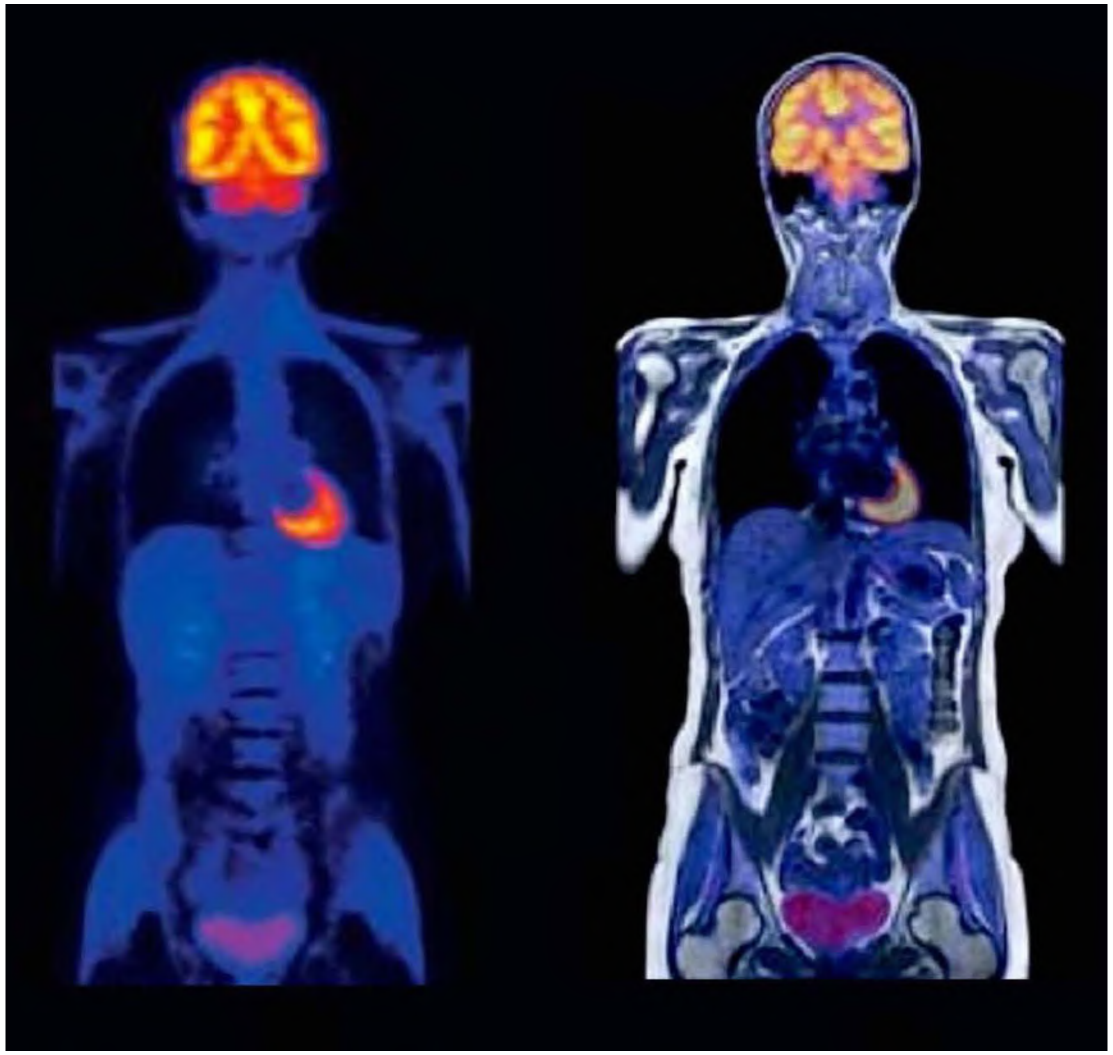
Positron Emission Tomography



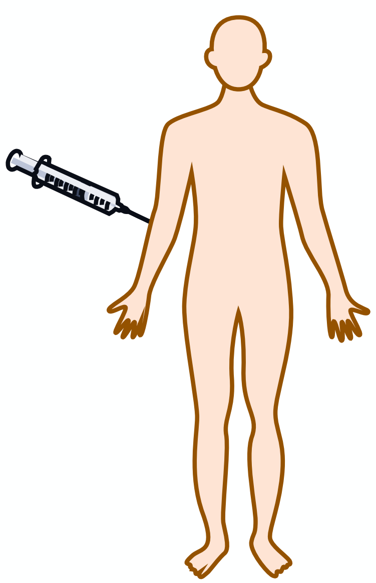
Robert Pipal

MacMillan Group Meeting

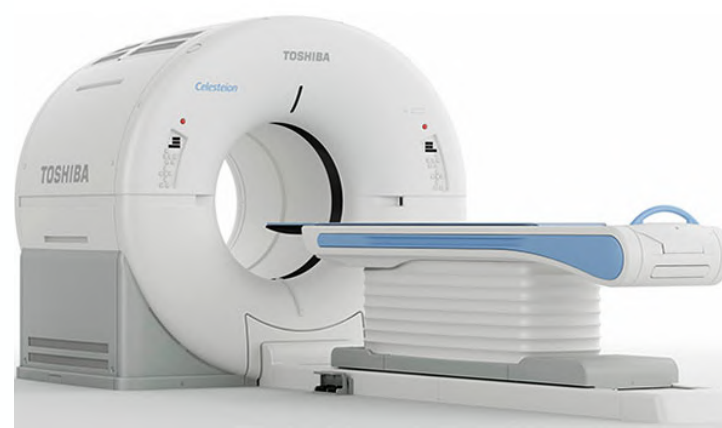
May 22, 2018

What is Positron Emission Tomography?

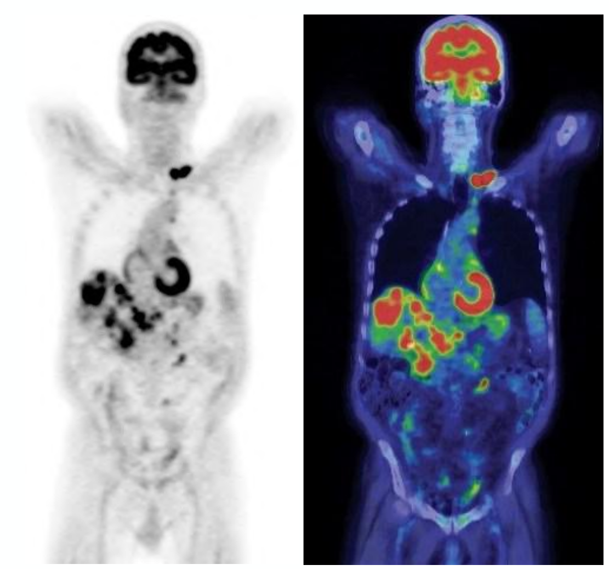
Positron Emission Tomography (PET): a molecular imaging technique which utilizes positron-emitting radiotracers, often used to observe *in vivo* metabolic processes as an aid to diagnose various diseases.



Radiotracer Delivery



PET Scanning



PET Image with CT Overlay

- an estimated 1.945 million clinical PET and PET/CT scans were performed in the US in 2017
- 13% increase in use compared to 2015

IMV Medical Information Division. 2018 PET Imaging Market Summary Report. Des Plaines, IL.

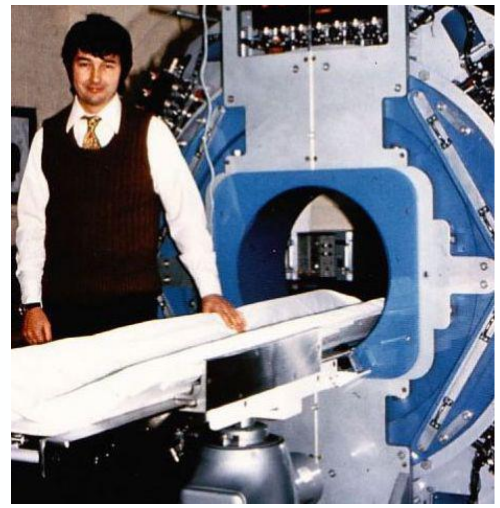
History of Positron Emission Tomography

1932: first observation of positrons by Carl Anderson

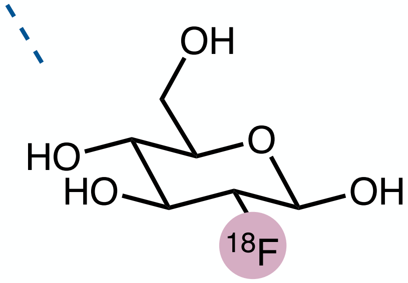
1936: development of the cyclotron

1945: first use of β⁺-emitting nuclides in humans using [^{11}C]-carbon monoxide

1973: X-ray CT developed



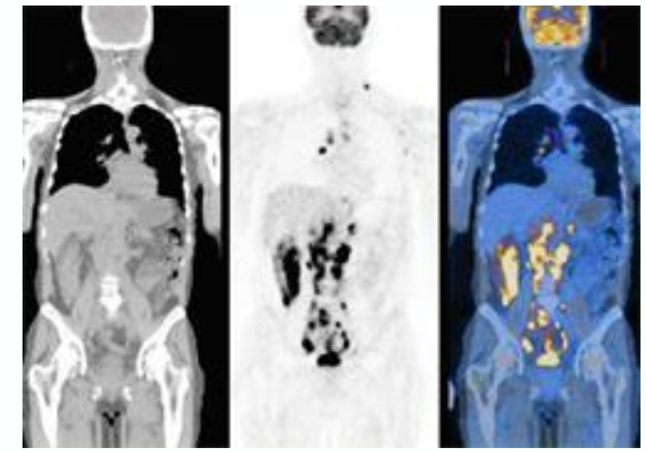
1975: first clinically used PET scanner developed



1976: development of [^{18}F]-fluorodeoxyglucose

1979: MRI introduced

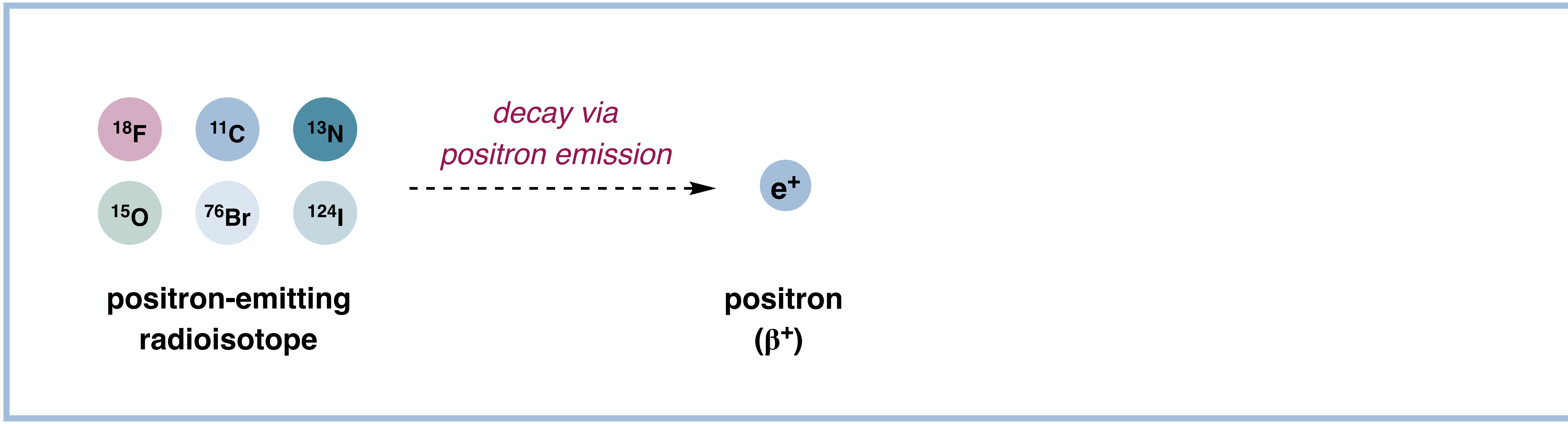
1997: first PET-MRI prototype developed



2000: first PET-CT prototype developed

Portnow, L. H.; Vaillancourt, D. E.; Okun, M. S. *Neurology* **2013**, *80*, 952.

Introduction to Positron Emission Tomography

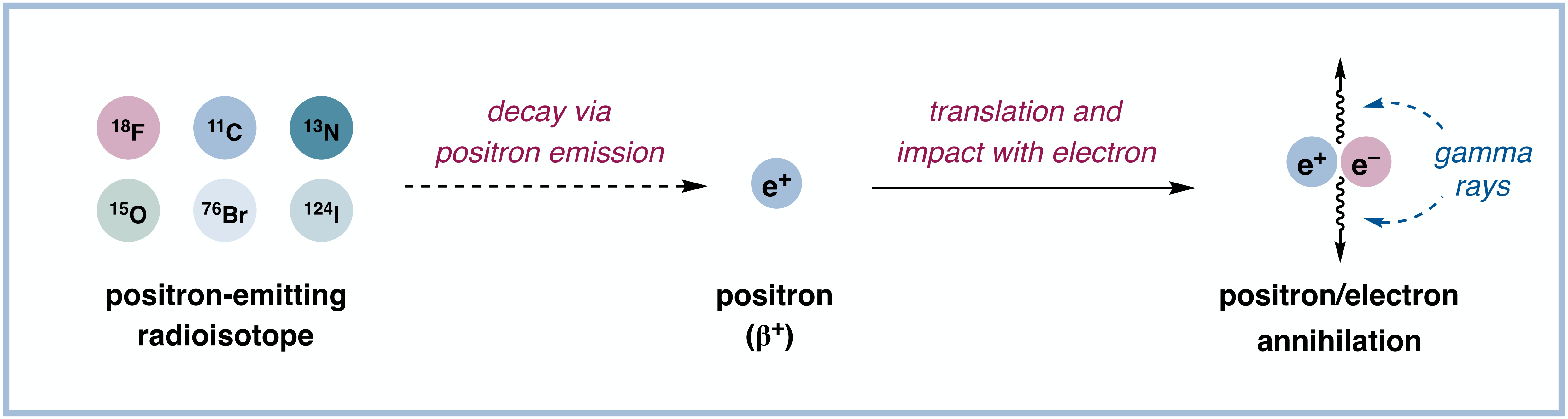


nuclide	half-life	β^+ efficiency	E_{max} (β^+)
^{18}F	110 min	96.7%	634 KeV
^{11}C	20.4 min	99.8%	961 KeV
^{13}N	10 min	99.8%	1190 KeV
^{15}O	2.0 min	99.9%	1732 KeV

- efficiency of positron emission and half-life dependent on radionucleus

Piel, M.; Vernaleken, I.; Rösch, F. *J. Med. Chem.* **2014**, *57*, 9232.

