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Production and Quality Control of Fluorine-18 Labelled Radiopharmaceuticals

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International Atomic Energy Agency

PRODUCTION AND QUALITY CONTROL OF FLUORINE-18 LABELLED RADIOPHARMACEUTICALS

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PRODUCTION AND QUALITY CONTROL OF FLUORINE-18 LABELLED RADIOPHARMACEUTICALS

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 2021

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FOREWORD

Fluorine-18 fluorodeoxyglucose ([^{18}F]FDG), the first radiopharmaceutical used in clinical applications of positron emission tomography (PET), was a revolutionary development in nuclear medicine that combined biochemistry, medicinal chemistry and radiochemistry. In the 1970s, it was used in brain imaging to aid clinical diagnosis; soon, the detection of malignancies and cardiac diseases also became possible. In the decades that followed, [^{18}F]FDG became widely available owing to the worldwide cyclotron network and automated synthesizers in clinics in many Member States. This paved the way for the development of additional ^{18}F-labelled radiopharmaceuticals adapted for other biological pathways and molecular and cellular mechanisms applicable to human diseases. The production and quality control of ^{18}F radiopharmaceuticals have been revolutionized from the industrial point of view, with the introduction of national and international safety, legislative and legal requirements.

In response to numerous requests from Member States, in 2020 the IAEA organized a number of consultancy meetings aimed at planning and preparing a publication on the production and quality control of ^{18}F radiopharmaceuticals. The present publication is a result of those meetings and provides information on practical production routes and on quality control and radiopharmaceutical aspects of ^{18}F tracers. It is expected to be of interest to radiopharmacists and radiochemists, as well as graduate students in the field of ^{18}F radiochemistry and radiopharmacy.

The IAEA wishes to thank the participating experts for their valuable work and scientific contributions, especially P.H. Elsinga (Netherlands) for compiling and carrying out the technical editing of this publication and J. Vera Araujo (Bolivarian Republic of Venezuela) for her editorial support. The IAEA technical officer responsible for this publication was A.R. Jalilian of the Division of Physical and Chemical Sciences.

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1. INTRODUCTION

1.1. BACKGROUND

Following its clinical use initiation in the 1970's, ^{18}F has become the most utilized positron emitting radionuclide in clinical studies. This is based on the well-established production route using $[^{18}O]H_2O$ target, development of various automated synthesizers and, most of all, increased clinical application of [^{18}F]FDG, the first clinically approved PET agent in the 1990's

One of the driving forces for this publication was to demonstrate the great potential for the optimization of existing ^{18}F radiopharmaceuticals production, as well as for providing centres worldwide interested in the development and production of new ^{18}F radiopharmaceuticals, approved protocols and guidelines on the design, production and quality control (QC) of ^{18}F-based radiopharmaceuticals. The focus will be given ^{18}F-labelled radiopharmaceuticals that have already paved their way into the clinics. Status and history of ^{18}F radiopharmaceutical production

[^{18}F]FDG has been widely used in oncology, brain disorders and heart conditions, and various clinical trials have also been conducted worldwide. Figure 1 shows the worldwide clinical trials using [^{18}F]FDG since 1970's.

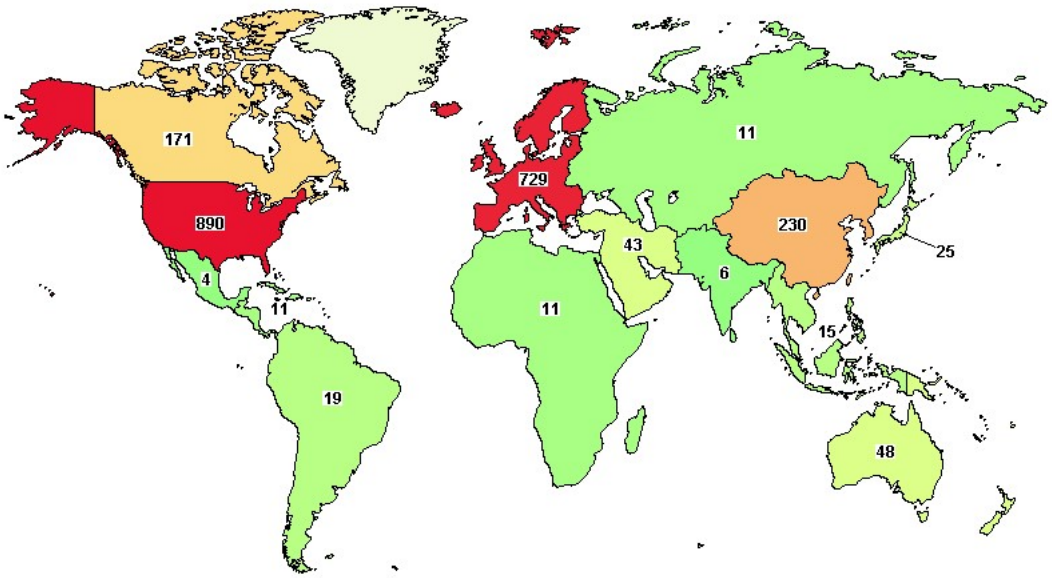

FIG. 1. Worldwide clinical trials involving [^{18}F]FDG since the 1970s (reproduced from Ref. [1] with permission).

Based on the success and clinical and regulatory approvals of [^{18}F]FDG, many other ^{18}F radiopharmaceuticals were developed for the detection and imaging of several metabolic, pathologic and physiologic patterns linked to various human diseases. Numerous review articles describing the application of various ^{18}F-radiopharmaceuticals have been described [2].

The research and development of new ^{18}F-radiopharmaceuticals is thriving and not only in oncology, but also in order to address new challenges in human health such as neurology, infection..

1.2. OBJECTIVE

This publication is intended to provide broad information (with ample references for those who wish to go deeper) on practical production routes and optimal QC and radiopharmaceutical aspects of ^{18}F tracers (including some non-traditional ones), to achieve the best possible products in high quantity and quality for medical interests in clinical applications and for fulfilling the regulatory aspects.

1.3. SCOPE

This publication briefly describes the recent advances in ^{18}F radiopharmaceuticals production and QC, with focus on [^{18}F]fluoride based nucleophilic chemistry reactions, providing guidelines and methods for development and/or modifications to their existing synthesis and QC setups.

This publication is made from the contributions of consultants who have significant experience in the field of ^{18}F radiochemistry and radiopharmacy, from both academia and industry sides, with recognized expertise in pharmaceutical chemistry, radiochemistry, radiopharmacy, installations and design and, last but not least, regulatory and legislative aspects. Consequently, this publication contains an overview on production of ^{18}F using a regular ^{18}O-water target by means of a cyclotron generated proton beam. Production of ^{18}F based on other targets (such as gas targets) and routes (such as deuteron reactions) are only briefly discussed in this publication, due to limited availability or lack of these routes worldwide.

The targeted readers of this publication are radiopharmacists, radiochemists, as well as cyclotrons/radiopharmacies decision makers interested in providing their network clinics with new ^{18}F radiopharmaceuticals for clinical applications. The technologists already working in Member States radiopharmaceutical labs wanting to enhance the utility of the existing synthesisers and setting up QC procedures would also highly benefit from this publication. Due to presentation of some new ^{18}F radiochemistry methods that are new or on the verge of entering application in clinical grade radiopharmacy production, many graduate students and scientists from Member States would also benefit.

1.4. STRUCTURE

This publication contains nine sections covering ^{18}F radiopharmaceutical production from ^{18}F radioisotope production to the final radiopharmaceutical production, formulation as well as general aspects. Section 3 provides information on the production of ^{18}F. Section 4 focuses on workup of [^{18}F]fluoride and automation of ^{18}F productions as well as purification, isolation and QC aspects of ^{18}F compounds. Section 5 describes general principles and concepts of [^{18}F]fluorinations that would be helpful for chemists and pharmacists that are new to the field or are interested to learn more on the underlying basic science. Examples of specific tracer applications, based on their molecular targets, are described in section 6. In section 7 regulatory considerations are described, since these are one of the most important aspects in the radiopharmaceutical production and utilization chain, also emphasized in many Member States recommendations. Section 7 also emphasizes GMP related to the production of ^{18}F compounds (facility design) as well as radiation safety aspects. From a more practical point of view, section 8 describes specific protocols for the production of most frequently used clinical ^{18}F radiopharmaceuticals as a technical source. Finally, section 9 takes a look at future trends in

^{18}F radiopharmaceuticals. A large and comprehensive collection of relevant references is provided at the end of the publication, for those who wish to delve deeper.

2. IAEA ACTIVITIES

The International Atomic Energy Agency (IAEA) is supporting 172 Member States, as of December 2020, on various peaceful applications of nuclear science and technology with focus on human welfare and health. IAEA supports Member States through various mechanisms, including research and development and implementation of projects. At least half of the Member States have ongoing national projects on the production and application of radiopharmaceuticals.

2.1. COORDINATED RESEARCH PROJECTS

The IAEA has several mechanisms for knowledge transfer to recipient Member States, among which coordinated research projects, CRPs, are one of the most efficient . In CRPs, both developed and developing countries are brought together to work together towards a common goal. Typically the technology involved is already in place for specific developed Member States and the knowledge and idea can be transferred to less developed Member States with appropriate background and potential for sustainability. Usually applications that are 'close to routine', such as radiopharmaceuticals under clinical trials or near to approval, are target objectives of these CRP projects. In 2008, a new project on the dissemination of knowledge for the routine production and QC of new ^{18}F radiopharmaceuticals was planned with the title 'Development of ^{18}F labelled Radiopharmaceuticals (beyond [^{18}F]FDG) for use in Oncology and Neurosciences' F22048, and implemented from January 2009 to December 2013. Fifteen participants from 15 Member States from America, Europe, Asia and Oceania (Fig. 2) took part in this project and shared the technical experience on the subject, which is to be disseminated to all the Member States [3]. It is noteworthy that several other CRPs with focus on clinical applications of [^{18}F]FDG are also in progress[1].

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[1] These CRPs are:

- PET/CT in the Evaluation of Locally Advanced Breast Cancer, Coordinated Research Project, E13044, from February 2018, to February 2021 (PET/CT in the Evaluation of Locally Advanced Breast Cancer | IAEA)

- Prognostic Value of Arterial [^{18}F]FDG PET Imaging - The PIAF Trial, Coordinated Research Project, E13048, from May 2019, to May 2024 (Prognostic Value of Arterial [18F]FDG PET Imaging - The PIAF Trial | IAEA)

- Standardizing Interpretation Criteria for Early Response Evaluation with [^{18}F]FDG PET/CT in Paediatric Lymphoma, Coordinated Research Project, E12017, from July 2013, to November 2018, (Standardizing Interpretation Criteria for Early Response Evaluation with [18F]FDG PET/CT in Paediatric Lymphoma | IAEA).

FIG. 2. Geographical distribution of participating countries in IAEA CRP on ^{18}F radiopharmaceuticals as of May 2021.

