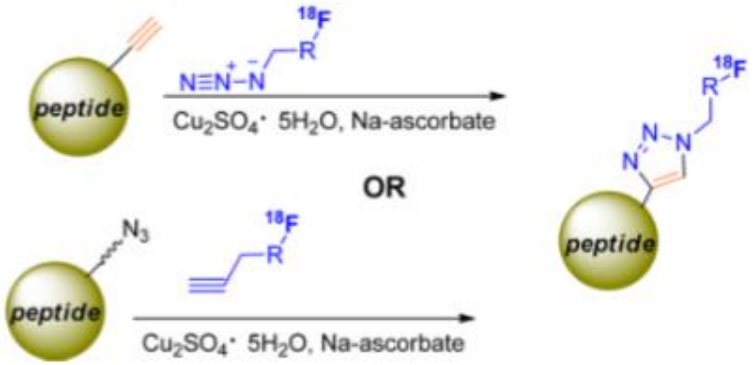
May alter the physical/physiological properties of the biomolecule

Barns et al., React. Chem. Eng., 2022, 7, 2265–2279

Indirect radiolabelling

Copper (I)-catalyzed Azide-alkyne cycloaddition (CuAAC)

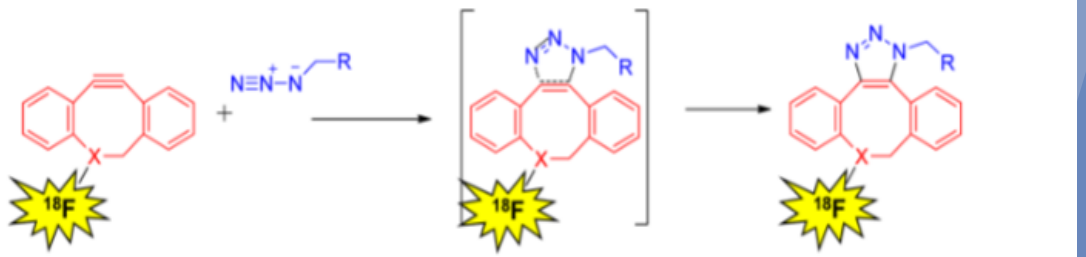




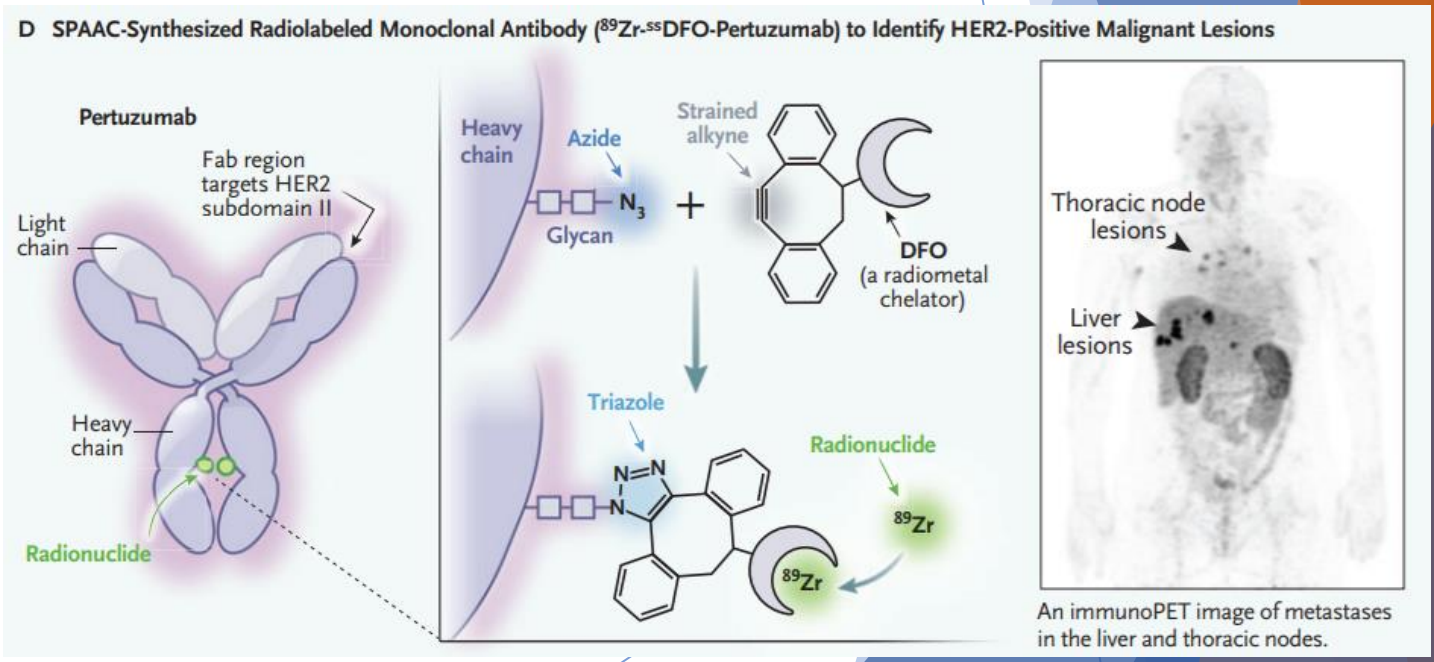
No need protecting groups,
Not sensitive to water/Oxygen
Fast