Introduction to Positron Emission Tomography

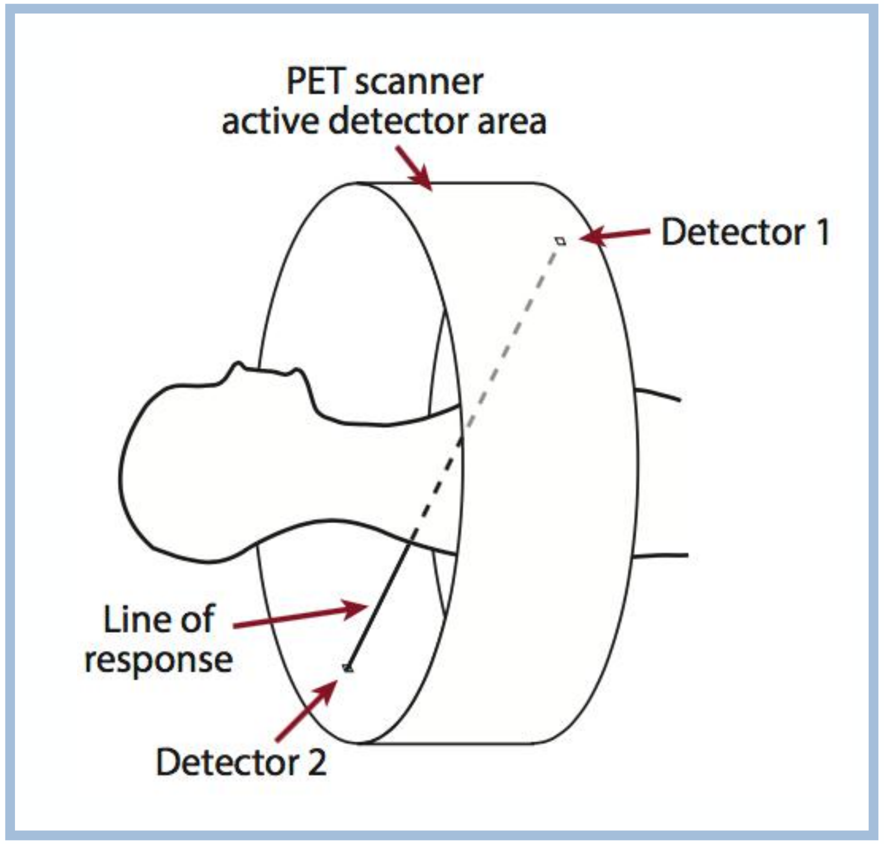


- positron travels 1 mm to 2 cm before annihilating with electron
- annihilation event produces two gamma rays, 180º from each other, each with energy of 511 KeV

PET utilizes these generated photons to observe the location of the radionucleotide in a given system

Piel, M.; Vernaleken, I.; Rösch, F. *J. Med. Chem.* **2014**, *57*, 9232.

Introduction to Positron Emission Tomography

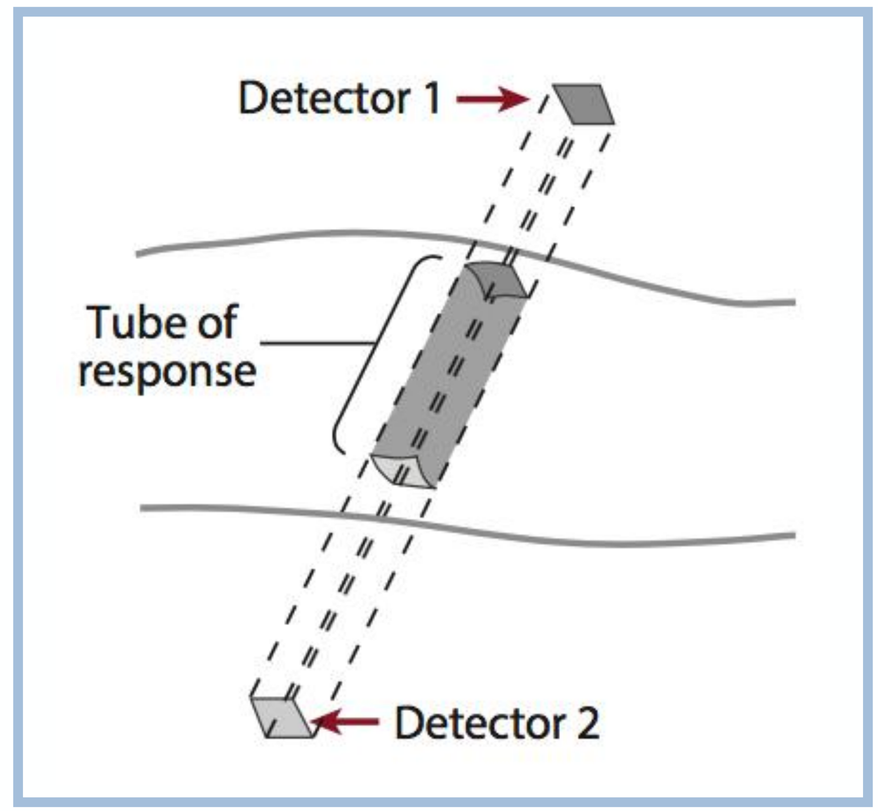


- PET scanner lined with small detectors which contain scintillator crystals coupled to photomultipiers

- gamma photon is absorbed by scintillant, relays to photomultipier, and records time and energy of photon

- photons with similar time of detection (1-10 ns) and energy are paired, and a line of response is drawn

- differential timing of detection can be used to localize the annihilation along the line of response



Vaquero, J. J.; Kinahan, P. *Annu. Rev. Biomed. Eng.* **2015**, *17*, 385.

Introduction to Positron Emission Tomography

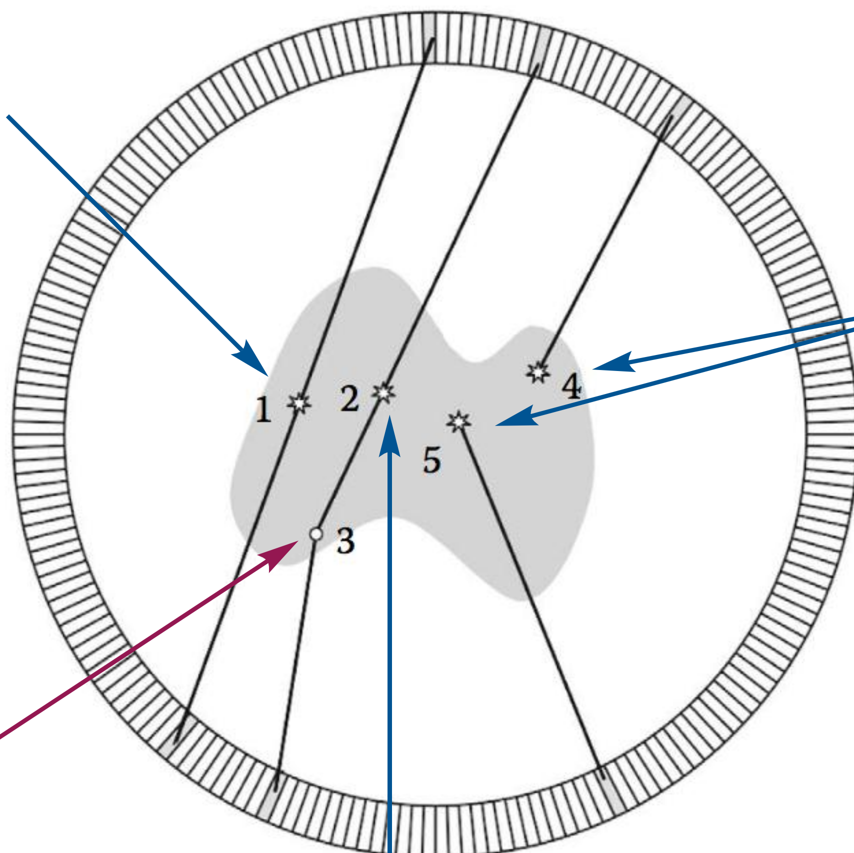
Causes of Noise in PET

true event

two γ-rays come from same positron-electron annihilation

random event

two γ-rays come from different positron-electron annihilation events



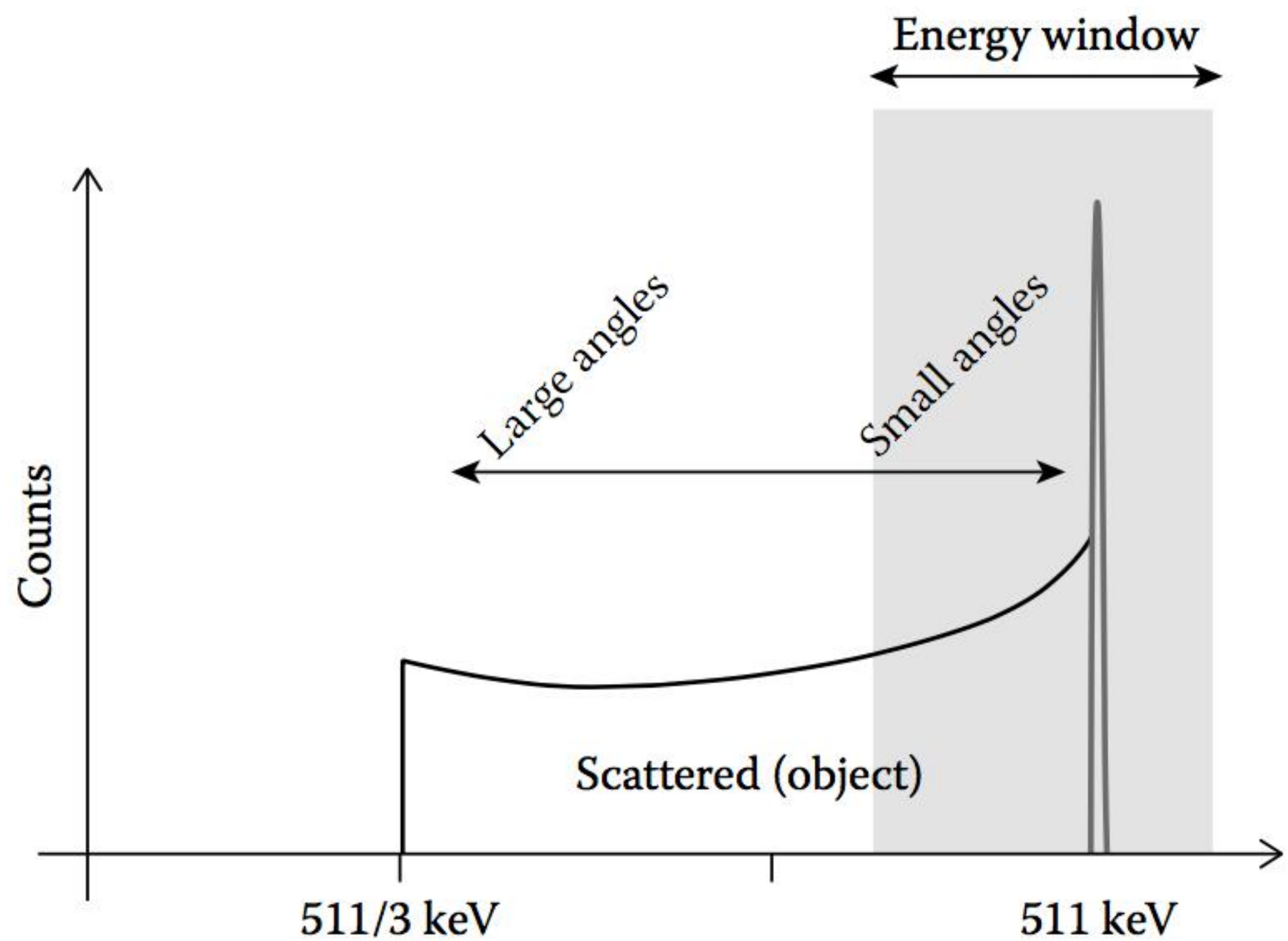
Compton scattering:
photon is scattered by collision with an electron, decreased energy of photon

- shortening the timing window or using a scintillator with short decay time can reduce random count rate

scatter event

two γ-rays come from different positron-electron annihilation events, but Compton scattering occurs

Farncombe, T.; Iniewski, K. *Medical Imaging*; CRC Press: Boca Raton, FL, 2014.



- Compton scattering reduces energy of gamma photons
- increasing the lower threshold of the energy window gives better resolution

Piel, M.; Vernaleken, I.; Rösch, F. *J. Med. Chem.* **2014**, *57*, 9232.