2.2. TECHNICAL COOPERATION

Based on the developments in the production and application of ^{18}F radiopharmaceuticals in recent decades, a large demand from the Member States to IAEA for support arose. Several national and regional projects have been initiated since the early 2000's. Figure 3 depicts the international distribution of IAEA TC projects on cyclotron produced radiopharmaceuticals (all involving FDG and other ^{18}F radiopharmaceuticals)

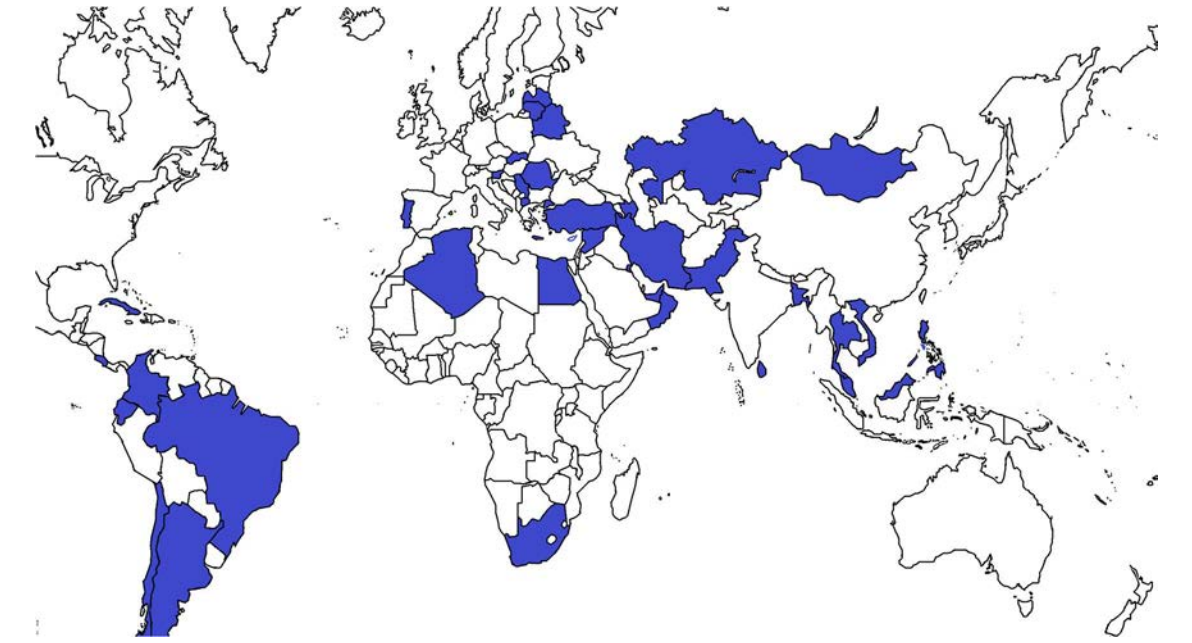

FIG. 3. Overview of Member States distribution benefiting from IAEA Technical Cooperation projects on ^{18}F radiopharmaceuticals and cyclotrons as of May. 2021 (1990-2020).

2.3. CYCLOTRON DATABASE

The vast distribution and rapidly growing demand for the production and application of ^{18}F radiopharmaceuticals is, in part, due to the huge worldwide network of cyclotrons, ranging from small clinical cyclotrons to larger machines in the energy range of 8–30 MeV. The IAEA cyclotron database contains pertinent information of this rapidly growing network, its data being updated based on Member States' and industry inputs[2]. The database now contains details on the production and contact details for 1279 cyclotrons (from 20 companies) in 95 Member States (Fig. 4).

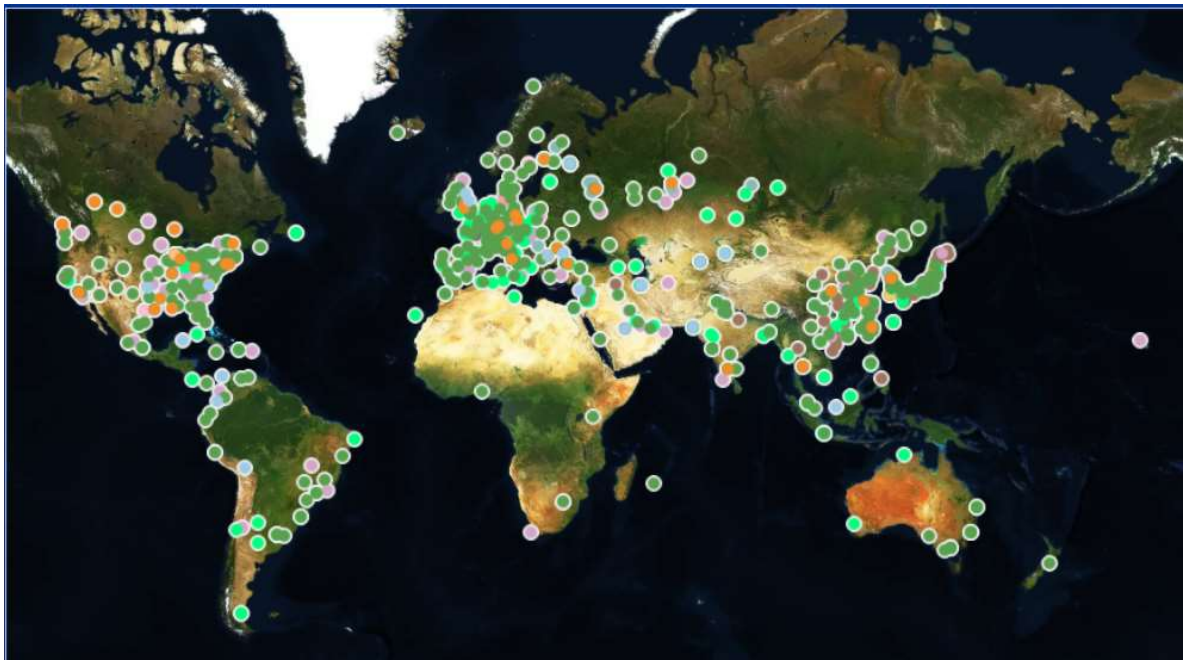

FIG. 4. Geographical map overview on the IAEA cyclotron database input as of May 2021.

2.4. IAEA PUBLICATIONS

In response to requests from Member States in recent decades on the information, and recommendations for installation of medical cyclotrons and PET radiopharmaceuticals, especially [^{18}F]FDG, several publications have been prepared and are now freely available online.

2.5. WORLDWIDE STATUS OF ^{18}F RADIOPHARMACEUTICAL-BASED CLINICAL TRIALS

A major criterion to understand the importance and acceptability of ^{18}F radiopharmaceuticals, is at the information of recorded clinical trials worldwide. Websites such as ´clinicaltrial.gov´ provide interesting information in this regard. Figure 5 shows the worldwide distribution of

[2] This database can be accessed at https://nucleus.iaea.org/sites/accelerators/Pages/Cyclotron.aspx

trials in different regions. In the United States alone, 870 trials have been recorded, more than any other region or countries around the world. This number is followed by 610 studies in the European Union and then 240 in China.

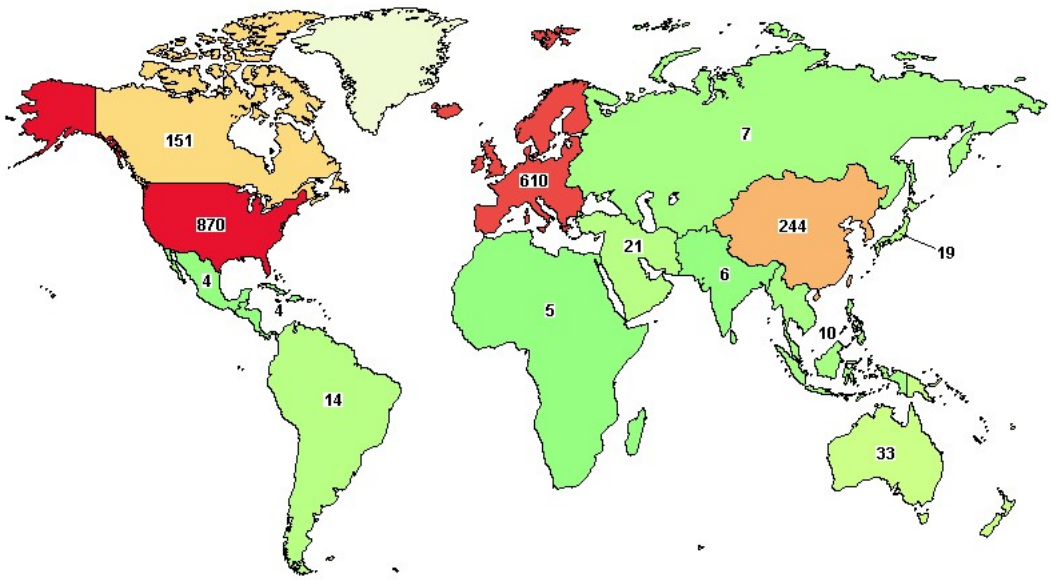

FIG. 5. Worldwide distribution of clinical trials on ^{18}F radiopharmaceuticals (reproduced from Ref. [1] with permission).

The worldwide status of all ^{18}F radiopharmaceuticals trials ever recorded (Fig. 6), demonstrates that around 1981 trials have been conducted; from those, 743 have been concluded with the results published, while many more (nearly a quarter) are still in recruitment status (473 studies), showing the importance and huge ongoing interests on these agents.

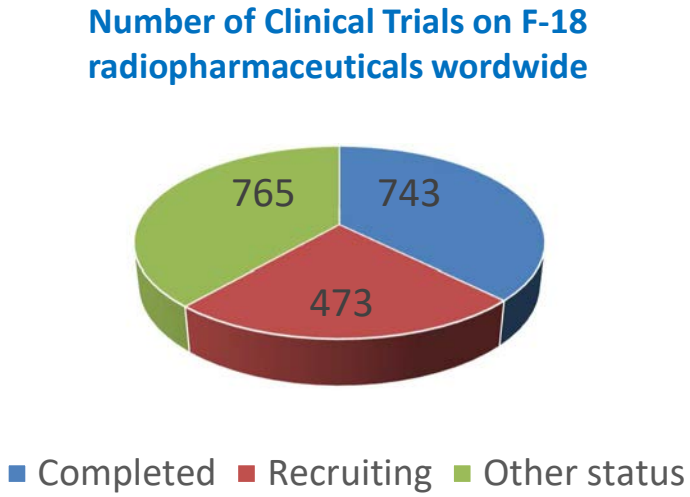

FIG. 6. Worldwide clinical trials based on ^{18}F radiopharmaceuticals (reproduced from Ref. [1] with permission).

Interestingly, scrutiny of the human subjects' age groups recruited to participate in the studies world-wide (Fig. 7) reveals that the different age groups span neonates to geriatrics (0>65 years

old). This emphasizes the safety and non-invasive nature of PET radiopharmaceuticals, especially short-lived radioisotopes such as ^{18}F.

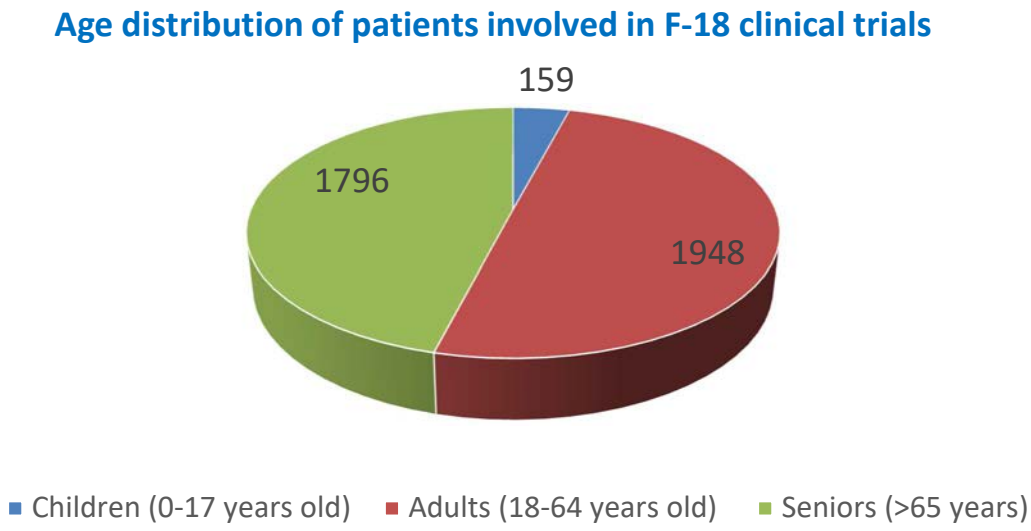

FIG. 7. Subject age distribution in worldwide clinical trials on ^{18}F radiopharmaceuticals (reproduced from Ref. [1] with permission).