Use of copper-toxic in vivo

Strain-promoter alkyne-azide cycloaddition (SPAAC)



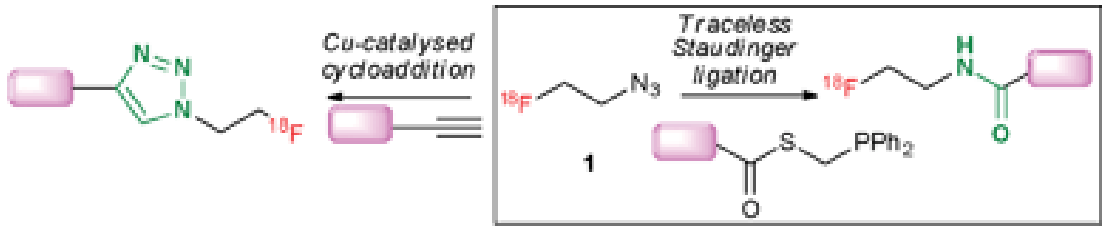
Fast, No need Cu



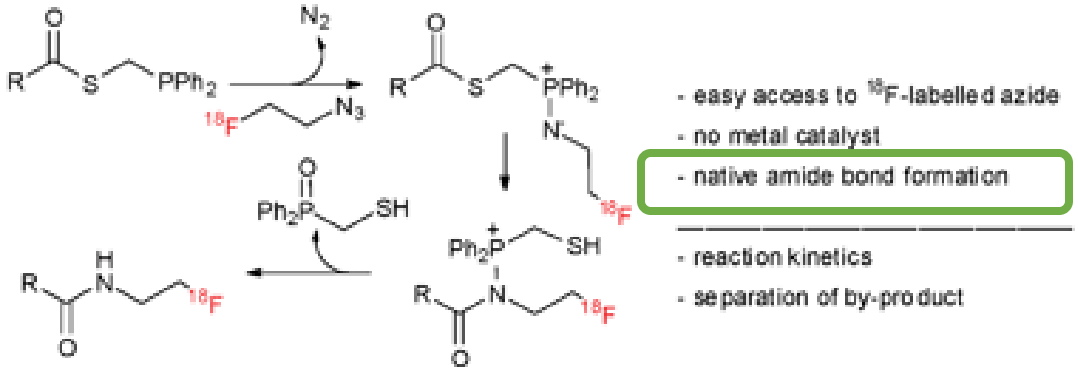
ClinicalTrials.gov number, NCT04692831

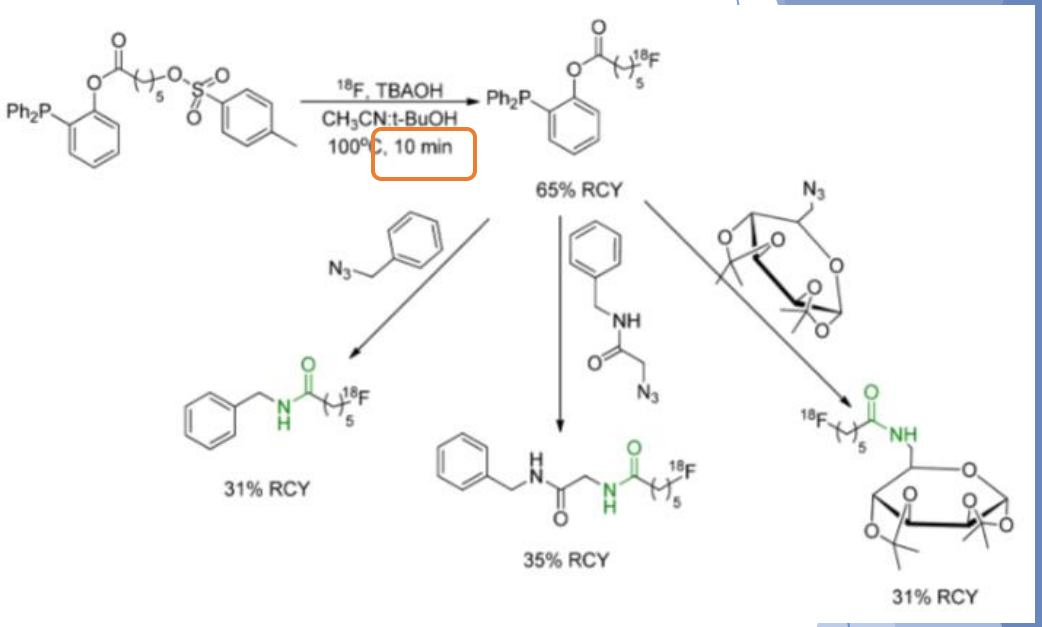
Indirect radiolabelling

Staudinger ligation