Positron Emission Tomography

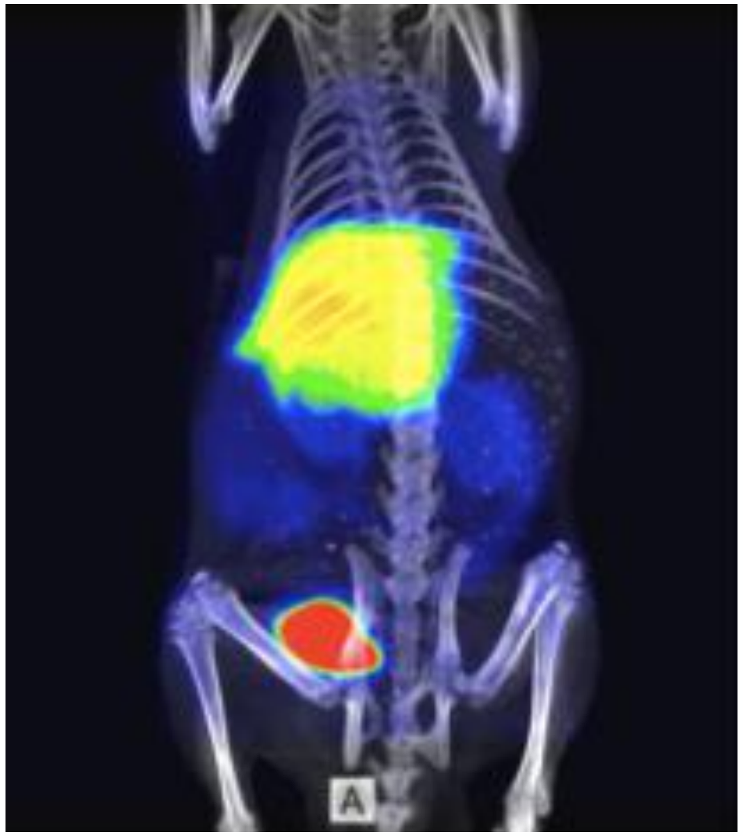
- **Methods for Radiotracer Synthesis**

  Carbon-11 labeling

  Automated synthesis

  Fluorine-18 labeling

  Oxygen-15, Nitrogen-13, and radioactive metals



- **Applications of PET**

  Cancer visualization

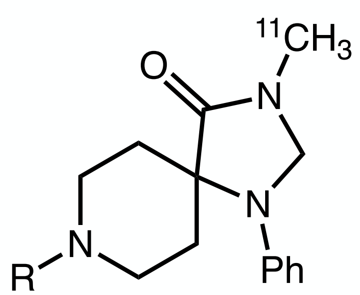
  Alzheimer’s disease diagnosis

  Drug research & development

Labeling with Carbon-11

Why Use Carbon-11 for Radiolabeling?

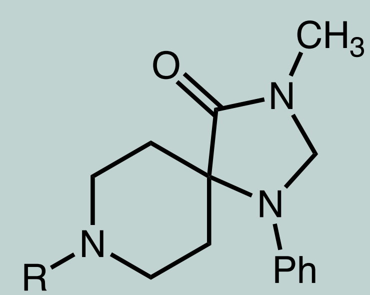
- well-suited for studying rapid kinetics in the brain
- short half-life allows several experiments to be run per day
- used more often in drug R&D than ^{18}F due to need for isotopic substitution



same drug properties

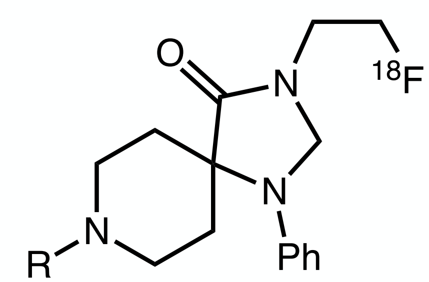
^{11}C labeling ←

(preferred)

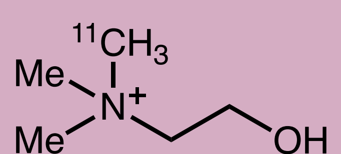


drug candidate

^{18}F labeling →



altered drug properties



[^{11}C]-choline

currently only carbon-11
FDA-approved radiopharmaceutical

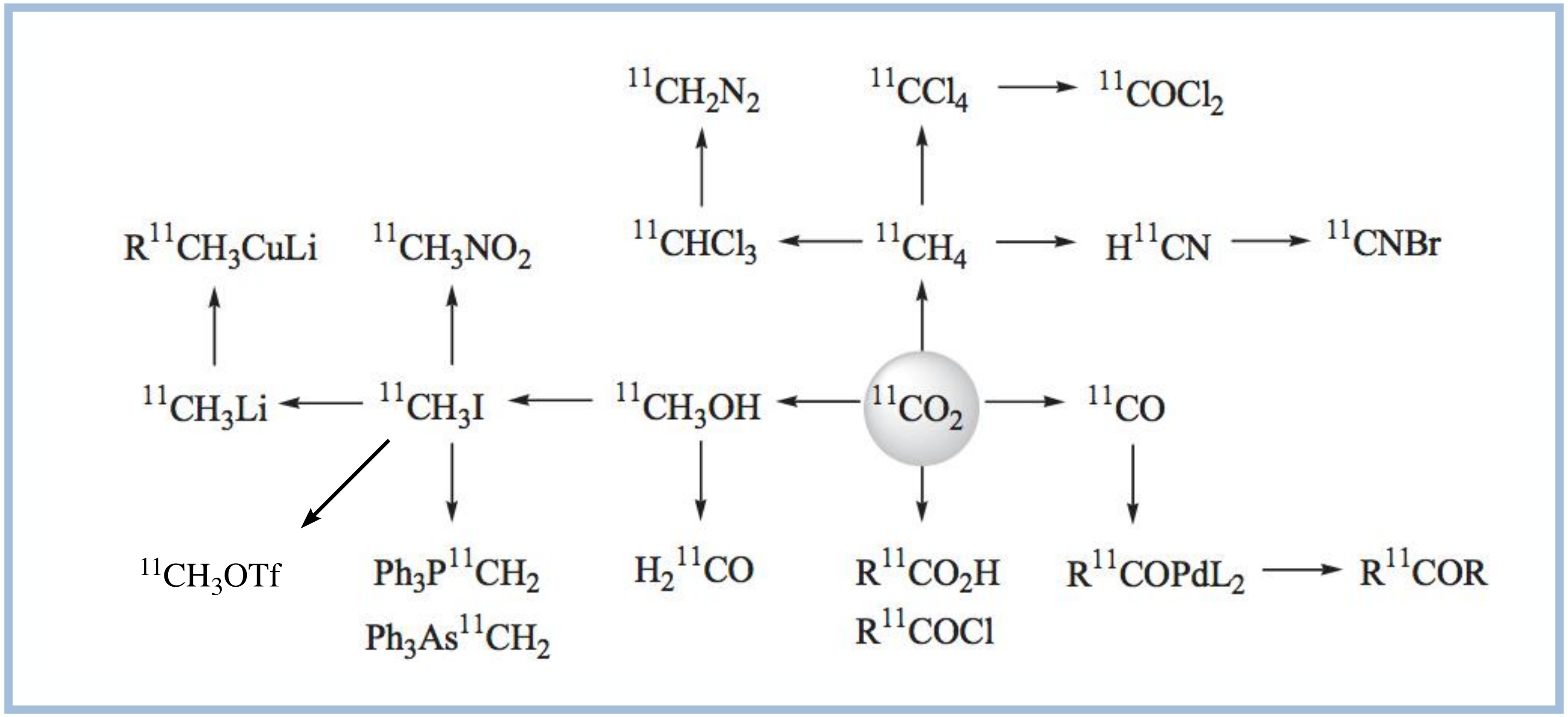
Major Challenges in Carbon-11 Labeling

- scale of reaction ~1 μmol
- reaction and purification in under 20 minutes
- limited variety of carbon-11 building blocks
- radiolabel must meet cGMP requirements

Piel, M.; Vernaleken, I.; Rösch, F. *J. Med. Chem.* **2014**, *57*, 9232.

Methods for Carbon-11 Installation

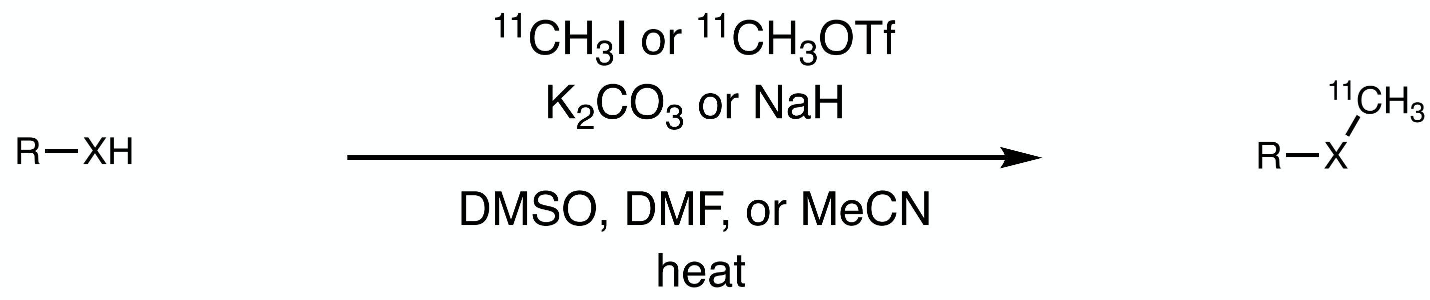
- [^{11}C]-carbon dioxide is the most important primary labeling precursor for generating ^{11}C-labeled compounds, from which a variety of secondary labeling precursors can be prepared



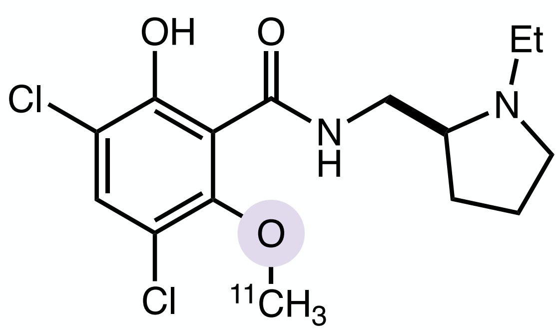
vast majority of carbon-11 labeled compounds are generated via heteroatom methylation using [^{11}C]-methyl iodide

Wuest, F.; Berndt, M.; Kniess, T. *Ernst Schering Res. Found. Workshop.* **2007**, *62,* 185.

Methods for Carbon-11 Installation: Heteroatom Methylation

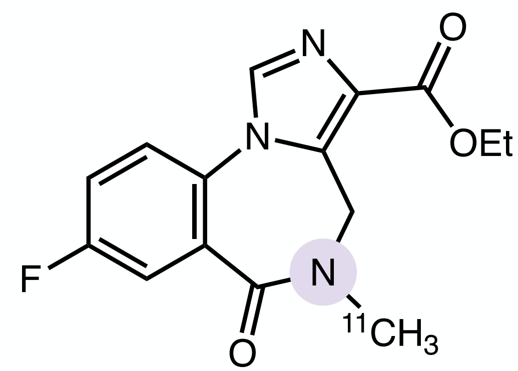


O-methylation



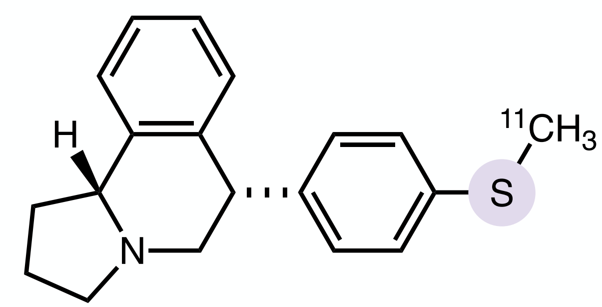
[^{11}C]-raclopride

N-methylation



[^{11}C]-flumazenil

S-methylation

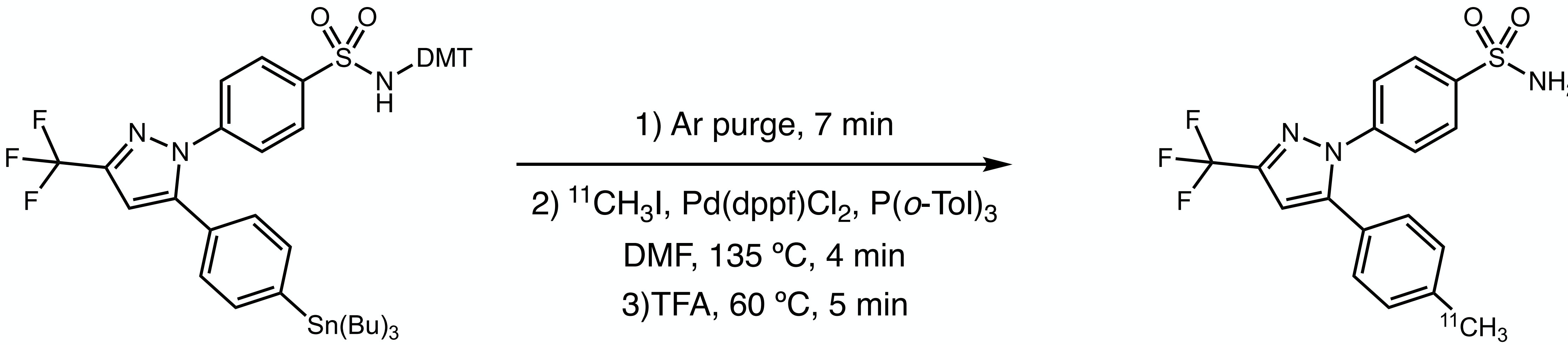


[^{11}C]-McN-5652

- reaction times around 5-10 minutes
- use up to 10,000X more of the desmethyl precursor relative to $^{11}CH_3I$ or $^{11}CH_3OTf$
- can be performed on a solid support to simplify and automate synthesis

Li, Z.; Conti, P. S. *Advanced Drug Delivery Reviews.* **2010**, *62,* 1031.