Regarding the gender distribution, statistically almost equal numbers of clinical trials on ^{18}F radiopharmaceuticals for men and women were recorded. Figure 8 shows the gender distribution.

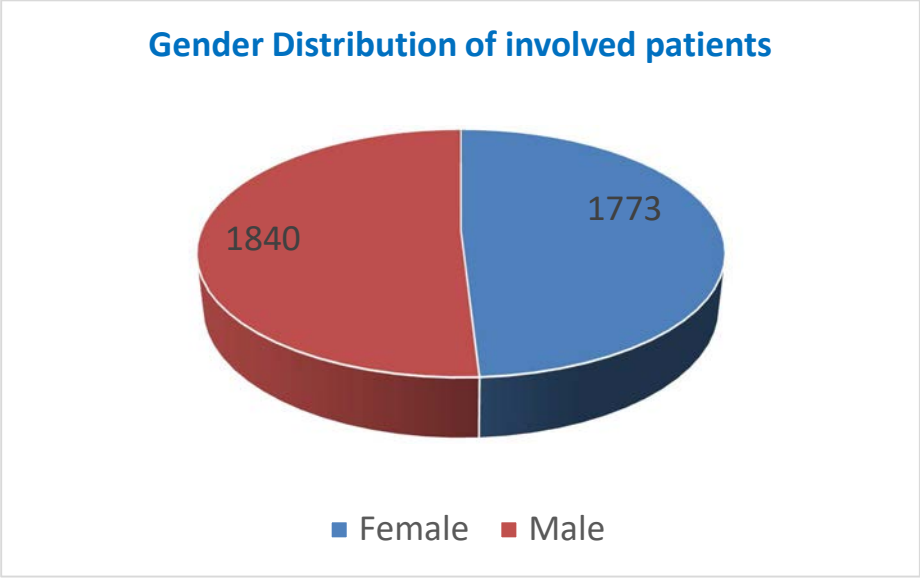

FIG. 8. Gender distribution among worldwide ^{18}F radiopharmaceuticals clinical trials (reproduced from Ref. [1] with permission).

The production and application of ^{18}F radiopharmaceuticals (excluding FDG) is also rapidly growing in all geographical regions (Fig. 9), with North America and Europe leading. Apart from FDG that is widely produced in almost 68 Member States, [^{18}F]FLT, is the most widely used tracer in clinical trials. [^{18}F]FDOPA is the second leading ^{18}F radiopharmaceutical in this

list; it was initially used for neuroreceptor imaging and has recently found applications in various malignancies. [^{18}F]FMISO and [^{18}F]FAZA are the other two ^{18}F radiopharmaceuticals used in trials based on their direct or indirect application in human malignancies.

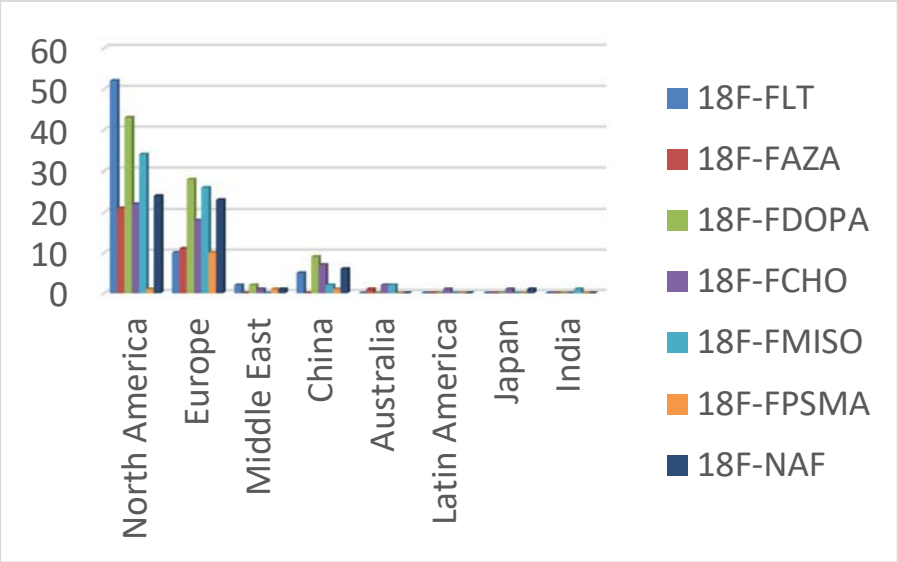

FIG. 9. Regional distribution of clinical trials on selected ^{18}F radiopharmaceuticals (reproduced from Ref. [1] with permission).

3. ^{18}F CHARACTERISTICS AND PRODUCTION

3.1. INTRODUCTION

F-18 as a radionuclide for medical imaging has a long history. Blau et al. [4] reported the first use of ^{18}F as sodium fluoride (Na[^{18}F]F) for imaging skeletal metastases in 1962. The synthesis of [^{18}F]FDG (also commonly referred as FDG) using cyclotron produced fluorine gas [^{8}F]F_2 in 1976 at the Brookhaven National Laboratory in the USA was a major breakthrough in radiopharmaceuticals sciences [5]. However, its initial application for brain imaging using a conventional gamma camera yielded very poor quality images, thereby causing a temporary setback in the use of this radionuclide for diagnostic imaging studies.

The development of positron emission tomography (PET) was a major innovation in nuclear imaging instrumentation which led to the revival of interest in FDG for brain imaging. The utility of FDG for diagnosis, staging, therapy monitoring and recurrence evaluation of different types of cancers was soon realized, which increased the demand for the production of ^{18}F for the synthesis of FDG.

Fusion of computed tomography (CT) with PET was yet another milestone in nuclear medicine, as both anatomic and functional information could be obtained from a single imaging procedure. This was milestone was followed by the development of PET fused with magnetic resonance imaging (MRI). Today, PET imaging using FDG is an integral part of cancer management.

Thanks to the continuous developments in the field of PET radiopharmaceuticals chemistry, the demand for ^{18}F has been increasing steadily. There are >1000 cyclotrons in the world producing ^{18}F on a daily basis for the preparation of FDG and other radiopharmaceuticals[3].

3.2. ADVANTAGES OF ^{18}F AS A DIAGNOSTIC RADIONUCLIDE

The are several favourable factors responsible for making ^{18}F as the most popular radionuclide for positron imaging. Some of these are related to the inherent nature of the element fluorine, some are related to the radionuclide ^{18}F and its versatile chemistry, and others are related to the imaging instrumentation. These factors are:

(a) Fluorine is a favourable element for drug development due to its physical properties such as high electronegativity, small van der Waals radius (1.47 Å), and ability to form a strong bond with carbon;

(b) The C-F energy bond is 112 kcal/mol compared to C-H bond energy of 98 kcal/mol. It is more thermally stable and oxidation resistant and hence yields highly stable products. This property is especially useful because the radiopharmaceuticals produced with ^{18}F are highly stable despite the deposition of significant amount of radiation energy due to the decaying ^{18}F;

(c) F-18 decays predominantly by positron emission (97%) and the rest by electron capture (3%), making it one of the best PET imaging radionuclides with high abundance (196%) of 511 keV photons;

(d) F-18 decays by emission of low energy positrons, maximum β^+ energy of 0.635 MeV with a maximum range in water of 2.4 mm enabling high resolution images using coincidence imaging;

(e) The 109.8 min half-life of ^{18}F is near ideal for clinical studies: 5–10 mCi (175–370 MBq) of ^{18}F activity can be injected to patients while keeping the radiation dose to the patients within acceptable limits;

(f) The 109.8 min half-life permits the distribution of the radiopharmaceuticals to several nuclear medicine centres by flight and road transport;

(g) The ability to produce several curies/gigabecquerels of ^{18}F in low-medium energy cyclotrons using ^{18}O-enriched water targets ensure commercial viability of this radionuclide;

(h) More than 1 000 cyclotrons are available worldwide, which provide warranty for isotope availability;

(i) The development of nucleophilic synthesis route has enabled the production of high specific activity ^{18}F radiopharmaceuticals, especially FDG;

(j) Automated synthesis modules (ASU) have been developed which have the ability to handle large quantities of radioactivity and capable of remote synthesis with high synthetic yields;

(k) The utility of FDG for imaging a variety of cancers in addition to the study of cardiac and brain diseases have made it a highly sought after radiopharmaceutical giving it the status of the ‘molecule of the previous millennium’;

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[3] The IAEA database has the most current ongoing list of cyclotrons worldwide, which can be accessed at https://nucleus.iaea.org/sites/accelerators/Pages/Cyclotron.aspx

(l) The development of PET by coincidence imaging technique to detect the two photons emitted due the annihilation of the positron allows high resolution imaging;

(m) The development of fusion imaging such as PET-CT and PET-MR give both anatomic and functional imaging in a single imaging study;

(n) The aA large number of ^{18}F radiopharmaceuticals other than FDG, can be synthesized and are useful for imaging different types of cancers, thus or other pathologies enlarging the scope of PET-CT imaging [3].

3.3. PRODUCTION OF ^{18}F

3.3.1. Reactor production of ^{18}F

F-18 was first prepared in a nuclear reactor using lithium carbonate as target [4]. Two concurrent nuclear reactions take place, leading to the production of ^{18}F. The first nuclear reaction, $^{6}Li(n,\alpha)^{3}H$ releases a tritium particle (^{3}H) which reacts with ^{16}O to form ^{18}F via the nuclear reaction $^{16}O(t,n)^{18}F$. Despite the nuclear reaction, $^{6}Li(n,\alpha)^{3}H$ having very high neutron cross section (~936 barns), the amount of the final product (^{18}F) formed is low because of the two successive nuclear reactions. Millicurie/megabecquerel quantities of ^{18}F have been produced by this method. This mode of production is now abandoned.

3.3.2. Cyclotron production of ^{18}F

Several nuclear reactions are reported for the production of ^{18}F using cyclotrons (Table 1) [6–15]. However, the most common nuclear reaction used for the production of ^{18}F is $^{18}O(p,n)^{18}F$ which also has the highest cross section among all the nuclear reactions [16, 17]. Initially gas targets containing enriched $^{18}O_2$ was used for the preparation of fluorine $[^{18}F]F_2$-gas [18, 19]. FDG was first synthesized using the electrophilic route using $[^{18}F]F_2$-gas produced in the cyclotron [5]. Fluorine-18 was also produced by irradiation of neon gas with deuterons [8, 9]. However, both these methods of production are not very popular at present.

TABLE 1. NUCLEAR REACTIONS USED FOR THE PRODUCTION OF ^{18}F
(courtesy of M.R.A. Pillai, Molecular Group of Companies)

Nuclear Reaction	Target	Energy Range (MeV)	%Natural Abundance
$^{18}O(p,n)^{18}F$	$H_2{}^{18}O$	18-4	0.2
$^{18}O(p,n)^{18}F$	$^{18}O_2$	18-4	0.2
$^{20}Ne(d,\alpha)^{18}F$	Ne, pure Ne+0.1% F_2	15-0	90.5
$^{16}O(^{3}He,p)^{18}F$	H_2O	15-1	99.7
$^{16}O(^{3}He,n)^{18}Ne$: ^{18}F	H_2O	40-15	99.7
$^{16}O(\alpha,np)^{18}F$	H_2O	40-20	99.7
$^{16}O(\alpha,2n)^{18}Ne$: ^{18}F	H_2O	52-10	99.7
$^{20}Ne(^{3}He,\alpha p)^{18}F$	Ne	40-10	90.5

The major part of ^{18}F used in the synthesis of radiopharmaceuticals is prepared by irradiating ^{18}O-water targets. This route of production yields 'no carrier added (nca)' fluoride ($[^{18}F]F^{-}$) in water which is used in nucleophilic synthesis routes. This nucleophilic substitution route is the preferred method as radiopharmaceuticals produced will be of high molar activity.

3.3.3. Production of ^{18}F by (d,α) reaction using Neon gas targets

F-18 radionuclide was first reported when it was produced using the ^{20}Ne(d,α)^{18}F nuclear reaction was developed at the Radiation laboratory of University of California and published in 1937 [20].