The Traceless Staudinger Ligation with ^{18}F-Labelled Azide **1**





Versatility

Total synthesis time: 50 min (90°C) and 70 min (40°C)

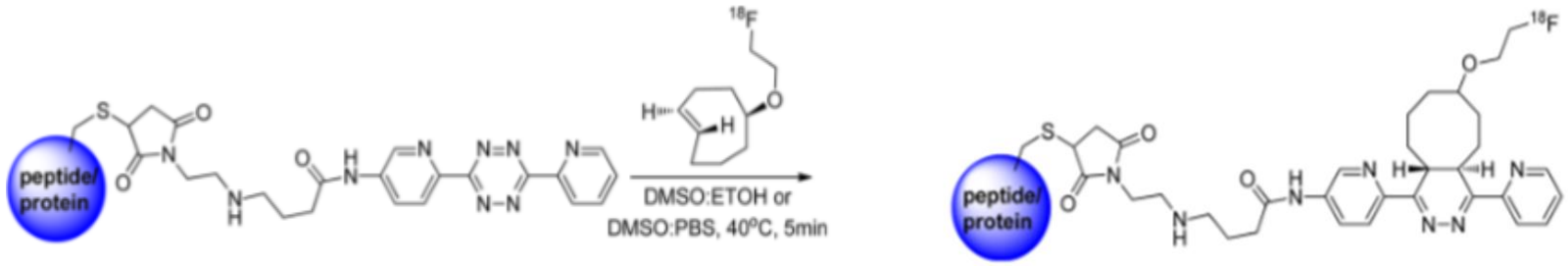
Does not work in DMSO and DMF

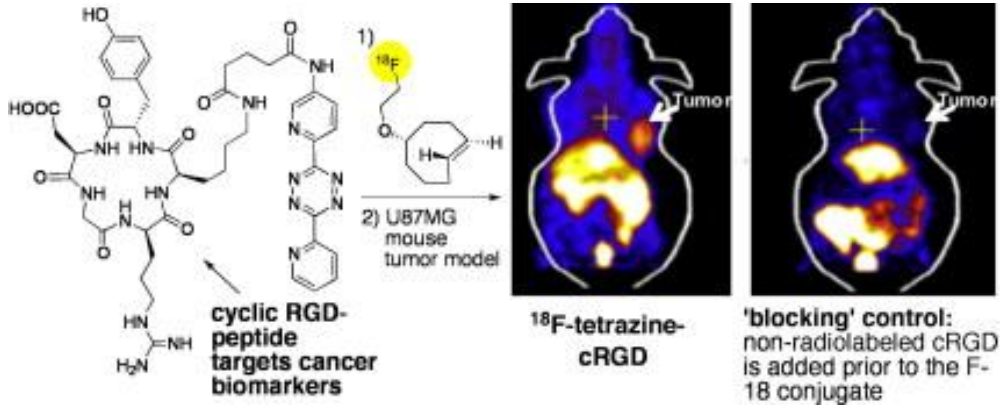
Carroll et al., *Org. Biomol. Chem.*, 2011,9, 136-140