Methods for Carbon-11 Installation: Stille Coupling

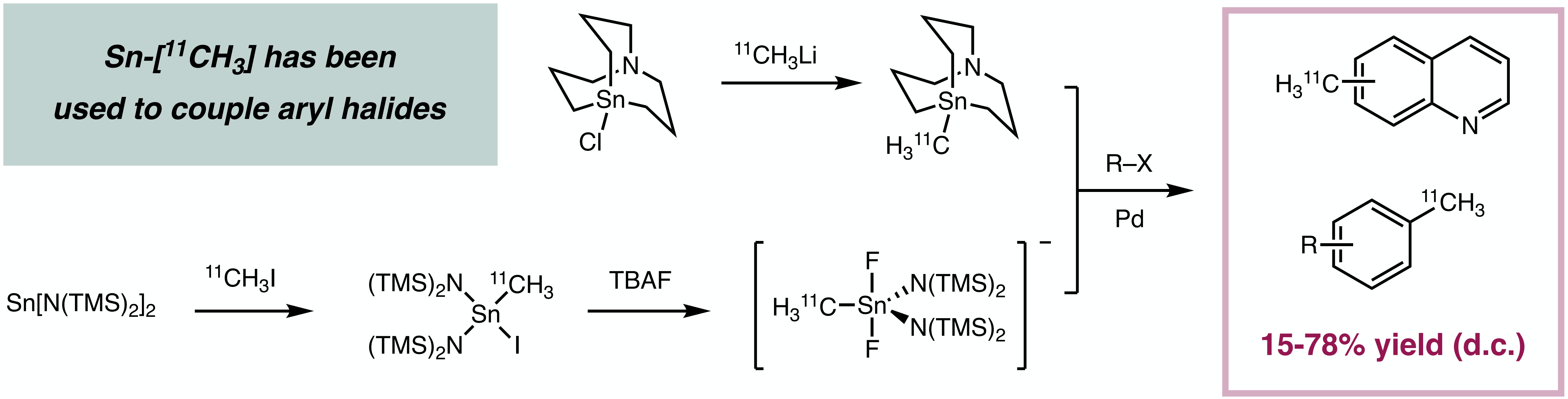


most commonly used method for aryl-[$^{11}CH_3$] installation

[^{11}C]-celecoxib

8% yield (decay-corrected)

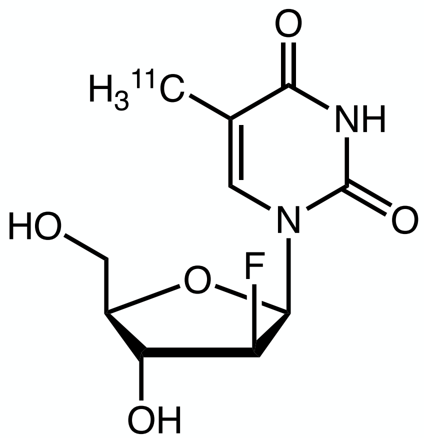
Sn-[$^{11}CH_3$] has been used to couple aryl halides



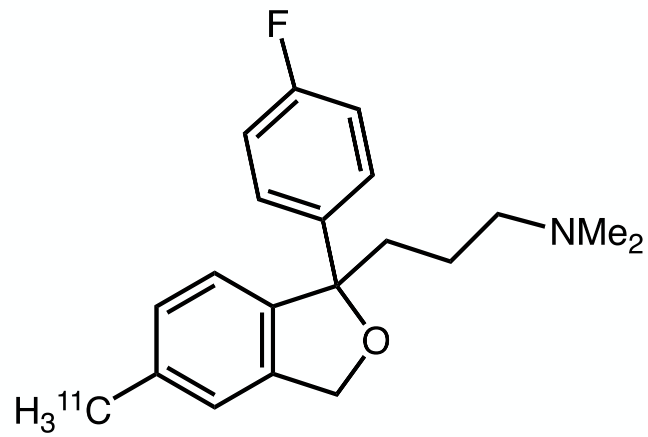
Prabhakaran, J. *et. al. J. Label Compd. Radiopharm.* **2005**,*48*, 887.

Li, Z.; Conti, P. S. *Advanced Drug Delivery Reviews.* **2010**, *62,* 1031.

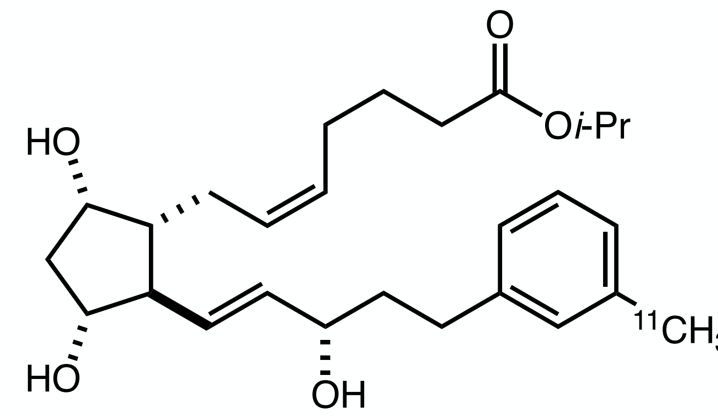
Methods for Carbon-11 Installation: Stille Coupling



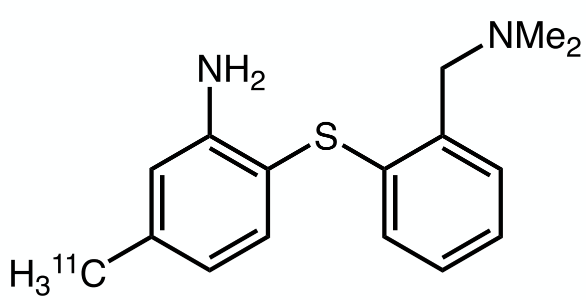
[^{11}C]-FMAU
28% RCY (d.c.)



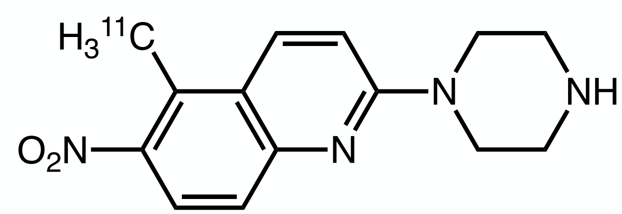
[^{11}C]-citalopram analogue
65-90% RCY (d.c.)



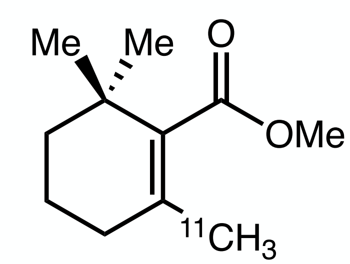
[^{11}C]-methyl PGF$_{2a}$-analogue
34% RCY (d.c.)



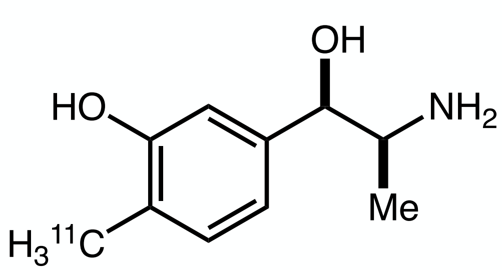
[^{11}C]-MADAM
10-30% RCY (d.c.)



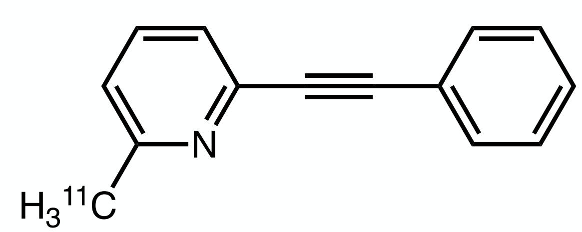
[^{11}C]-methyl-6-nitroquipazine
***2 steps*, 60-80% (d.c.)**



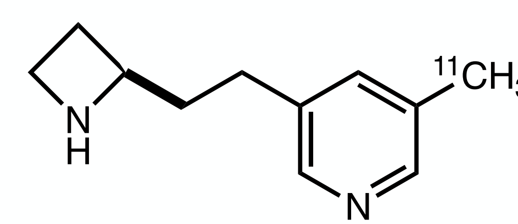
[^{11}C]-cyclohexene derivative
85% RCY (d.c.)



[^{11}C]-methylmetaraminol
***2 steps*, 20-25% RCY (d.c.)**



[^{11}C]-MPEP
13% RCY (d.c.)

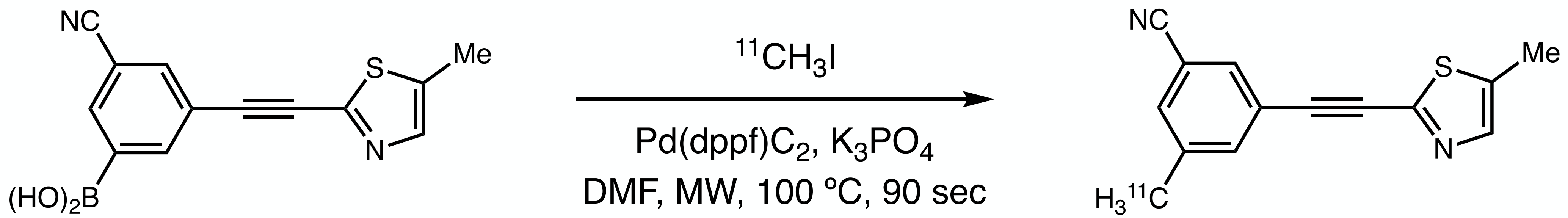


[^{11}C]-methyl-A-85380
***2 steps*, 39% RCY (d.c.)**

Schubiger, P. A.; Lehmann, L.; Friebe, M. *PET Chemistry*; Springer: Berlin, Heidelberg, 2007.

Methods for Carbon-11 Installation: Suzuki Coupling

[^{11}C]-Methylation with Arylboronic Acids

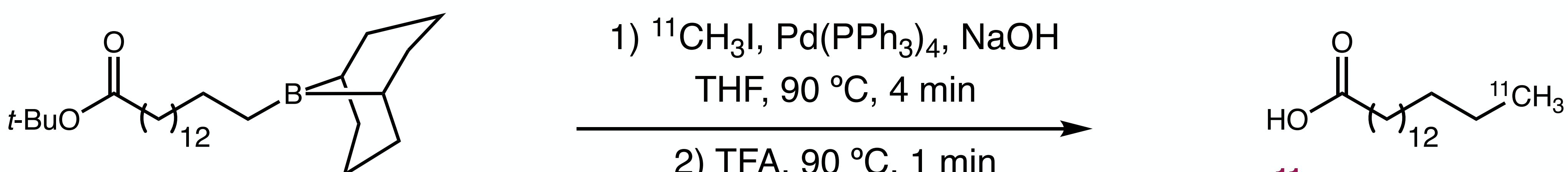


- other examples include phenylboronic acid derivatives
- aryl pinacol boronates also suitable coupling partners

[^{11}C]-M-MTEB

29% yield (not decay-corrected)

[^{11}C]-Methylation with Alkylboronates



alkyl borane precursor

[^{11}C]-palmitic acid

75% yield (d.c.)

Hamill, T. G. *et. al. Synapse.* **2005**, *56,* 205.

Hostetler, E.D. *et. al. J. Org. Chem.* **1998**, *63,* 1348.

Radiotracer Synthesis Enabled by Automation

advances in automated synthesis help solve challenges associated with short half-life radionuclides

automated synthesis module used in ^{18}F-labeling

Advantages of Automated Synthesis:

- increases reliability of synthesis
- reduces radiation exposure
- rapid and simpilified synthesis

methyl electrophiles are prepared via automation

$$^{11}CO_2 \xrightarrow{Ni/H_2} {}^{11}CH_4 \xrightarrow{I_2} {}^{11}CH_3I \xrightarrow{AgOTf} {}^{11}CH_3OTf$$

Schubiger, P. A.; Lehmann, L.; Friebe, M. *PET Chemistry*; Springer: Berlin, Heidelberg, 2007.

Labeling with Fluorine-18

Why Use Fluorine-18 for Radiolabeling?

- radiotracer may be trasported due to relatively long half-life (110 minutes)
- synthesis can be carried out in multiple steps, and over a longer time

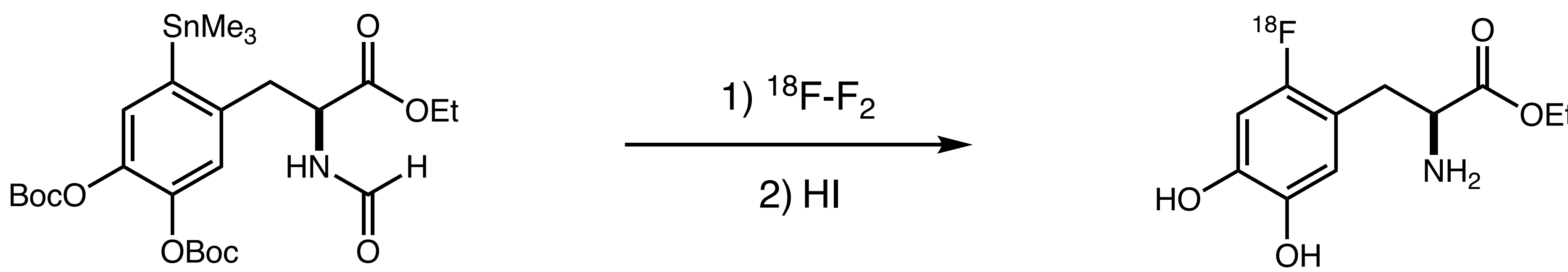
Fluorine-18 can be generated in two different ways:

$^{18}O(p,n)^{18}F$ → $[^{18}F]\text{-}F^{-}_{(aq)}$ **nucleophilic ^{18}F source** → ***preferred fluorine source*** S.A. = 2.7 Ci/μmol

$^{20}Ne(d,\alpha)^{18}F$ → $[^{18}F]\text{-}F_2$ **electrophilic ^{18}F source** → low specific activity due to carrier fluorine-19 in synthesis S.A. = 2.7-16.2 mCi/μmol

Jacobsen, O.; Kiesewetter, D. O.; Chen, X. *Bioconjugate Chem.* **2015**, *26*, 1.