This route of production of [^{18}F]F_2 obtained from this nuclear reaction was the starting material for [^{18}F]FDOPA electrophilic production for more than 25 years until the optimized nucleophilic synthesis became available and largely replaced it. This method achieves [^{18}F]F_2 production from Neon gas (with natural abundance in ^{20}Ne) with a low amount of added [^{19}F]F_2 gas (0.1–0.3%). Product recovery is low around 50–70%, due to the high fluorine gas reactivity. To increase recovery, target passivation including heating is needed. The isotope recovery depends on the beam conditions, on the amount of carrier and on duration of irradiation. Fluorine gas is highly toxic and very aggressive and hence can affect the target and the transfer lines.

The ^{20}Ne(d,α)^{18}F nuclear reaction excitation functions and saturation yield curves for this reaction are reported in Fig. 10 [13]. The targetry used for production is made of aluminium and has a cylindrical form. Aluminium is easily machined, has high thermal conductivity and is easy to passivate.

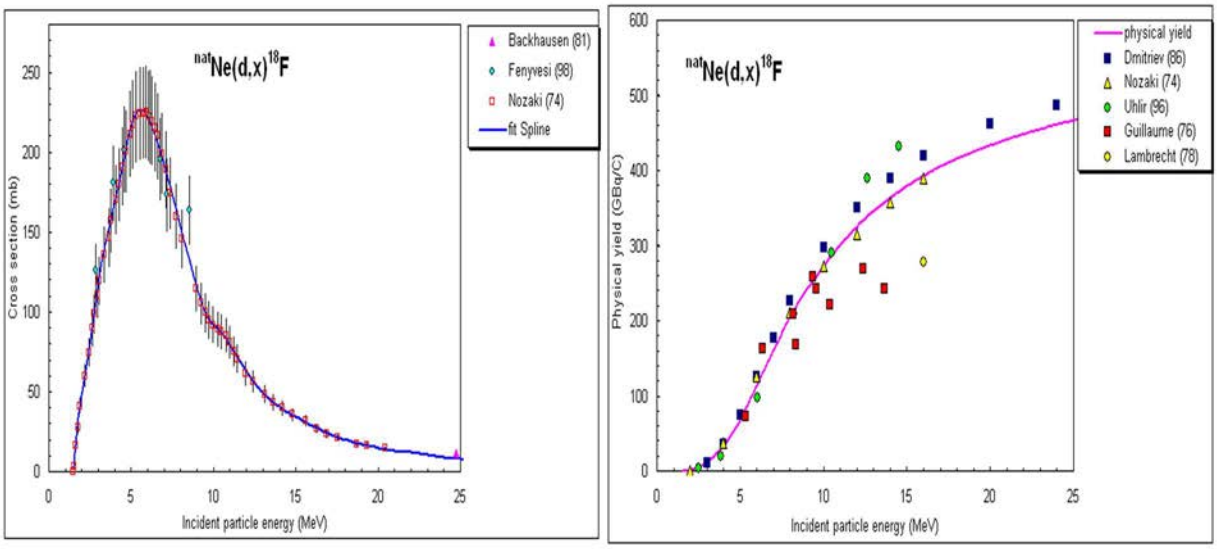


FIG. 10. Cross section and yield curves for the ^{20}Ne(d,α)^{18}F reaction.

Other advantages in the use of aluminium targetry is the significant reduction in the co-production of undesirable long-lived radionuclides. The passivation process is an important step to maximize product recovery and needs to be done repeatedly, in order to maintain high target performance. Target window foils used are made of Havar® and coating techniques were developed to increase the performance and thus reduce degradation.

There are several drawbacks in the production of [^{18}F]F_2 using the ^{20}Ne(d,α)^{18}F nuclear reaction. The first and foremost is the requirement of a deuteron beam in the cyclotron, which needs additional investment. The deuteron energy obtained from a cyclotron will be just half of the proton energy due to the increased mass of deuteron. Thus, an 18 MeV proton cyclotron will only give 9 MeV deuterons. The cross section of the nuclear reaction peaks at 6 MeV; however, the cumulative yield of produced ^{18}F increases continually up to 25 MeV (Fig. 10). The cyclotrons used for PET radionuclide production have a maximum proton beam energy of

20 MeV. Hence, the maximum deuteron energy is reduced to 10 MeV and, after degradation of the beam energy in the Havar® foil, it could be only 8–9 MeV. The yield of production at this energy level is only 50% of the saturation yield. This is far less compared to the yields obtained with $^{18}O(p,n)^{18}F$ nuclear reaction used in the $[^{18}F]F^-$ aqueous target. The second and equally important drawback is that the $[^{19}F]F_2$ produced is carrier added. The radiopharmaceuticals produced are of low molar activity and hence will be inferior for clinical use. Because of the above reasons, this production pathway is not commonly used, and its application is now limited to research purposes. However, it is important to mention that radiolabelling chemistry with $[^{18}F]F_2$ gas is less cumbersome as compared to the nucleophilic reaction with $[^{18}F]$fluoride ions.

TABLE 2. IRRADIATION PARAMETERS FOR $[^{18}F]F_2$ PRODUCTION
(courtesy of E. Cazzola, Sacro Cuore Hospital).

Target gas composition	Ne + 0.3 % F_2
Beam Energy	8.4 MeV
Filling pressure	10 bar
Target volume	600 cm^3
Irradiation current	35 μA
Irradiation time	60 min
Activity EOB	10.2 GBq

3.3.4. Production ^{18}F by (p,n) reaction using water targets

Enriched ^{18}O-water ($H_2[^{18}O]O$) in the form of a liquid target is used to produce ^{18}F, which undergoes the nuclear reaction $^{18}O(p,n)^{18}F$. The cross section of this nuclear reaction up to 20 MeV proton energy is given in Fig. 11 [6, 15]. The threshold energy for this nuclear reaction is 2.3 MeV, where the nuclear reaction starts and peaks at 6 MeV protons. However, there is significant cross section up to 18 MeV. The broad cross section for this reaction permits the production of large quantities of ^{18}F using medium energy cyclotrons having energy up to 20 MeV. A major advantage of this route of production is that there are no competing nuclear reactions yielding radionuclide impurities while using higher energy protons.

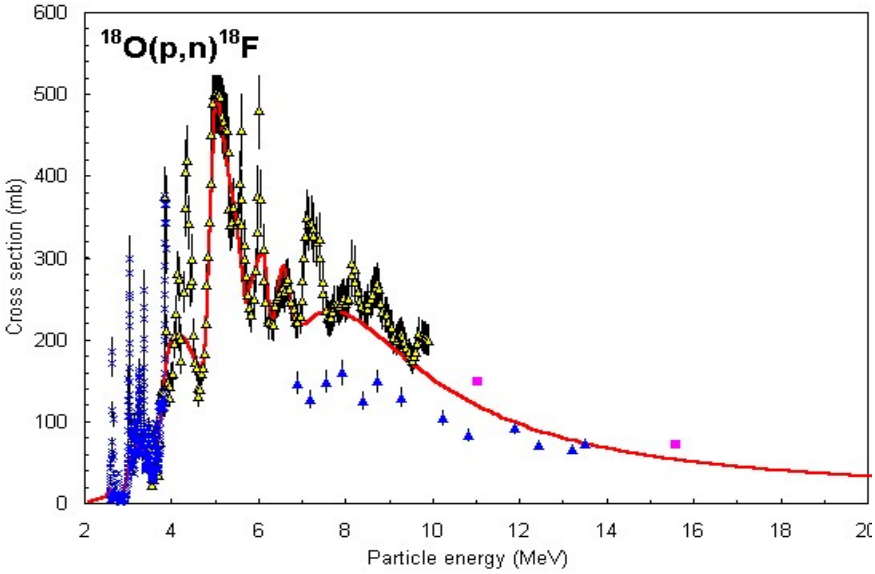


FIG. 11. Cross section for the $^{18}O(p,n)^{18}F$ reaction (reproduced from Ref. [21]).

Figure 12 gives the cumulative yield while using proton energy from 20–4 MeV [13]. Though the production of ^{18}F can be done at low proton energies, the yield increases as the proton energy increases. The theoretical yield is almost double using a 20 MeV proton beam compared to a 10 MeV proton beam. Commercial manufacturers of PET radiopharmaceuticals prefer to use cyclotrons having beam energy of 16.5–20 MeV, as it allows the production of very large quantities of ^{18}F for distribution of radiopharmaceuticals to several nuclear medicine centres.

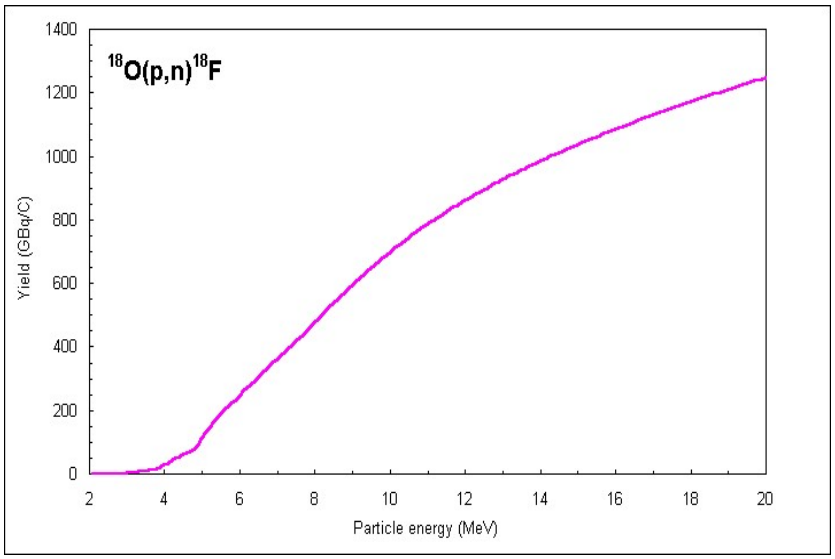


FIG. 12. The yield curve for the $^{18}O(p,n)^{18}F$ reaction from 2-20 MeV (reproduced from Ref. [21]).

Other than the proton energy, another important criterion is the beam current that enhances the production yields. Commercial cyclotrons up to 300 µA beam current are now available in the market for routine production of ^{18}F. Table 3 lists the cyclotrons in the market and the typical yield of production of ^{18}F.

TABLE 3. TYPICAL YIELDS OF ^{18}F PRODUCED WITH DIFFERENT COMMERCIAL CYCLOTRONS
(Courtesy of M.R.A Pillai, Molecular Cyclotrons)

Cyclotron type	Beam Energy MeV		Yield of ^{18}F (GBq/Ci)
GE Pet trace 800 series	16.5	60 mA	240.5/6.5
		100 mA	407/11
		130 mA	524.4/14.2
		160 mA	648.5/17.5
IBA Kiube	18	100 mA	370/10
		150 mA	592/16
		180 mA	740/20
		300 mA	1110/30
Advanced Cyclotron	19	300 mA	1110/30

3.4. TARGET

Isotopically enriched water ($H_2[^{18}O]O$) is used as target to produce [^{18}F]fluoride. The enrichment of $H_2[^{18}O]O$ water is an important factor. O-16 present in the target water will also undergo a nuclear reaction, $^{16}O(p,\alpha)^{13}N$ producing ^{13}N which is also a short lived ($T_{1/2}$ 10 min) PET radionuclide. O-18 water with enrichment >98% is recommended for use.

The reuse of ^{18}O-enriched water after purification is reported. However, one needs to be extremely careful as reused water could interfere in the production process and reduce the production yields of radiopharmaceuticals considerably. This is thought to be due to the chemical impurities, both organic and inorganic, present in the recovered water.