Pretze et al., Tetrahedron letters, 2010, 51, 6410-6414

Indirect radiolabelling

Tetrazine-trans-cyclooctene ligation





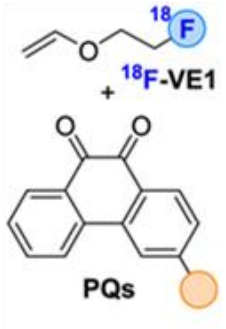
^{18}F-tetrazine-cRGD

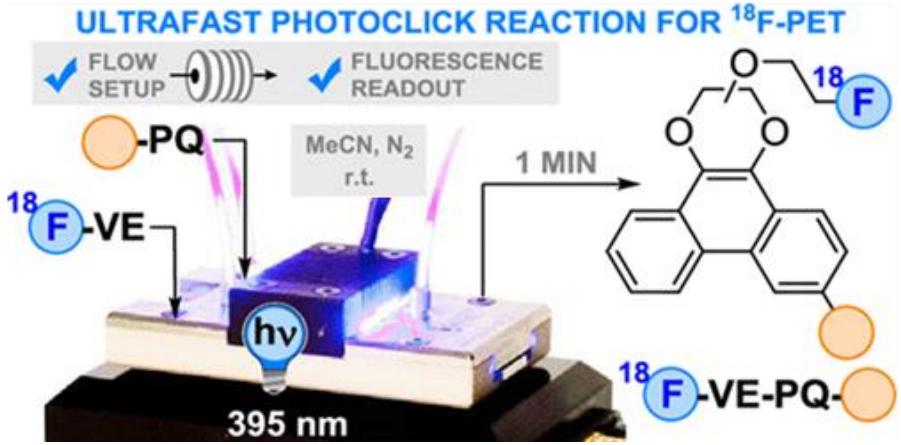
'blocking' control: non-radiolabeled cRGD is added prior to the F-18 conjugate

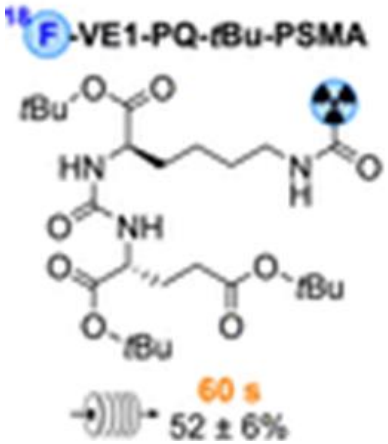
Reaction	RGD derivative (amount µM)	Labelling Yield (%)
TTCA	78	90
CUAAC	940	30
Conjugation 18F-SFB	1800	70

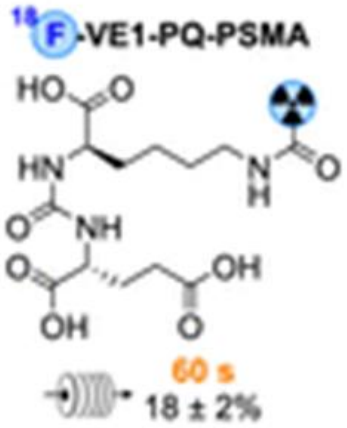
Conti et al., Bioorganic & Medicinal Chemistry Letters, 2011 ,21, 5011-5014

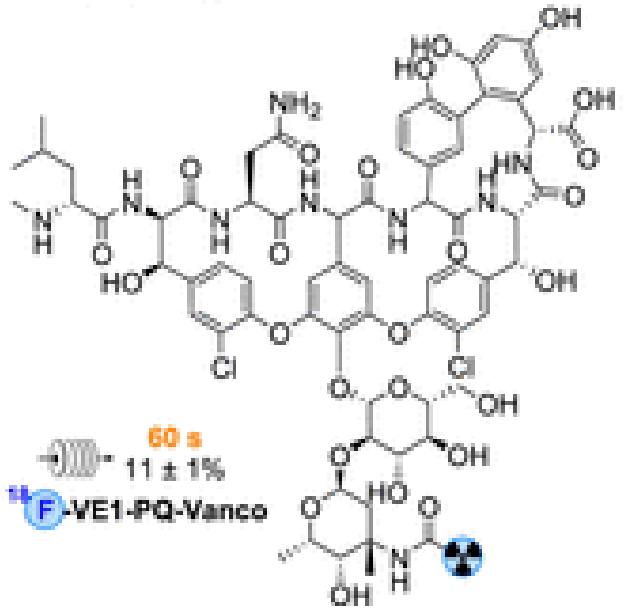
Indirect radiolabelling











60 s @395 nm light→ RCC 11 ± 1% without the need of protecting groups.

Fu et al.Journal of the American Chemical Society 2021 143 (27), 10041-10047
Spoelstra et al., Eur J Nucl Med Mol Imaging 51, 2583–2596 (2024)

Conclusions

- Traditional nucleophilic 18F-radiofluorinations is challenging
- New methods have emerged to facilitate radiofluorination such as the use of metals as catalysts.
- 18F-AlF chemistry has busted the radiolabeling of biomoleculs like peptides
- Late-stage deoxyfluorination has enable the synthesis of several new PET tracer into clinic
- The use of click chemical reactions for fast labelling of biomolecules has been heavily exploited.

umcg

Thank you!

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