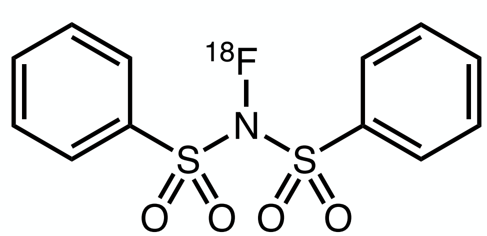
Methods for Fluorine-18 Installation: Electrophilic Fluorination



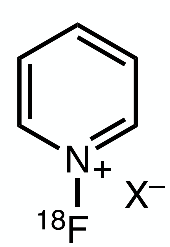
organotin precursor **[^{18}F]-DOPA**

- [^{18}F]-DOPA important for studying Parkinson's disease
- though reaction gives poor specific activities, still best way to make [^{18}F]-DOPA

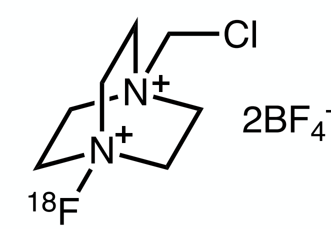
a variety of electrophilic fluorinating reagents have been prepared and used for radiolabeling:



[^{18}F]-NFSI



[^{18}F]-N-fluoropyridinium

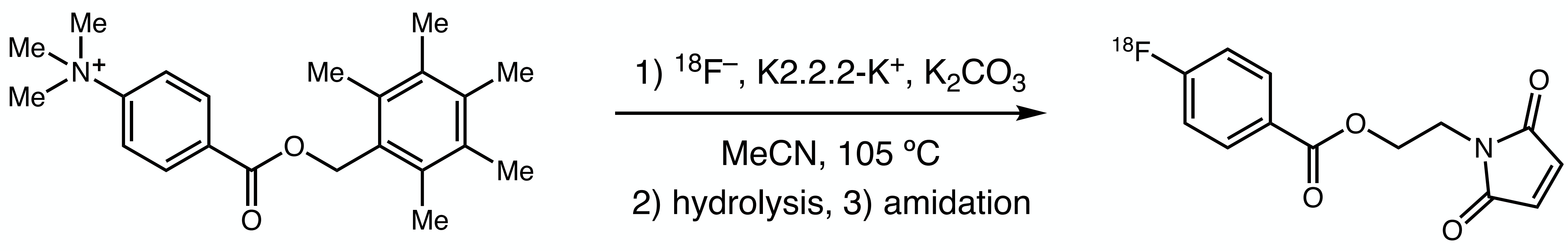


[^{18}F]-Selectfluor

Jacobsen, O.; Kiesewetter, D. O.; Chen, X. *Bioconjugate Chem.* **2015**, *26*, 1.

Methods for Fluorine-18 Installation: Nucleophilic Fluorination

[^{18}F]-Fluorination via S_NAr

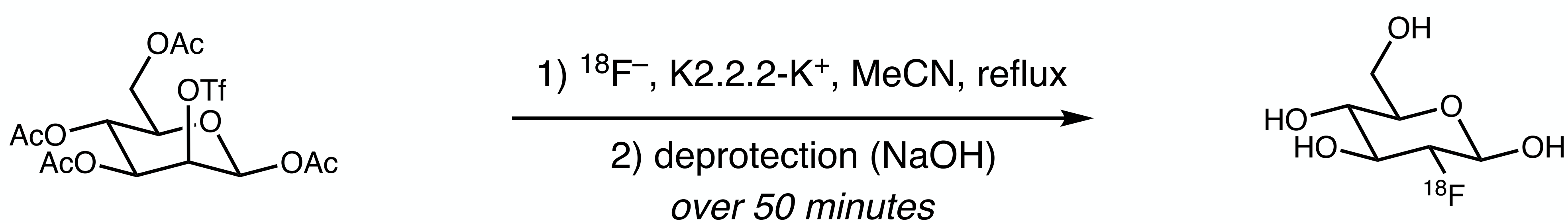


- require electron deficient arene, electron neutral possible
- examples demonstrated with I, Br, Cl, F, and NO_2 LGs

[^{18}F]-FBEM

20% RCY (not decay-corrected)

[^{18}F]-Fluorination via S_N2



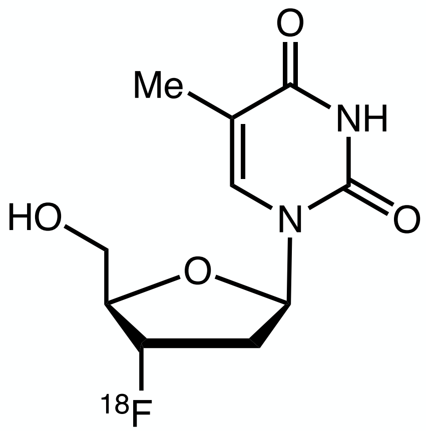
- usually performed in polar organic solvent
- relatively short reaction times producing moderate yields

[^{18}F]-fluorodeoxyglucose

50% RCY

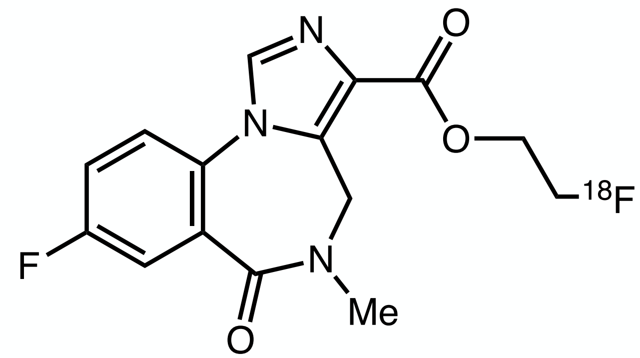
Li, Z.; Conti, P. S. *Advanced Drug Delivery Reviews.* **2010**, *62,* 1031.

Methods for Fluorine-18 Installation: Nucleophilic Fluorination



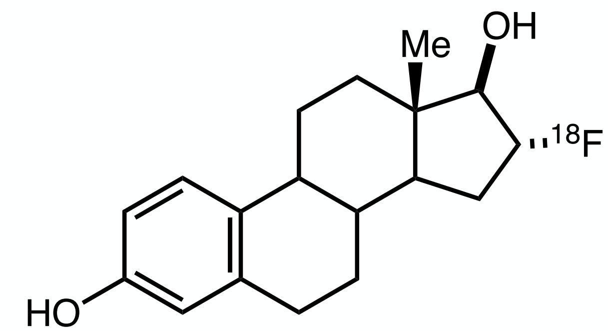
[^{18}F]-FLT

***2 steps,* 17% RCY**



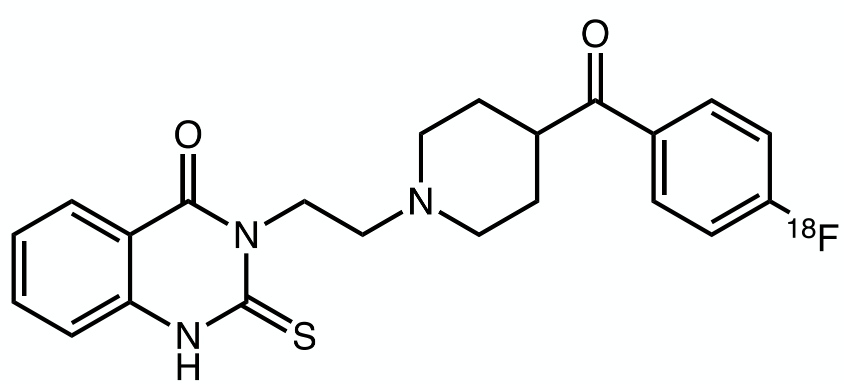
[^{18}F]-fluoroethylflumazenil

20% RCY



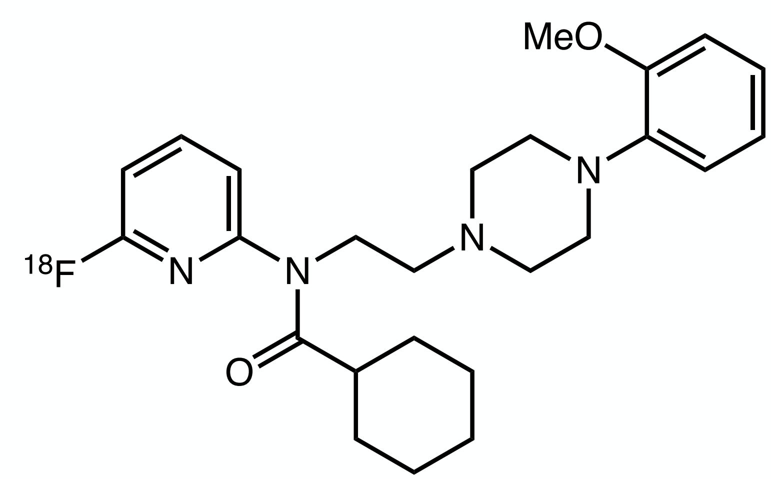
[^{18}F]-FES

***2 steps,* 76% RCY**



[^{18}F]-altanserin

23% RCY



[^{18}F]-fluoro-WAY-1000635A

60-90% RYC

Li, Z.; Conti, P. S. *Advanced Drug Delivery Reviews.* **2010**, *62,* 1031.

Methods for Fluorine-18 Installation: Nucleophilic Fluorination

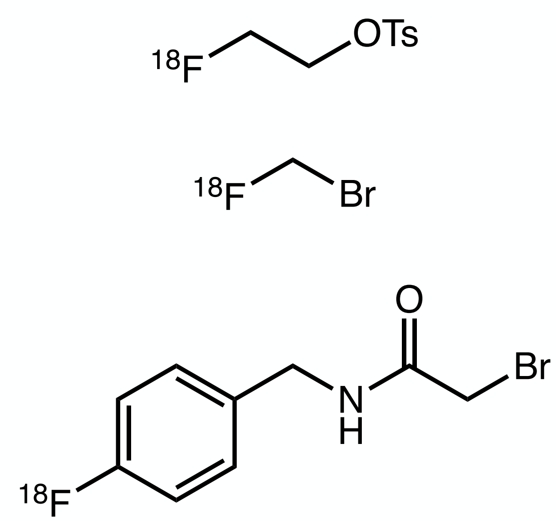
Radiolabeling with $^{18}F^{-}$ generally requires harsh reaction conditions and reactive LGs…

How can peptides, proteins, and other complex molecules be radiolabeled?

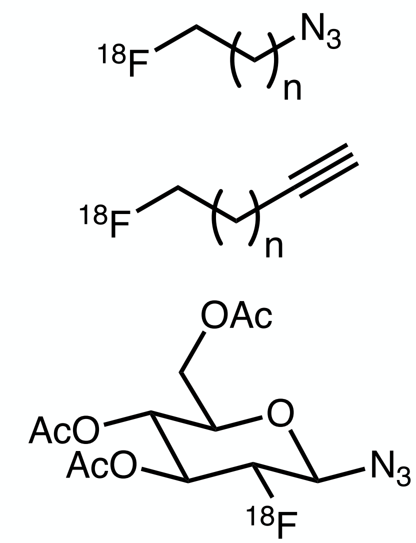
Solution:

radiolabeling can be accomplished using ^{18}F-labeled prosthetic groups

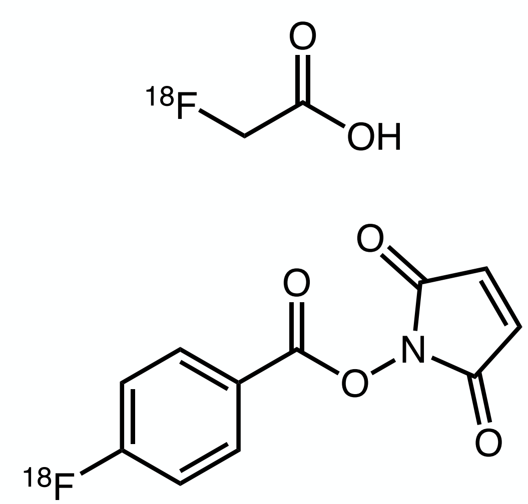
alkylation



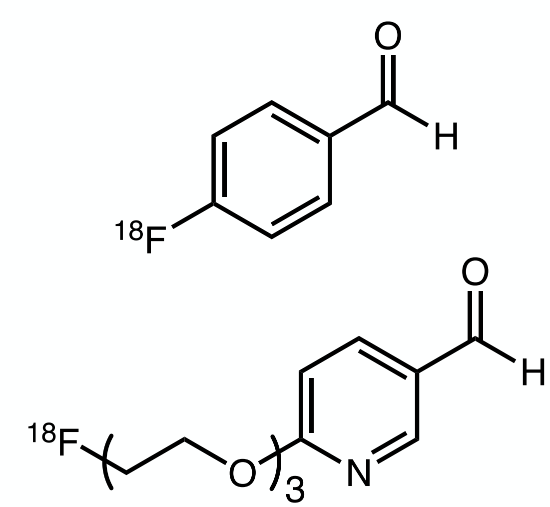
click chemistry



amidation



condensation



Li, Z.; Conti, P. S. *Advanced Drug Delivery Reviews.* **2010**, *62,* 1031.

Utilizing Other Positron-Emitting Radionuclides

Oxygen-15 (half-life = 2.04 min)

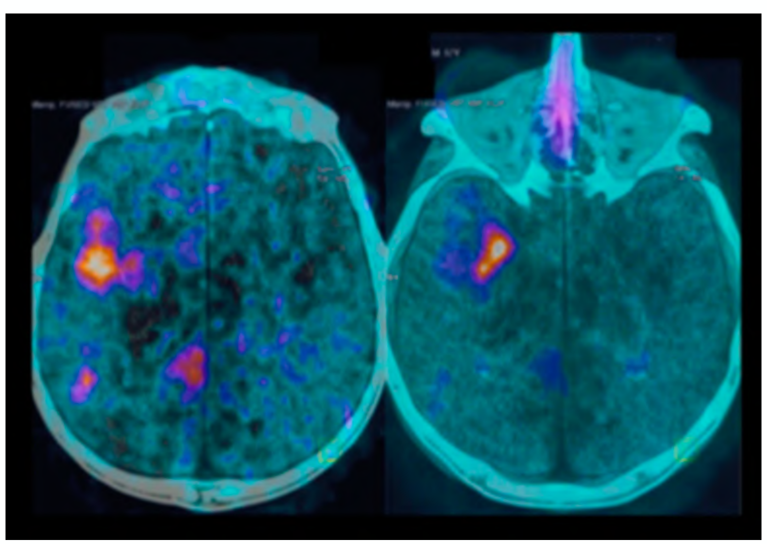
$^{14}N(d,n)^{15}O \longrightarrow [^{15}O]\text{-}O_2 \xrightarrow{H_2/Pt} \mathbf{[^{15}O]\text{-}H_2O}$

Nitrogen-13 (half-life = 9.97 min)

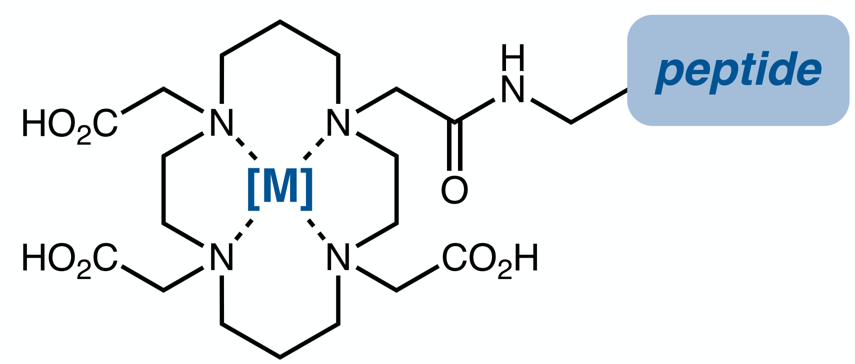
$^{16}O(p,\alpha)^{13}N \longrightarrow [^{13}N]\text{-}NO_x \xrightarrow{\text{reduce}} \mathbf{[^{13}N]\text{-}NH_3}$

- limited utility in radiolabeling due to short half-life
- $[^{15}O]\text{-}H_2O$ and $[^{13}N]\text{-}NH_3$ primarily used to study blood flow

β^+-emitting metals such as Cu-64 and Ga-68 are also used



PET-CT scan using $^{64}Cu^{2+}$ to visualize cerebral tumor



TETA metal chelator allows for conjugation of metal to complex molecules

Chakravarty, R.; Chakraborty, S.; Dash, A. *Mol. Pharmaceutics* **2016**, *13*, 3601.