The targetry, where the nuclear reaction takes place, is a metal container holding the enriched water, isolated from the vacuum through a window foil. Schematic representation of a targetry is given in Fig. 13. Targets are the least mature parts by companies, and they are under continuous development. To comply with the increasing request of ^{18}F anions, the targets were

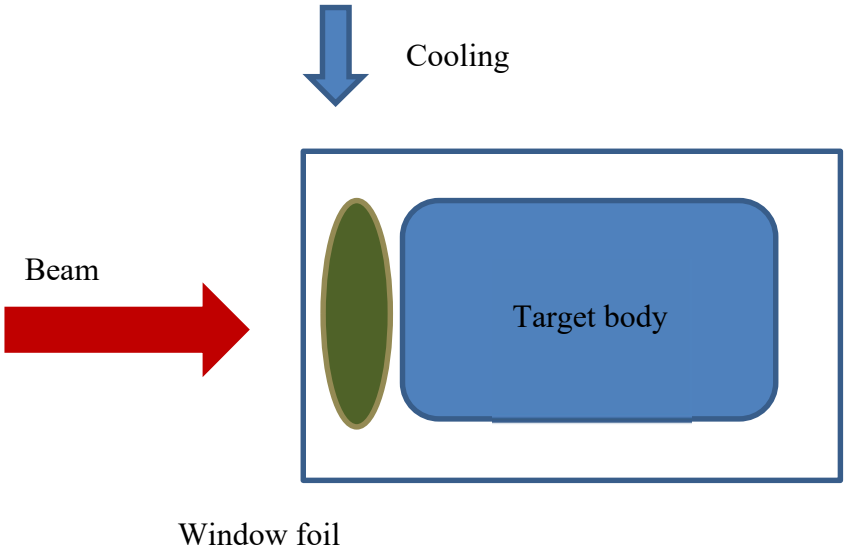


FIG. 13. Simplified target drawing (courtesy of E. Cazzola, Sacro Cuore Hospital).

modified in terms of materials and design. These aspects enhance productivity, reduce the release of impurity and increase the maintenance interval without compromising operator's safety.

Enriched $H_2[^{18}O]O$ target is irradiated in a suitably designed metallic targetry. The use of enriched $H_2[^{18}O]O$ water for irradiation is the major cost component in the production of ^{18}F radiopharmaceuticals. Though the cost of enriched water has been coming down over the past two decades it is desirable to use the minimum required amount of target for each irradiation.

Metallic targets are designed in such a way that the smallest possible volume of enriched water can be used and at the same time producing the highest amount of ^{18}F. Though initial target volumes used to be as low as 0.5 mL, most of the commercial sites now prefer to use higher volume targets (2.5–4 ml) to maximise the production yields.

A number of factors are considered while designing a targetry for the production of $[^{18}F]$fluoride ion which are listed below:

(a) The target volume should be as small as possible, however, with the highest possible yields. Decreasing the target volume while maintaining the $[^{18}F]$fluoride production quantity will reduce the material cost per run and use of $H_2[^{18}O]O$. Due to the increasing demand for radiopharmaceuticals, the quantity of $[^{18}F]$fluoride needed is also increasing. Hence, target volumes up to 4 ml are used by different cyclotron manufacturers;

(b) The target should withstand prolonged bombardment as most cyclotron centres use bombardment time of 2 to 3 hours. Irradiation beyond three hours has no meaning because of saturation of the yields;

(c) The target cooling should be efficient in order to prevent boiloff or cavitation of the target water. A highly focused beam or inefficient target cooling can lead to complete target voiding, drastically reducing the yield;

(d) Radiolysis in target water should be minimal during irradiation. Intense irradiation on small volume targets increases the likelihood of radiolytic loss of target water;

(e) The target body used should be inert, so that it does not react with the $[^{18}F]$fluoride ions and not reduce the reactivity of the fluoride ion. Reactivity can be lost because of several reasons.

All the above factors are interconnected and hence selection of the right metal for the preparation of the target and efficient target design will rely on optimal combination of all of these elements. A target has several components. These include the metallic target body, the foil to seal the target through which the beam enters, the grid holding the foil and the target plug. The components used for the preparation of the target can have significant effects on:

(a) The temperatures inside the target during irradiation;

(b) The chemical form of the formed radionuclide;

(c) The radionuclide impurities that will be formed during irradiation;

(d) Maintenance frequency of the target components.

The design of a target varies from cyclotron to cyclotron. Figure 14 shows a water target used in one of the commercial cyclotrons (Siemens HP Cyclotron): assembled water targetry (top

row); schematic diagram of the target components (middle); and targetry components (bottom) i. Metallic plug, ii. Tantalum target body, iii. Havar foil and iv. Copper grid (middle row). This target can be attached to a target carousal which rotates as needed to allow the impinging of the protons after passing through the grid and the foil in the ^{18}O-enriched water (Fig. 15). The cooling water also circulates around the carousal and removes the heat by conduction.

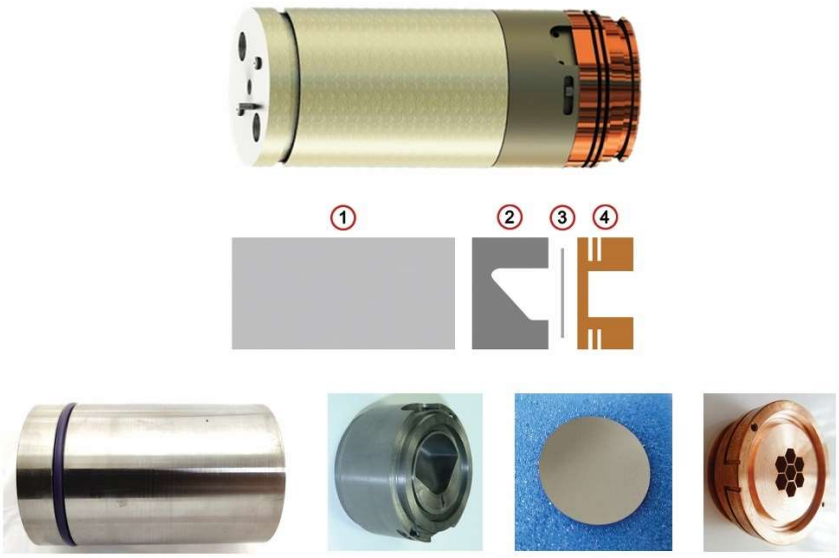


FIG. 14. Cyclotron water target (courtesy of K. Dilshad, Molecular Cyclotrons).

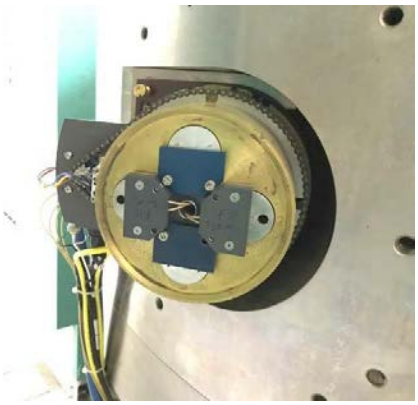


FIG. 15. A target carousel having two targets and two Faraday cups used in a Siemens HP cyclotron (courtesy of M. Anees, Molecular Cyclotrons).

3.4.1. Target plug

The enriched water lines are connected to the cavity inside the metallic target through the target plug. Similarly, the piping carrying the argon or helium gas used for pressurizing the ^{18}O water as well as used to push the irradiated water to the hot cell enters through the metallic body.

3.4.2. Target body

The target body contains the cavity to hold the ^{18}O-enriched water. The target body is made of metals. The desirable properties of the target body are:

(a) Ability to withstand high temperature formed during irradiation;
(b) Chemically inert under gas plasma conditions;
(c) High thermal conductivity to enable heat transfer;
(d) It should not get radioactive either by reaction with the protons or with the secondary neutrons produced during irradiation.

The size of the cavity determines the volume of water that can be filled. The cavity is machined inside the target to contain the ^{18}O-enriched water. Different designs of cavity are possible and investigated by cyclotron manufacturers. An extra volume than required to contain the liquid is built in the metallic target body. Additional volume is introduced to be filled with inert gas for building pressure inside the water target.

Several metals such as silver, aluminium, nickel, titanium, niobium and tantalum are used for the preparation of the target body. Niobium and tantalum targets are finding increasing usage these days as most cyclotron manufacturers prefer targets made of these metals.

Silver was one of the preferred metals for the construction of water targets in the first generation of cyclotrons. Silver has the highest thermal and electrical conductivity of all metals. It can be easily machined. It does not react with air, water or many acids under normal conditions. However, silver gets tarnished with exposure to active oxidizing species such as ozone. This reaction can be seen in water targets after high current irradiations and hence results in impurities and requires frequent rinsing/cleaning or replacement of the target parts. When the beam current increases, the silver body target is affected by degradation and silver particles can be released. Figure 16 shows the accumulation of silver in used water recovery vial and QMA cartridge.

FIG. 16. Silver release problem in silver body target. Recovered $H_2[^{18}O]O$ (left), QMA Cartridge with silver ion contamination (right) (courtesy of E. Cazzola, Sacro Cuore Hospital).

Niobium metal targets are frequently in use these days. Niobium metal is very inert at room temperature. Niobium has a very low activation cross section with both protons and neutrons.

However, niobium has poor chemical resistance at elevated temperatures and can easily be attacked by oxygen, halogens and even carbon. Despite the above problems, niobium is still useful for making water targets for ^{18}F production.

Tantalum is also widely used for making water targetry for the production of ^{18}F. Tantalum is one of the refractory metals that offers properties compatible for the preparation of targets. It can be handled easily at room temperature. Tantalum is resistant to most of the common acids. Tantalum's corrosion resistance is unusually good in most commercial combinations of acids. The comparative properties of the different metals used for targetry preparation is given in Table 4.

TABLE 4. THE PROPERTIES OF DIFFERENT METALS USED FOR TARGETRY PREPARATION *(courtesy of E. Cazzola, Sacro Cuore Hospital).*

Material	Easy to machine	Chemical inertness	Thermal conductivity ($W.m^{-}1.°C^{-}1$)
Silver	Good	Good	4.27
Titanium	Good	Excellent	0.31
Niobium	Good	Excellent	0.54

Regarding the body target shape, filling volume and target orientation some considerations need to be done: The target chamber will be shaped to host a maximum volume of enriched water, to allow maximal beam interaction, to facilitate target emptying, and to minimize bubble formation. The last issue is critical to control target pressure increase, which is responsible for target overpressure and explosion.

The majority of cyclotrons use a vertical target, where the angle between the beam and water is 90°. This configuration is used on MINItrace and PETtrace cyclotron from GE. It has to be noted that this target shape has some problems in heat dissipation and beam to target material interaction is not optimal. TR19 cyclotron from ACSI performs a 20° angle target to maximize the beam interaction and heat exchange, so to reach 150 μA beam target current as is shown in Fig. 17.

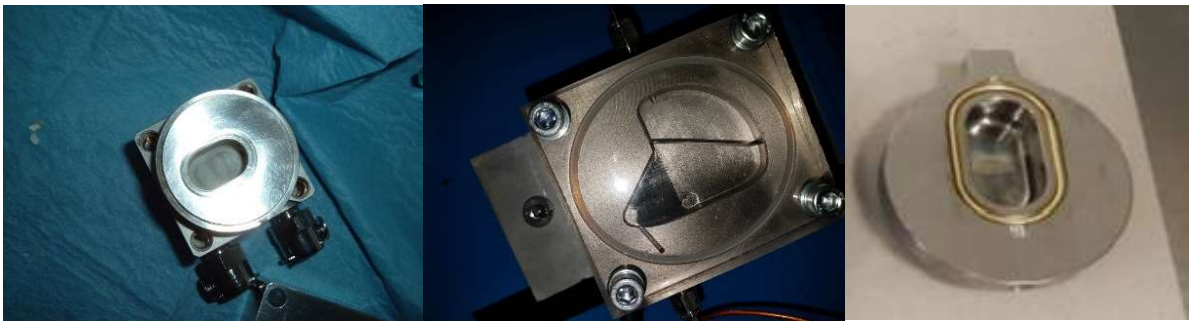


FIG. 17. Vertical target body of MINItrace, angled target body shape from ACSI, PETtrace vertical body target (courtesy of E. Cazzola, Sacro Cuore Hospital).

3.4.3. Target foil

The cavity of the target is closed by using a thin metallic foil that allows the penetration of the beam to the liquid target. Very thin metallic sheets are used for this purpose to reduce the loss of beam energy, especially while using low energy cyclotrons. The target foil material should

maintain strength at elevated temperatures encountered by the target. The most commonly used material to make target foils are Havar, aluminium, niobium and titanium of which the last three are also used as target bodies.

Target foils have a heat load due to the energy deposited by the passing beam. The strength of the foil when it heats up during irradiation is an important consideration while selecting the material for the foil. The heat can be dissipated in different ways, depending on the choice of foil material and thickness.

Titanium is the most resistant metal that can be used for window foil since it has high thermal conductivity. This factor combined with its high melting point makes this metal very interesting for this application. However, its density needs to be considered for beam energy degradation contribution. [^{48}V]Vanadium is produced during the target beam irradiation.

Currently, most manufacturers prefer to use Havar as foil material. Havar is a cobalt based alloy having very high strength at higher temperatures. When heated up to 510°C Havar retains 75% of its strength compared to its strength at room temperature which is an important desirable property. The incoming protons deposit ~1 MeV while passing through the foil and generate significant heat loads. The typical composition of Havar is Co (42%), Cr (19.5%), Ni (12.7%), W (2.7%), Mo (2.2%), Mn (1.6%), C (0.2%) and traces of Fe. Havar has a melting point of 1480 ºC. Generally, Havar foils of ~25 microns are used.

The Havar foil needs to be replaced at periodic intervals. Havar foils get highly activated due to the reaction of the different metals with the secondary neutrons and hence need to be disposed safely when removed during the rebuilding of the target. Table 5 gives the typical radionuclides formed in a Havar foil when irradiated in a 16.5 MeV cyclotron [22].

TABLE 5. LONG LIVED RADIONUCLIDIC IMPURITIES OBSERVED IN HAVAR FOIL AFTER LONG IRRADIATIONS IN A 16.5 MEV GE PET TRACE CYCLOTRON
(Courtesy of M.R.A Pillai, Molecular Cyclotrons)

Radionuclide	Half-life (d)
V-48	16
Cr-51	27.7
Mn-52	5.59
Mn-54	312.3
Co-56	77.3
Co-57	271.8
Co-58	70.8
Tc-95m	61
Tc-96	4.28
Re-183	70
Re-184	38

In order to reduce and eliminate the metal and isotope release, foil coating with Ta, Nb, Zr was investigated, especially for high target beam current. The coating procedures are mainly performed with sputtering technique and gave additional properties to Havar foil, like increased inertness and mechanical resistance.

3.4.4. Grids

During irradiation the pressure inside the water target goes up to 550–600 psi (40–43 bar). As the thickness of the Havar foil used is ~25 microns it could burst because of the high pressure. Metallic grids are used to support the Havar foils so that they can withstand the pressure developed inside the target during irradiation. This is especially important with low energy cyclotron targets used for the preparation ^{18}F where very thin foils are used to enhance production yields.

There are several designs for grids, including circular holes and hexagonal arrays. The grids with hexagonal design have higher transmission as compared with discs with circular holes. The target cooling water that circulates over the carousal removes heat from the metallic grid. The physical and chemical characteristics of the target components including grid are important. The grids are made of copper as it has very high thermal conductivity.

3.4.5. Target cooling

The last parameter in target optimization is the cooling, probably the most important one in terms of target development. During bombardment, high heat power is produced and an efficient system to remove heat is mandatory. The heat hot spots are located on target windows foils, enriched water and, due to the beam focus, on target body. An efficient cooling system needs to be present to prevent foil explosion, to minimize impurity release, to avoid water boiling and target degradation.

The target cooling system was originally divided in two parts, the helium frontal cooling and the water rear cooling. The frontal helium cooling is responsible for removing windows foil heat. This part allows current increase and preserves foil degradation. Water cooling is designed in order to remove the remaining heat production. Such target configuration has two windows target foils, one to isolate the frontal helium cooling from the vacuum chamber and the second one to isolate the He cooling from the enriched water. This means two foils need replacement during target maintenance, double foil break possibility and higher operator exposure during the foil replacement. Target cooling helium leaks are one of the most frequent problem in target operation. This aspect, combined with the decrease in worldwide helium availability, makes this cooling approach not convenient anymore. To simplify target cooling, while reducing operative cost and leak possibility, new targets were designed without frontal helium cooling. To allow this implementation an increased water cooling flow and target distribution system was developed [19].

Another very important aspect is the beam shape control (Fig. 18). To have an accurate representation of the beam target shape, fine tuning of the cyclotron should be allowed, so to maximize beam target current and to minimize preventive maintenance. Different methods to evaluate the beam shape are possible, starting from cheap and easy paper burn implemented on a GE cyclotron, passing to metal burn implemented on ACSI cyclotron up to the more complex and accurate method that allows to have a real time beam shape picture as developed by the Braccini group [23].

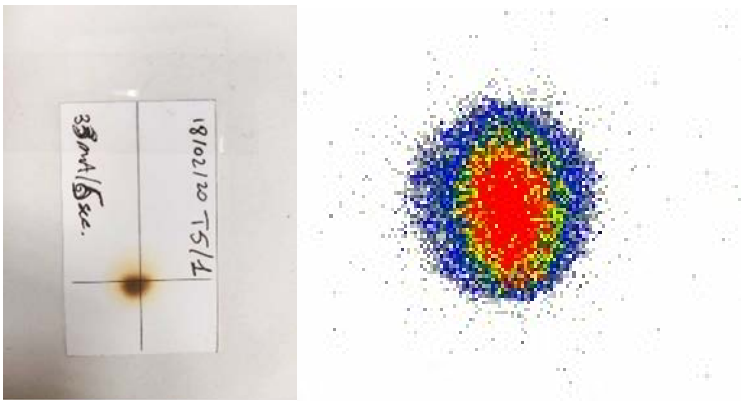


FIG. 18. Paper burn beam shape form PETtrace cyclotron, Metal burn beam shape from TR19 cyclotron (courtesy of E. Cazzola, Sacro Cuore Hospital).

3.4.6. Rebuilding the target

Rebuilding of target is required because radionuclides are built up on the target over time due to activation of the target, the Havar foil and the grid. There could be presence of organic impurities also on the target. These organic and inorganic impurities are removed by cleaning the target with acids and organic solvents. The procedure to rebuild the target varies based on the type of target and cyclotron manufacturer and needs to be followed meticulously on a periodic basis to get consistent yields of ^{18}F. In addition, the periodic rebuilding of the target carousal and replacement of the O-rings is also essential.

3.5. CYCLOTRON IRRADIATION OF $H_2[^{18}O]O$ WATER TARGETS

Figure 19 shows the sequence followed for the production of ^{18}F in a cyclotron using ^{18}O-enriched water target.

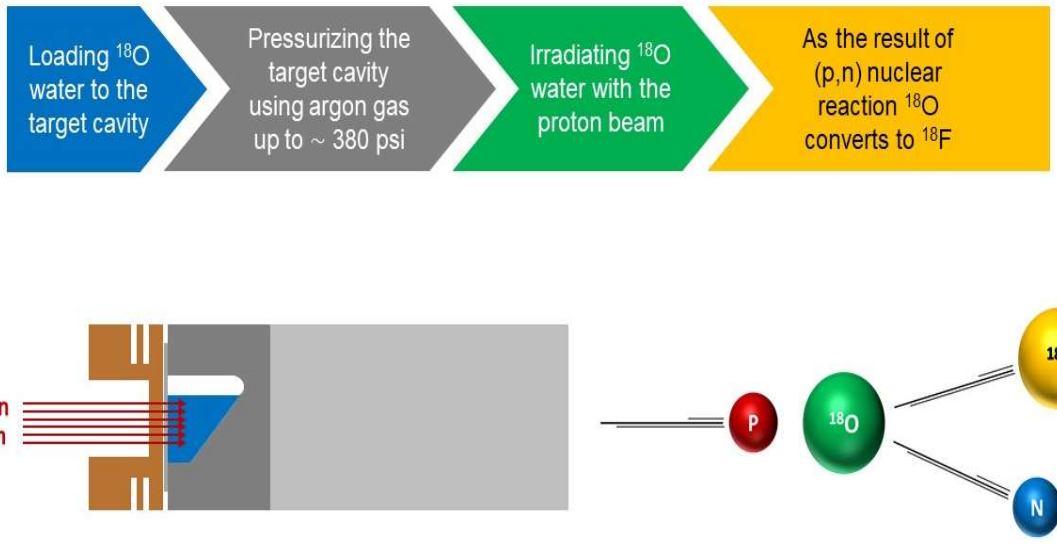


FIG. 19. The sequence for the production of ^{18}F using a ^{18}O-water target (top). Schematic of the targetry and the proton beam (bottom, left). Schematic representation of the nuclear reaction (bottom right). (Courtesy of N. Raviteja, Molecular Cyclotrons,).

3.5.1. Loading of ^{18}O enriched water

The enriched water is usually kept in 50–100 mL vials attached to the cyclotron panel from where it is loaded into the target using a syringe pump. At several centres the targetry is first rinsed by pushing ultra clean water followed by inert gas to dry the target and the line. This process might be repeated. It helps to remove some of the micro-contaminations present on the target as well as to check the delivery lines.

Enriched water is then pushed to the target using the same syringe pump and using argon or helium as the push gas. Every targetry has a fixed volume of enriched water to be loaded depending upon its design. After loading the water target a high push is done to push Argon to the remaining volume of the target. The pressure inside the target is kept between 300–350 psi (21–25 bar) during this step. Figure 20 shows the ^{18}O-enriched water loading station in a Siemens HP Cyclotron.

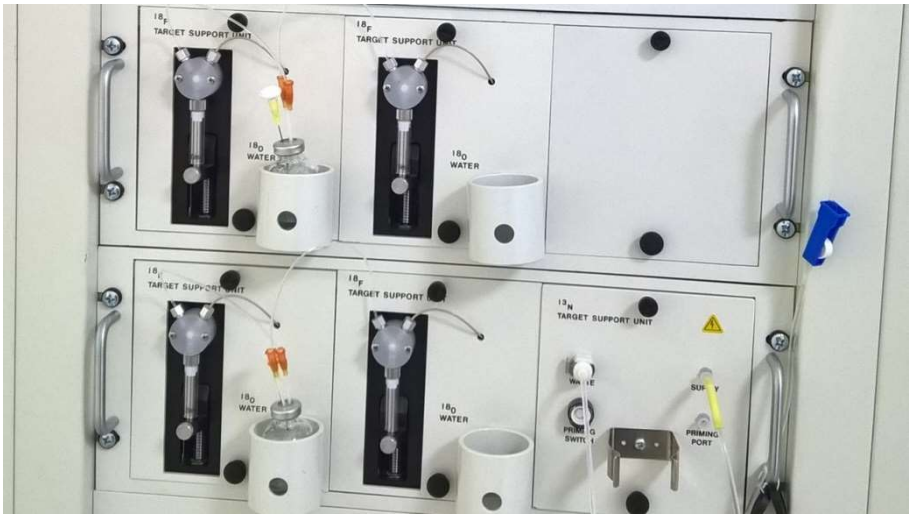


FIG. 20. ^{18}O-enriched water loading station (courtesy of M. Anees, Molecular Cyclotrons).

3.5.2. Irradiation of ^{18}O-water

There are two parameters to be varied during irradiation: the beam current and time of irradiation. Both these parameters are optimized depending on the amount of ^{18}F activity required. When maximum activity is needed, the beam current is taken to the maximum designed values. This varies from cyclotron to cyclotron. Recently manufactured cyclotrons have beam currents up to 300 μA available for ^{18}F production. The maximum irradiation time used is ~180 min as longer irradiation times do not show much benefit. While the above protocol is followed by commercial distributors of [^{18}F]FDG, shorter irradiation times at low beam currents are usually done to obtain the desired activity in single user sites.