Positron Emission Tomography

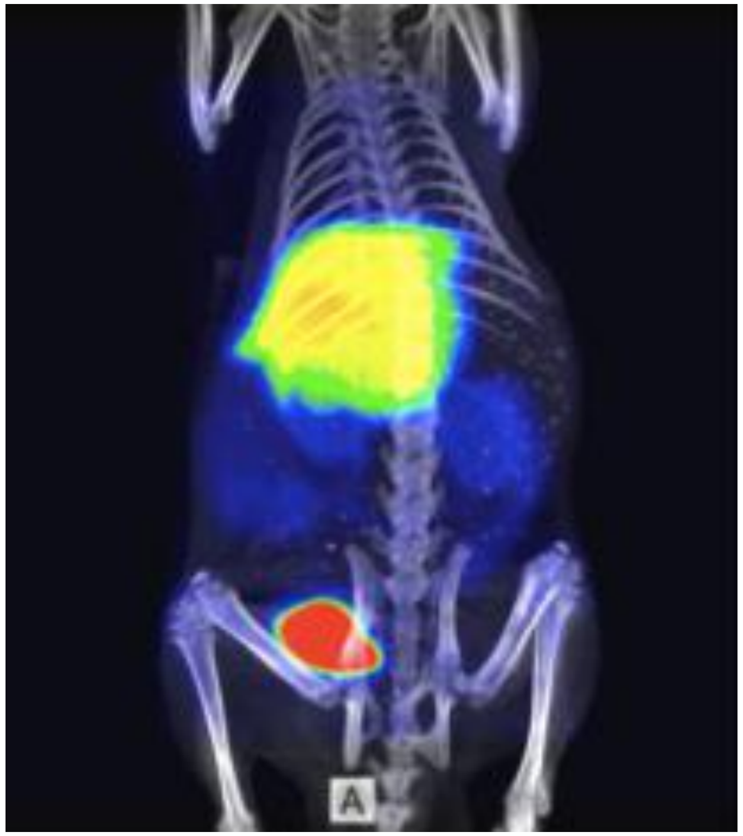
- **Methods for Radiotracer Synthesis**

    Carbon-11 labeling

    Automated synthesis

    Fluorine-18 labeling

    Oxygen-15, Nitrogen-13, and radioactive metals



- **Applications of PET**

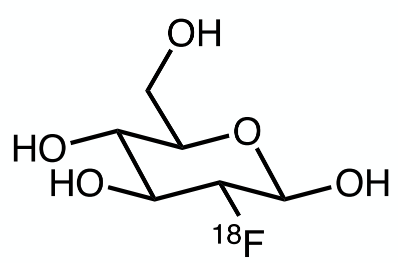
    Cancer visualization

    Alzheimer's disease diagnosis

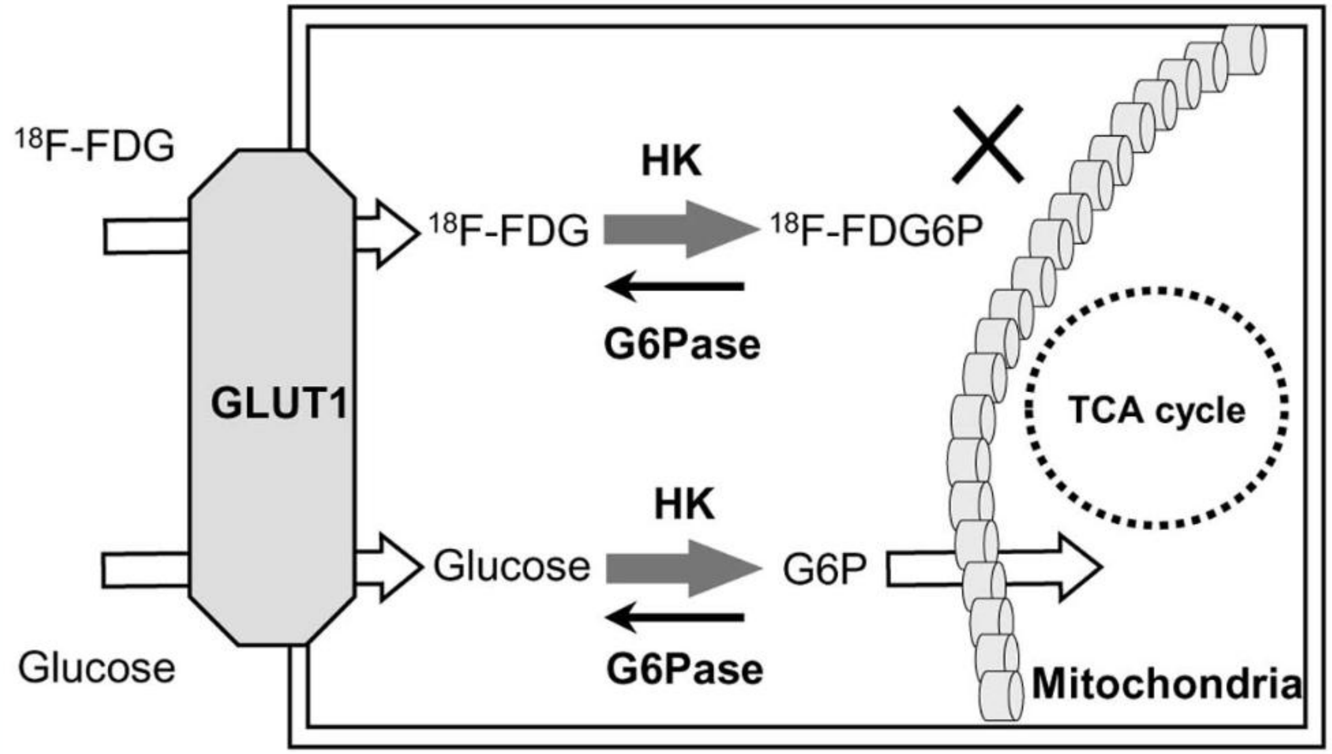
    Drug research & development

PET Imaging in Oncology

- 96% of PET studies in 2011 used [^{18}F]-FDG
- [^{18}F]-FDG can be used to quantify regional glucose metabolism in humans



[^{18}F]-fluorodeoxyglucose

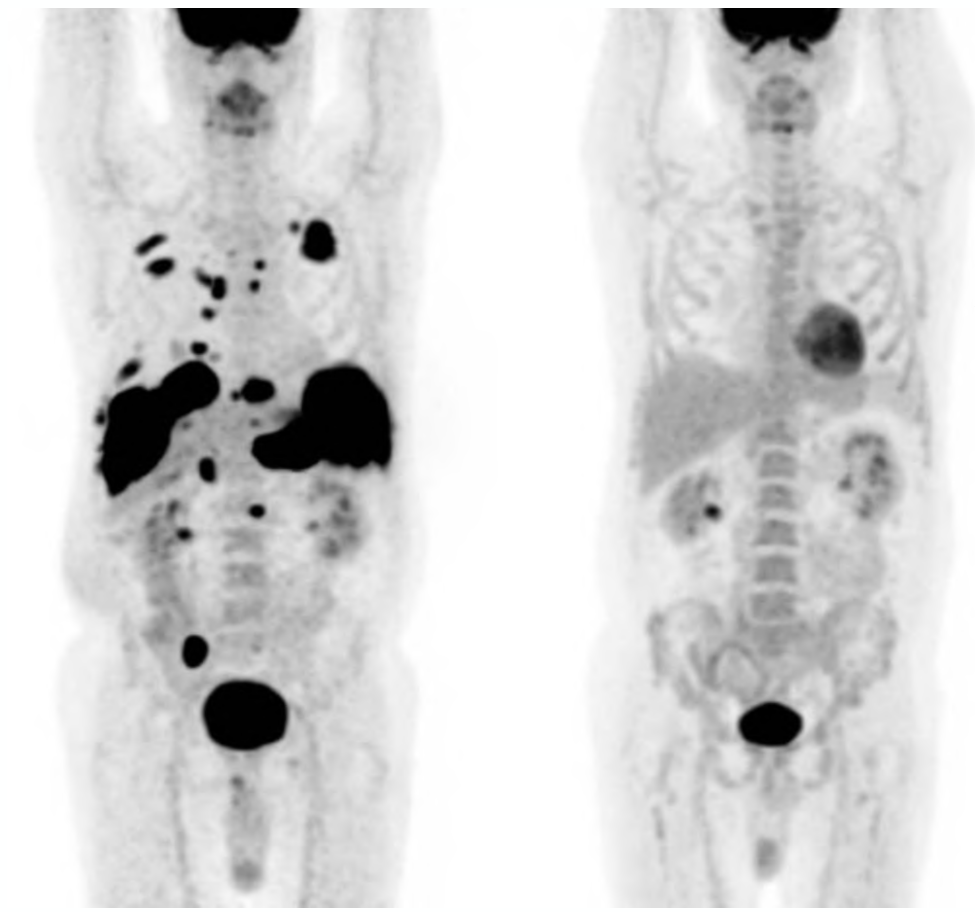


- radioactivity concentration within cells is proportional to rate of [^{18}F]-FDG phosphorylation
- uptake visualized in high glucose-using cells: brain, brown fat, kidneys… ***and cancer cells***

Farwell, M. D.; Pryma, D. A.; Mankoff, D. A. *Cancer* **2014**, *120*, 3433.

PET Imaging in Oncology

- discovered in 1920's, cancer cells have abnormally high rates of glycolysis (Warburg effect)
- increased expression of glucose transporters for faster glucose uptake



patient with B-cell lymphoma

[^{18}F]-FDG PET scans before and after 3 cycles of chemotherapy

[^{18}F]-FDG-PET imaging changed physicians' intended management in 36% of patients:

- 29% nontreatment to treatment
- 7% treatment to nontreatment

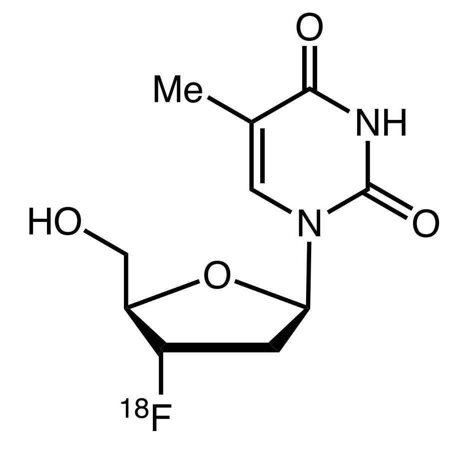
most commonly used for diagnosing: lymphoma, melanoma, head and neck cancer, lung, colorectal, breast, esophageal, cervical, thyroid, and pancreatic cancers

Farwell, M. D.; Pryma, D. A.; Mankoff, D. A. *Cancer* **2014**, *120*, 3433.