3.5.3. Activity transfer

Once the irradiation is completed the beam is shut down and the pressure in the target released using a vent valve. Once the pressure is released the liquid target is pushed through a thin capillary tube of ~1/16th inch diameter to the synthesizer placed inside the hot cell. It is ideal to minimize the distance and angles between the hot cell and the cyclotron. In most of the sites, hot cells will be built in one side of the cyclotron vault. The irradiated water is pushed to the

hot cell using either or helium or another inert gas. These transfer lines are sent through the trenches that are shielded either by using concrete or lead.

It is important to push the irradiated water in a controlled manner such that the pushing gas should minimize fractionation of the irradiated water within the transfer line. Transfer of water through this line often has associated problems with a delay in reaching the activity in the synthesizer or at times no delivery at all. In order to reduce the probability of this happening, it is important to keep the transfer lines clean. This is done by rinsing with pure deionized water from time to time and replacement on a periodic intervals [20].

Different materials for the transfer line are available on the market and they can be used depending on local considerations. Many different parameters need to be evaluated in order to find the appropriate delivery line, some of them are:

(a) Fluorine should not be released from the transfer line that affect the specific activity or molar activity (A_m) if [^{18}F]fluoride is not reduced;
(b) Resistance to radiation to avoid multiple replacements;
(c) Resistance to mechanical stress to facilitate the replacement;
(d) Cost.

Over the years transfer lines of different materials are investigated and details are given below:

(i) Tefzel (Ethylene-tetrafluoroethylene)

This fluorinated polymer is inert and resistant to high pressure up to 3 000 psi (210 bar), is flexible and robust. It is one of the materials that is most frequently used for delivery lines, it is easy to pass through hot cell and cyclotrons, but it can be rigid and fragile due to radiation exposure. It needs to be often replaced but is relatively cheap, easy to manage and the bolus transfer is not fractionated.

(ii) PEEK (polyethylene-ether ketone)

PEEK is a fluorine free polymer, biocompatible, inert, resistant at a temperature up to 100°C without physical modification, robust but difficult to pass between target and hot cell. It is not suitable for hard bends (risk of clogging if bended to hard), it is resistant to high pressure, but there is a risk of a non-uniform internal diameter due to production procedures. This uniformity problem can affect the delivery reproducibility in different lines and can lead to a split in the delivery bolus. It is an expensive material, but it is resistant to radiation degradation.

(iii) PP (Polypropylene)

Fluorine free polymer is less expensive and inert to chemicals. It is resistant to radiation damage and it shows a compact product bolus transfer profile.

3.5.4. Co-production of ^{13}N

While the production of ^{18}F is taking place, there will be a concurrent nuclear reaction leading to the production of ^{13}N ($T_{1/2}$ 10 min). The ^{16}O present in water undergoes a $^{16}O(p,\alpha)^{13}N$ nuclear

reaction producing ^{13}N. This nuclear reaction also has a reasonably high cross section in the 6–20 MeV energy (Fig. 21).

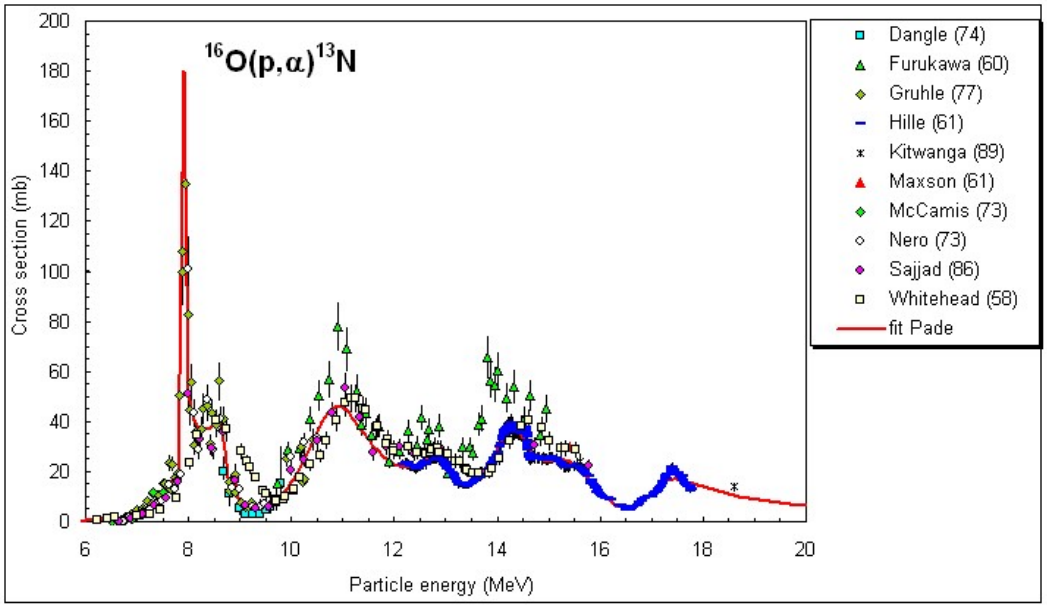


FIG. 21. Excitation function of the nuclear reaction $^{16}O(p,\alpha)^{13}N$ from 6–20 MeV protons (reproduced from Ref. [15]).

The ^{13}N formed will be in multiple chemical forms such as [^{13}N]nitrites, [^{13}N]nitrates and [^{13}N]N_2 gas. The [^{13}N]N_2 gas will mostly escape during the unloading of the water target to the hot cells. However, the negatively charged nitrite and nitrate ions get trapped on the anion exchange resin used to trap [^{18}F]F^-. However, unlike the fluoride anion, nitrite and nitrates have very high affinity for the anion exchange resin. It will not be stripped when the column is eluted with the eluent solution containing potassium carbonate and kryptofix. Millicurie/megabecquerel quantities of ^{13}N are expected to be formed depending upon the enrichment of the water used for irradiation. Even if this is not a major issue during the synthesis of ^{18}F radiopharmaceuticals, it still needs consideration during the preparation of Na[^{18}F]F.

3.5.5. Recovery of enriched water

Enriched water after irradiation is recycled in certain sites [24–26]. However, a thorough purification is essential for removing chemical impurities. Purification can be done by passing through ion exchange columns followed by distillation. Nevertheless, there will be a lowering of enrichment due to the inevitable contamination with natural water. If recycled enriched water is used, its purity and suitability need to be assured. Some suppliers also buy back the used water to be used for purification and further enrichment.

3.6. CONCLUSION

F-18 is currently the major medical radionuclide used in diagnostic nuclear medicine. There are over 1 000 cyclotrons in the world involved in the production of ^{18}F and a rough estimate is that on an average about 10 Ci (370 GBq) of fluorine is made per cyclotron each day. Single user institutions that use cyclotrons make much less, however, the cyclotrons involved in the distribution of radiopharmaceuticals make much higher amounts. This accounts to the

production about three million curie (1.2×10^8 GBq) of radioactivity. The cyclotrons used for this production have beam energy varying from 8–20 MeV and there is a preference to use higher energy (16–20 MeV) cyclotrons. The requirements for beam currents are also increasing with latest machines having proton beam current 300 μA or even more. Almost all these cyclotrons use water targets to make fluoride ($[^{18}F]F^-$) that is used for nucleophilic syntheses. It is expected that the application of ^{18}F will continue to increase in the future.

4. GENERAL ASPECTS OF ^{18}F RADIOCHEMISTRY

4.1. INTRODUCTION

Fluorine is the thirteenth element present in Earth's crust with 0.065% of abundance, however only twelve organic compounds containing fluorine have been found in nature [27]. Despite such tiny numbers of natural occurring molecules, an enormous number of synthetic fluorine compounds have been widely used in medicinal chemistry, because these modified molecules have quite unique proprieties. A clear example is the radiopharmaceutical $[^{18}F]$FDG, a blockbuster that has greatly contributed to raise nuclear medicine and molecular imaging as a good standard as a diagnostic technique.

Modification of molecules with ^{18}F is a common approach used in radiochemistry to synthesize targeted radiopharmaceuticals, because of its nuclear properties, chemical reactivity, and easy radioisotope availability. A huge number of new ^{18}F fluorinated molecules is published every year. Furthermore, different synthetic routes are studied, also for well-established ^{18}F radiopharmaceuticals to achieve shorter synthesis times, increased radiochemical yields (RCY) and simplification of production and QC protocols. Radiopharmaceutical production for human application, the process, reagents and solvents need to comply with all the requirements of national regulatory agencies and need to be validated for this application.

In this section, some common aspects with respect to preparations of $[^{18}F]$fluorinated radiopharmaceuticals are described. Many different approaches have been used to produce ^{18}F fluorinated radiopharmaceuticals. Most of them will be presented in section 5 but new developments on fluorination chemistry are constantly published. This section describes general considerations with respect to ^{18}F radiopharmaceutical synthesis.

$[^{18}F]$Fluorine can be used in three different strategies to prepare fluorinated compounds:

(a) Creation of a C-F bond through reaction of $[^{18}F]F^-$ nucleophilic fluorination;
(b) Creation of a C-F bond through reaction of $[^{18}F]F^+$ electrophilic fluorination;
(c) Creation of a C-C bond starting from a $[^{18}F]$Fluorinated synthon.

All those strategies contain some common elements like precursors and solvents that are discussed in the following sections. Furthermore, during the production of radiopharmaceuticals Automated Synthesis Units (ASUs) are used. Several approaches to automate production of radiopharmaceuticals are presented in this section, followed by general considerations and recommendations regarding QC of these produced radiopharmaceuticals.

4.1.1. Precursor

The definition of a precursor is “chemical precursor from radiochemical preparation is not radioactive substance obtained by chemical synthesis for combination with a radionuclide” as reported in a dedicated monograph in the EU Pharmacopoeia [28]. Some considerations are important in the selection of a chemical precursor.

— Solubility under reaction conditions;
— Appropriate leaving and protecting groups;
— Thermal stability.

Those are some properties that need to be considered to evaluate the precursor and its application in synthesis. Leaving and protecting groups properties need to be taken into account, to enable fast and easy [^{18}F]fluorination to obtain the desired molecule.

Depending on the application some other considerations need to be taken into account in order to decide for the appropriate radiopharmaceutical production process.

The precursor to be used either in R&D or clinical application is to be produced under different requirements and the specifications can be different. For R&D use, an appropriated purity level with a good chemical characterization is required for precursor.

Human applications need more strict requirements, like GMP production of pharmaceutical grade and a thorough characterization needs to be present, together with a toxicological study. Those characterizations allow the identification of all possible impurities generated on the precursor production and that can be present in the final product. This assessment needs to be implemented during validation of the QC analysis. Different production pathways can be studied, also for established radiopharmaceuticals, to offer the most efficient process; not only regarding yield, but also in terms of safety of precursors.

4.1.2. Solvent

The use of reaction media can be a limiting step for a radiochemical reaction; therefore, two different aspects need to be evaluated. Nucleophilic properties of [^{18}F]fluoride are increased in polar aprotic thermal stable organic solvent and due to the radiopharmaceutical application, solvent toxicity needs to be evaluated and residual solvent amounts are strictly defined by Regulatory Authorities. Dimethyl sulfoxide (DMSO), acetonitrile, dimethyl formamide (DMF), and tetrahydrofuran (THF) are the most commonly solvents used in radiopharmaceutical preparation for routine medical application (Table 6).

Different synthesis steps are dedicated to solvent management like water evaporation, reagents dissolution solvents content reduction and in the final formulation the preparation of a final solution that meets the residual solvent monograph [28].

Based on this monograph a dedicated QC analysis should be performed to ensure the presence of solvent below a monograph limit (see also section 4.3.2.5)

Each solvent that is used for human preparation is included in such monograph and classified based on its toxicity in three classes. DMSO, class 3 (low toxicity) solvent, is a good solvent

for nucleophilic reaction due to its polarity and the possibility to reach high reaction temperatures, those properties can make it difficult to remove the solvent by evaporation and DMSO has also slightly oxidant properties.