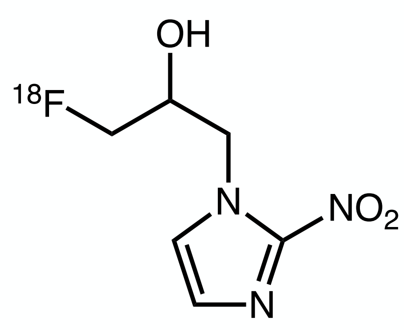
PET Imaging in Oncology

other characteristics of cancer can be targeted with radiotracers

- increased cellular proliferation is another hallmark of cancer
  - greater rate of uptake of nucleosides for DNA replication
- [^{18}F]-FLT valuable for monitoring tumor response to treatment



[^{18}F]-fluorothymidine (FLT)



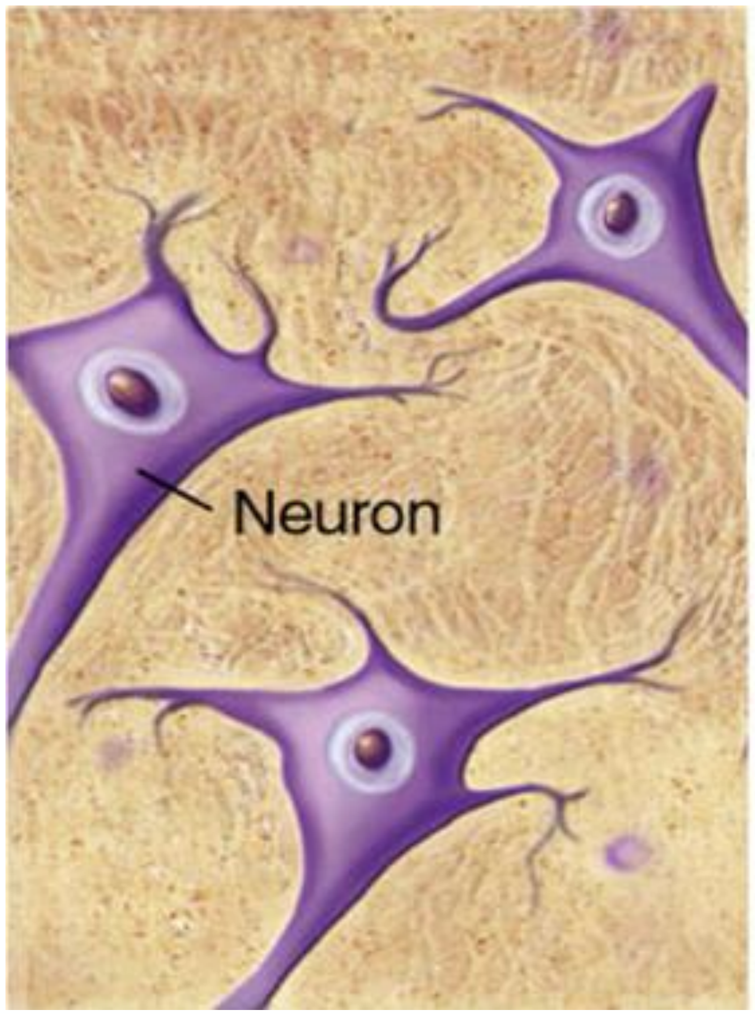
[^{18}F]-FMISO

- hypoxia (low oxygen concentration) is associated with cancer
  - [^{18}F]-FMISO remains in cells lacking oxygen
- nitroimidazole is reduced in hypoxic cells, slowing cellular clearance

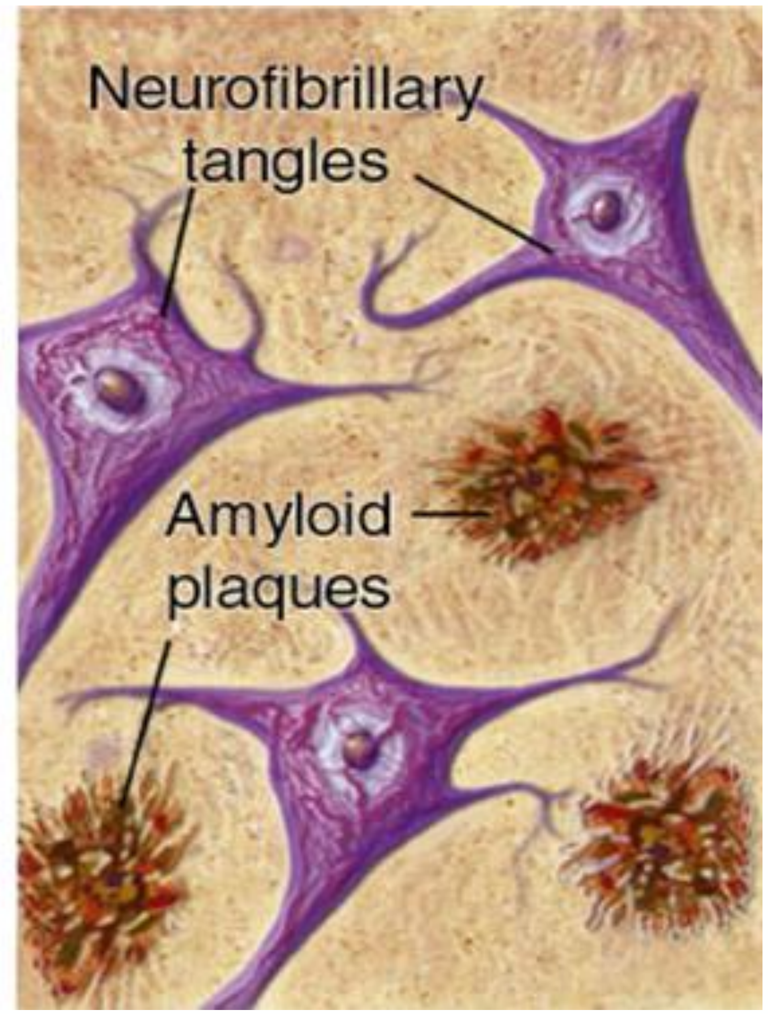
Farwell, M. D.; Pryma, D. A.; Mankoff, D. A. *Cancer* **2014**, *120*, 3433.

Characteristics of Alzheimer’s Disease

Normal



Alzheimer’s

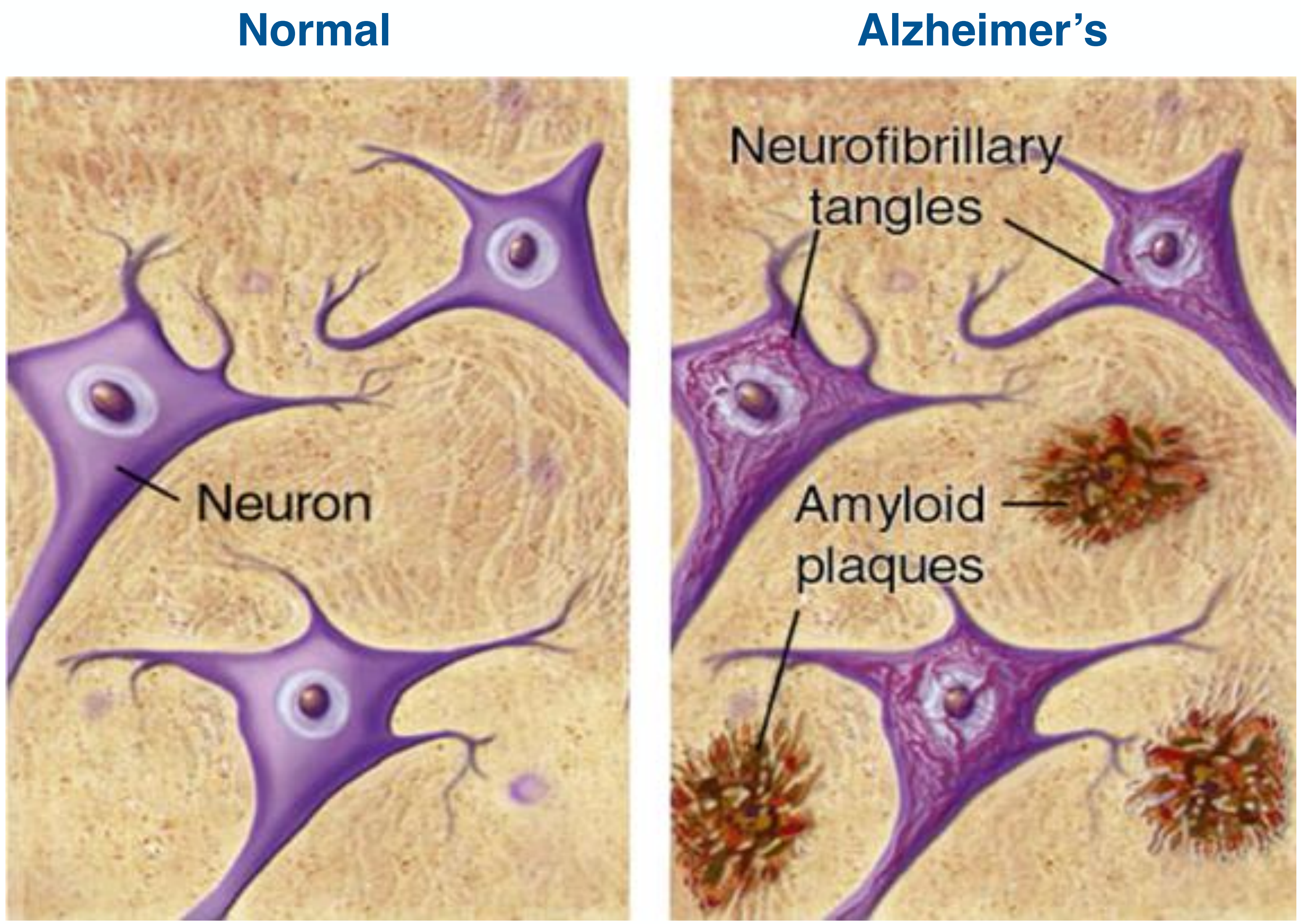


Two pathological hallmarks of Alzheimer’s disease (AD):

- senile plaques composed of amyloid β peptides
- neurofibrillary tangles (NFTs) composed of aggregated tau protein

Ariza, M. Kolb, H. C.; Moechars, D.; Rombouts, F.; Andrés, J. I. *J. Med. Chem.* **2015**, *58*, 4365.

Characteristics of Alzheimer's Disease



neuropathological changes are thought to begin >20 years before symptoms appear in AD

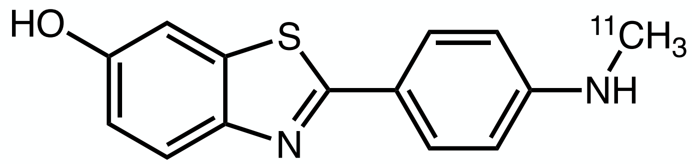
visualizing this would elucidate the link between neuropathology and emergence of clinical disorder, and opportunity for early diagnosis and intervention

Ariza, M. Kolb, H. C.; Moechars, D.; Rombouts, F.; Andrés, J. I. *J. Med. Chem.* **2015**, *58*, 4365.

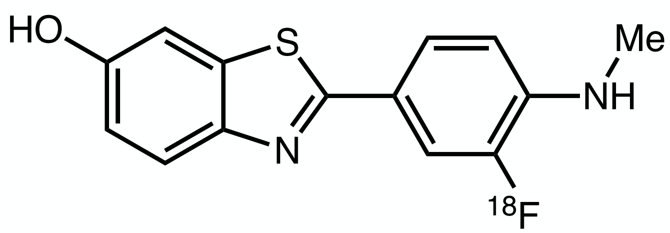
PET Imaging in Alzheimer's Disease

several PET radioligands have been developed to specifically bind to amyloid β-plaques or neurofibrillary tangles

amyloid β-plaque radiotracers

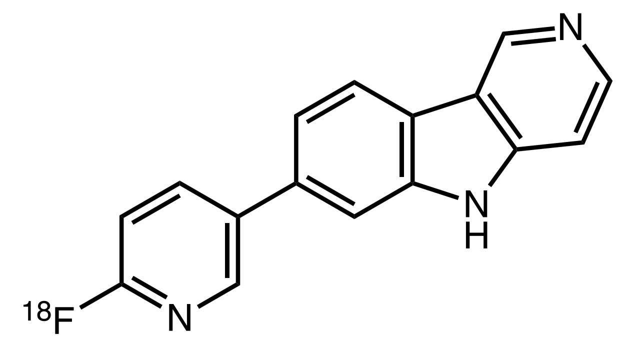


[^{11}C]-Pittsburgh compound B

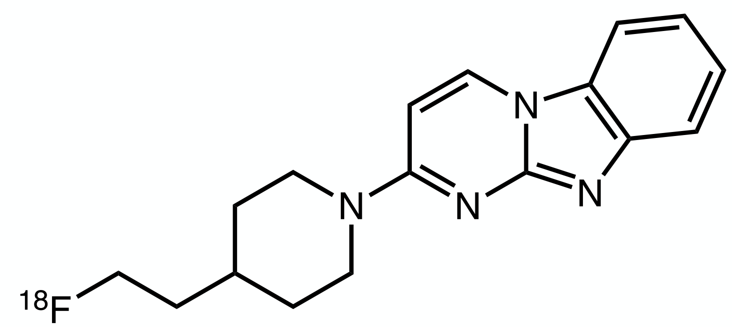


[^{18}F]-Flutemetamol

tau protein aggregate radiotracers



[^{18}F]-T807

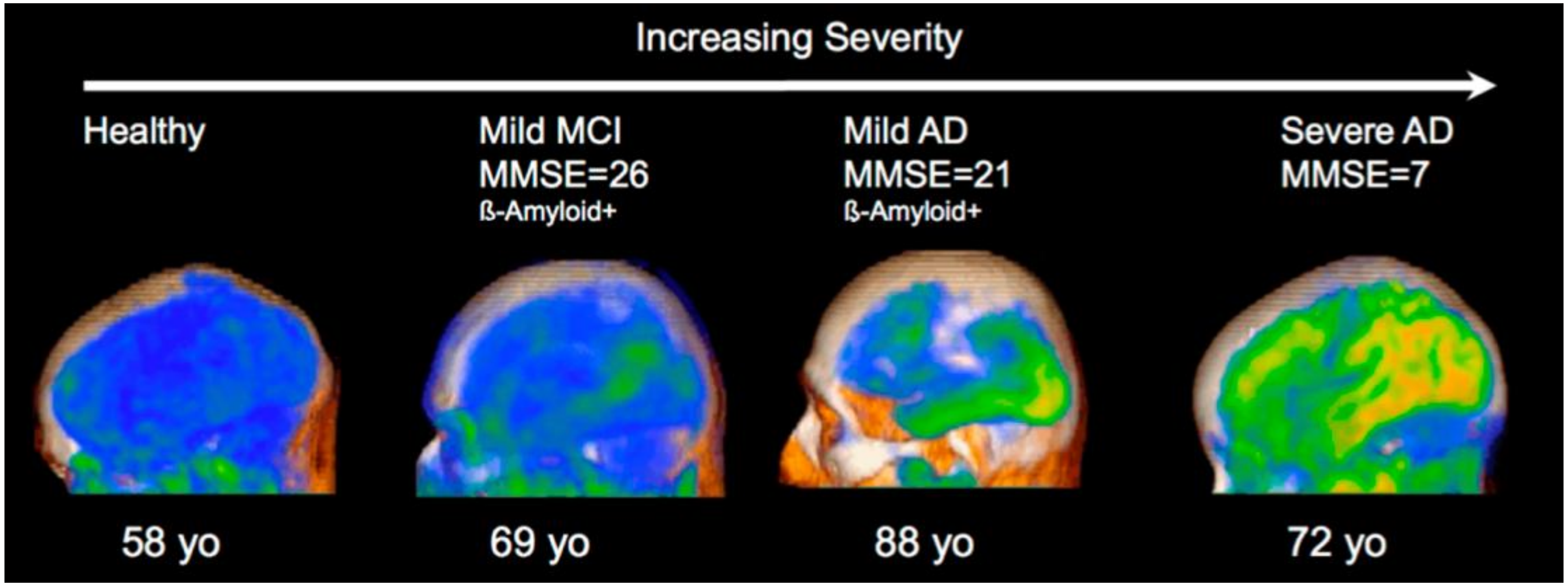


[^{18}F]-T808

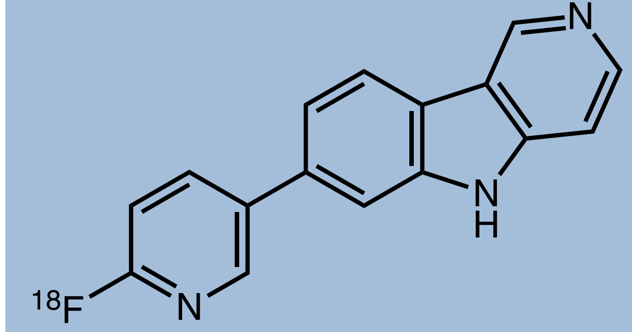
- amyloid imaging used as a diagnostic method for the exclusion of AD in cognitively impaired patients
- NFTs correlate with progressive neuronal degeneration and cognitive impairment
- both tracers may enable earlier diagnosis of AD and differentiation from non-AD dementia

Ariza, M. Kolb, H. C.; Moechars, D.; Rombouts, F.; Andrés, J. I. *J. Med. Chem.* **2015**, *58*, 4365.

PET Imaging in Alzheimer's Disease



PET-CT scan taken 80-100 min after injection with [^{18}F]-T807

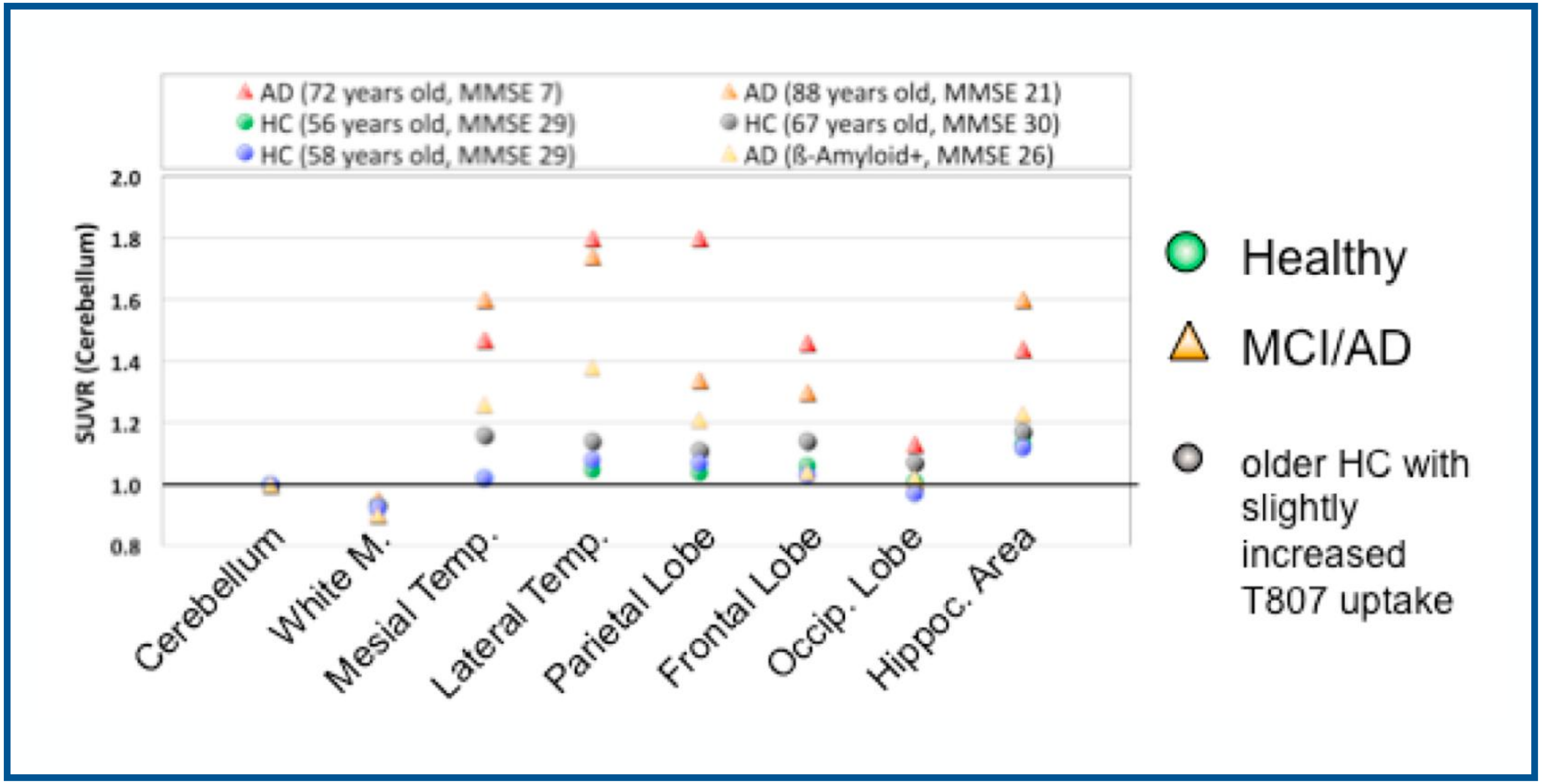


[^{18}F]-T807

- MMSE score represents cognitive impairment (24-30 signifies normal cognition)
- **increasing tau levels track with increased disease severity**

Vaquero, J. J.; Kinahan, P. *Annu. Rev. Biomed. Eng.* **2015**, *17*, 385.

PET Imaging in Alzheimer's Disease

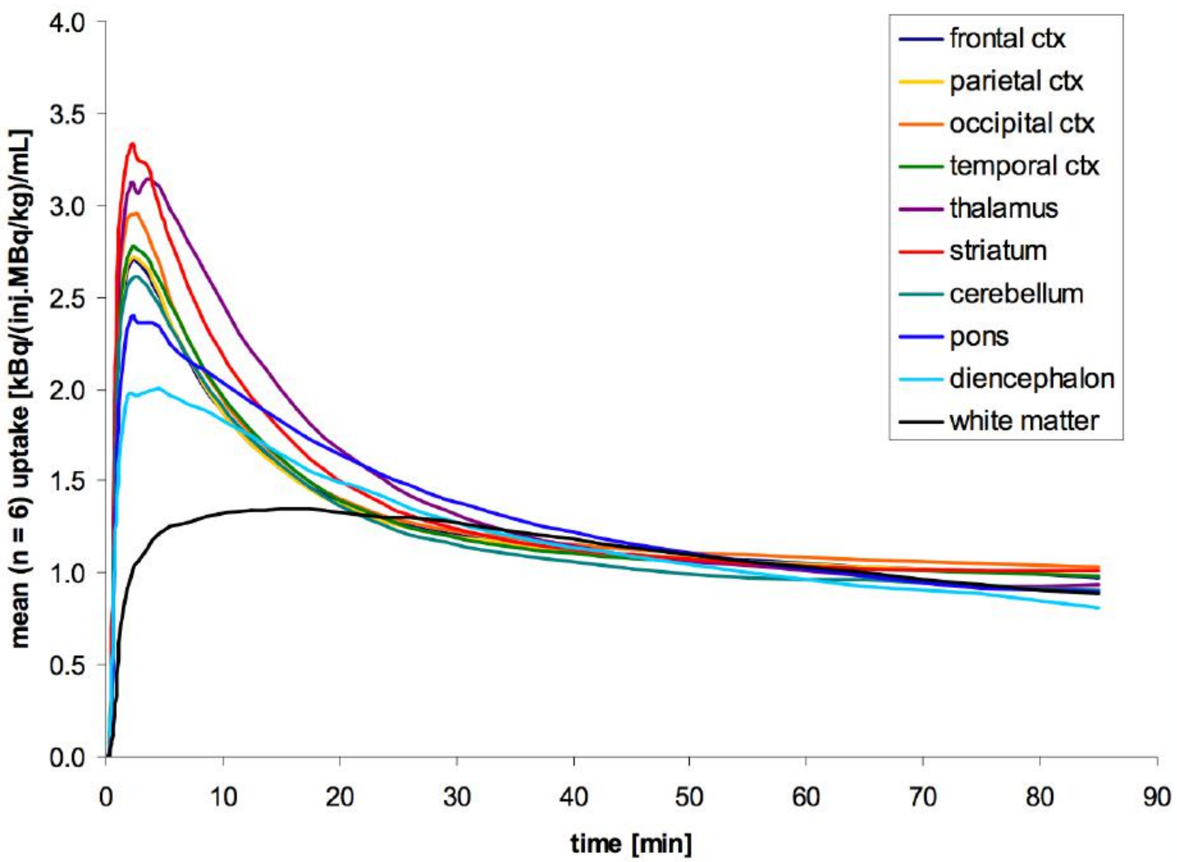


radiotracer distribution is consistent with tau aggregation pathology

Vaquero, J. J.; Kinahan, P. *Annu. Rev. Biomed. Eng.* **2015**, *17*, 385.

PET Imaging in Drug Research & Development

PET is a powerful tool in drug development which can be used to assess all aspects of a drug's behavior in vivo



directly adminstering radiolabeled drug candidate allows for insight on:

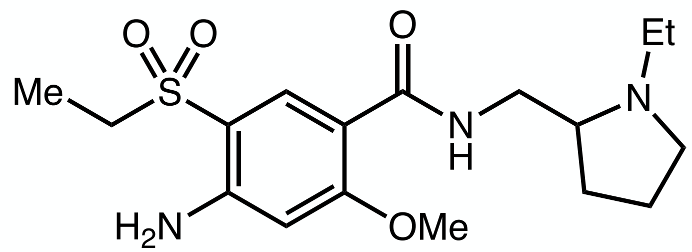
- uptake across the blood-brain barrier
- biodistribution
- tissue kinetics
- radiotracer metabolism

- requires isotopic labeling (carbon-11 usually used more often than fluorine-18)
- time-intensive and costly to develop new radiotracer

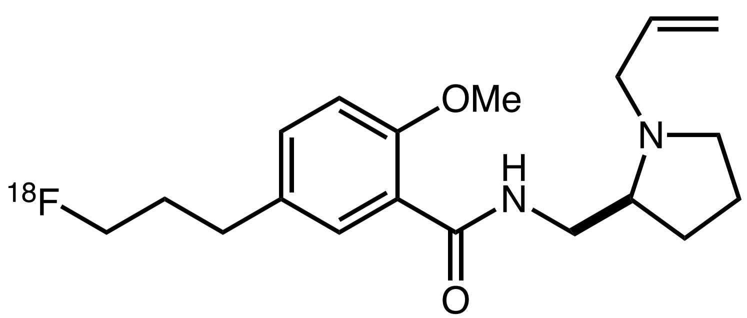
Piel, M.; Vernaleken, I.; Rösch, F. *J. Med. Chem.* **2014**, *57*, 9232.

PET Imaging in Drug Research & Development

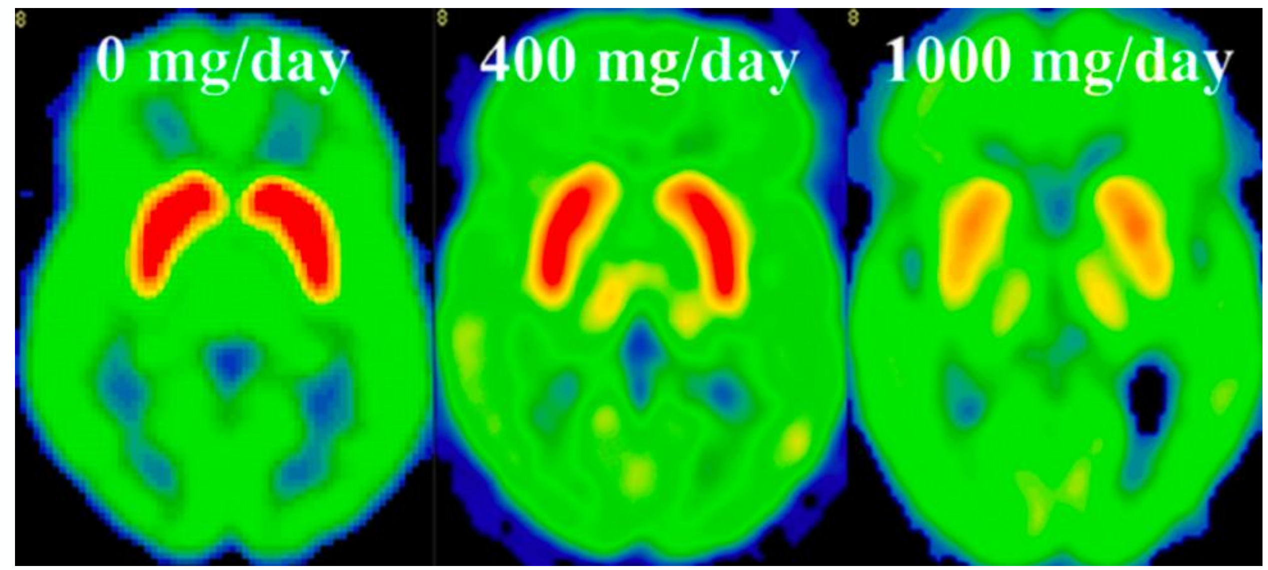
Indirect Quantification of Ligand Binding Potential



amisulpride



[^{18}F]-desmethoxyfallypride



reduced radiotracer binding at dopamine $D_{2/3}$ receptors after dosing with amisulpride

- used to determine target receptor density, K_D and binding potential of drug candidate
- gives information on extent of target interaction needed for pharmacological effect
- enables drug dose-finding in a small group of volunteers

Piel, M.; Vernaleken, I.; Rösch, F. *J. Med. Chem.* **2014**, *57*, 9232.

Positron Emission Tomography

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