Acetonitrile, class 2 (medium toxicity) solvent, has several good properties to make it the preferred solvent for routine productions. Because of its relatively low boiling point solvent, acetonitrile can be efficiently removed from the reaction mixture due to the azeotropic mixture with water. Reaction often proceeds at reaction temperatures close to the boiling point of acetonitrile. Substrate solubility is sometimes a problem. The solvent content needs to be carefully checked in the final product due to its toxicity.

In the last two decades new synthetic approaches have been investigated, such as click chemistry and use of protic solvents in nucleophilic fluorinations. In these cases, different considerations need to be done in solvent selection. Nevertheless, it is important to take into account the solvent evaluation and classification as described in the Monograph to obtain authorization for human application.

Tert-Butanol is one of the evaluated protic solvents to increase synthesis efficiency by increasing yield and fast reaction time as reported by Lee et al. [29] in FET synthesis application. This solvent is not classified in the Pharmacopoeia and needs to be toxicologically assessed to justify a limit on its evaluation.

TABLE 6. CHARACTERISTICS OF CHEMICAL SOLVENT USED IN [^{18}F]FLUORINATION *(courtesy of E. Cazzola, Sacro Cuore Hospital).*

Solvent	Boiling point (°C)	EDQ* Classification
Acetonitrile	81.8°C	3
DMSO	189°C	2
THF	66°C	2
t-BuOH	82°C	n.a.

* Estimated Doses of Concern

4.1.3. Fluoride ion extraction

^{18}F is produced as [^{18}F]F_2 gas (electrophilic) or [^{18}F]F^- (nucleophilic) in water solution. [^{18}F]F_2 gas can be used directly to perform an electrophilic reaction, for instance by trapping the gas on freezing solvent ($CDCl_3$) followed by direct substrate reaction or by radiofluorination of intermediates [30].

A different approach should be performed with the [^{18}F]F^- liquid production as considerable efforts need to be taken to convert this radioactive [^{18}F]fluoride into a reactive species in organic solvents for radiochemical synthesis. Metal target impurities need to be removed as well as water, often by SPE purification followed by azeotropic evaporation (acetonitrile/water) in the presence of cationic counterion.

The first step of the [^{18}F]fluorination procedure is the metal target impurity purification (Fig. 22). This procedure is widely performed on solid phase extraction (SPE) applying an anionic exchange method. [^{18}F]F^- is trapped on the anionic exchange cartridge while the metal cationic target impurity passes through. Many different solid phases were tested starting from quaternary 4-aminopyridinium resin [5] and then via AG1X8 to quaternary ammonium

chloride polymer (QMA), which is used in virtually all commercially available kits for radiopharmaceutical preparations. QMA trapping and [^{18}F]fluoride recovery with K_{222}/tetrabutyl ammonium (TBA) carbonate solution were standardized in all the radiopharmaceutical preparations for conventional reaction pathways. However, some different approaches are tested to overcome some problems caused by QMA application such as Chromafix (Ps-HCO_3) fluorine trapping and release with Cs_2CO_3 to be applied in protic solvent reactions.

The eluent solution (Fig. 22) can be different depending on the chosen reaction pathway: for nucleophilic reactions in aprotic solvents, carbonate in water or water/acetonitrile solutions are recommended, while using a protic solvent approach, not standardized conditions are yet identified.

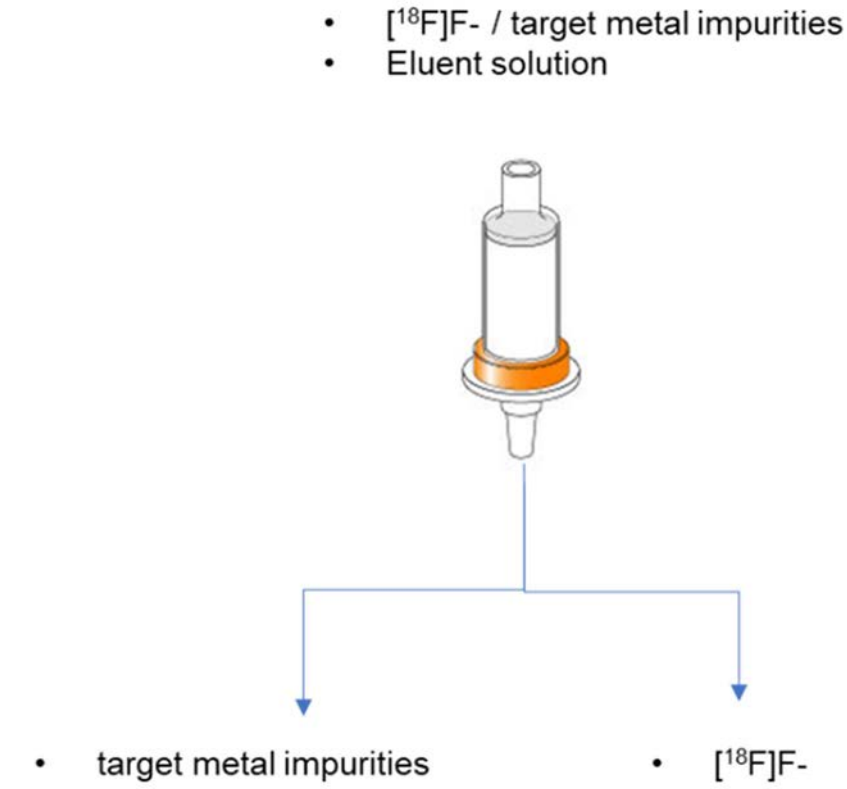


FIG. 22. [^{18}F]fluoride metal target impurity purification and recovery process (courtesy of E. Cazzola, Sacro Cuore Hospital).

After elution the two pathways using either protic or aprotic solvents start to be different. In the protic approach [^{18}F]fluoride is already released in a suitable condition for nucleophilic reaction but using aprotic solvents some additional steps need to be performed: nucleophilic reactions in aprotic solvents require a an evaporation step to ensure that [^{18}F]fluoride is dried preventing solvation by water and to minimize formation of side products. Dissolution in an organic solvent in the presence of a phase transfer catalyst is thereby a crucial step.

To allow an anionic form of fluorine to react in an organic solvent a phase transfer catalyst is required as shown in Fig. 23.

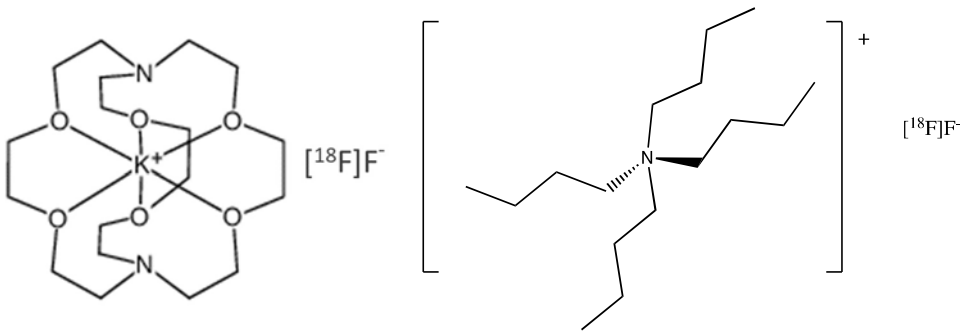


FIG. 23. K_{222} and TBA [^{18}F]fluoride complex structures (courtesy of E. Cazzola, Sacro Cuore Hospital).

Phase transfer catalysts are based on two different classes. Cationic organic compounds like quaternary amines that can form a neutral organic salt soluble in organic solvent with [^{18}F]fluoride. TBA amines are the best option compared to methyl analogs which are more thermal stable, however n-butyl groups are better soluble in acetonitrile.

Crown ethers can be used instead of quaternary ammine to chelate a cation which forms a neutral organic soluble complex with [^{18}F]fluoride. The crown ether K_{222} has a high affinity for the potassium cation and is largely used in radiochemistry. The K_{222}/K^+ complex is positively charged and is able to bind [^{18}F]F$^-$ in neutral organic soluble complex. In this complex the fluoride nucleophilicity is unaffected and can be therefore used as a nucleophilic agent.

TBA and K_{222} are performed in a similar way, where K_{222} seems to be less sensitive to metal target impurities while TBA [5] counterions can negatively affect the basicity of final reaction environment.

Different large and soft metal counterions were tested during the years like Cs, Ru, K. Csand Ru over time ran out of favour and the potassium cation was raised as the favourite counterion for nucleophilic substitution. Caesium became important in nucleophilic [^{18}F]fluorinations in protic solvents [29].

[^{18}F]Fluoride is an efficient nucleophilic agent, but the reactions conditions need to be controlled to avoid or minimize elimination side reactions that proceed under the same reaction conditions like substitution. Nucleophilic substitution and elimination are always in competition and the final basicity of reaction environment are critical. HCO_3^- and CO_3^{2-} are used on eluent solution to control the final pH in labelling step.

4.2. AUTOMATED SYNTHESIS MODULES

4.2.1. Background

Early syntheses of ^{18}F radiopharmaceuticals were conducted manually using small amounts of radioactivity and laboratory set-ups akin to mainstream organic chemistry. For example, the earliest syntheses of [^{18}F]FDG were conducted in specially designed reaction vessels (Fig, 24), generating ~15 mCi or 555 MBq of the radiopharmaceutical [31].

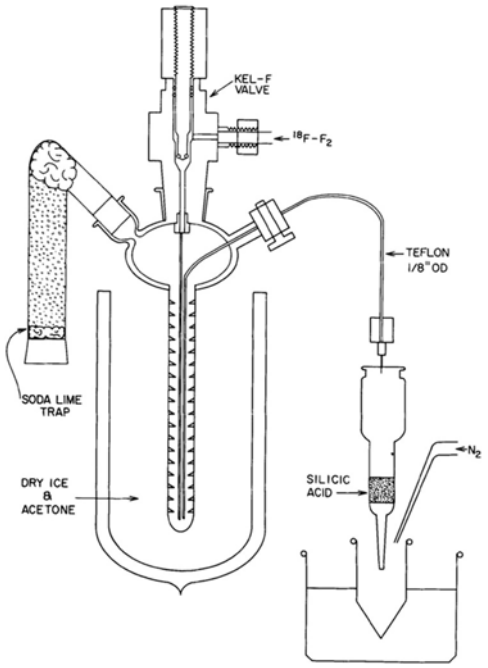


FIG. 24. Early laboratory set-up for the synthesis of [^{18}F]FDG (reproduced from Ref. [31] with permission of [© 1983 John Wiley & Sons, Ltd.]).

Successful clinical imaging with [^{18}F]FDG in the 1970s lead to more widespread use of the agent for neuroscience and ushered in an exciting time for PET. The 1980s saw continued development of [^{18}F]FDG and the discovery that it accumulates in tumours began the development of PET as a major clinical tool in cancer diagnosis that continues today. This, in combination with the introduction of other radiotracers into clinical use, such as [^{18}F]6-fluoro-L-DOPA, [^{18}F]NaF, [^{18}F]fluoroestradiol and [^{18}F]FMISO, created a need to use larger amounts of ^{18}F in radiosyntheses. The radiation safety implications of this meant that early PET radiochemists were already considering how to automate their chemistry.

These early radiopharmaceutical synthesis modules were homemade with hardwired components composed of laboratory equipment connected by valves and transfer lines (Fig. 25 [32]). Synthesis steps such as movement of fluids and temperature changes (heating and cooling) relied on manually controlled electrical switches that were located outside of the lead shielding surrounding the synthesis module, while manipulator arms could be used to move items around and retrieve doses [32]. Early advances in computers and laboratory robotics in the 1980s meant that modules quickly evolved, and control of the different units began to be done using programmable logic controllers, and ultimately computers (Fig. 26) [33].

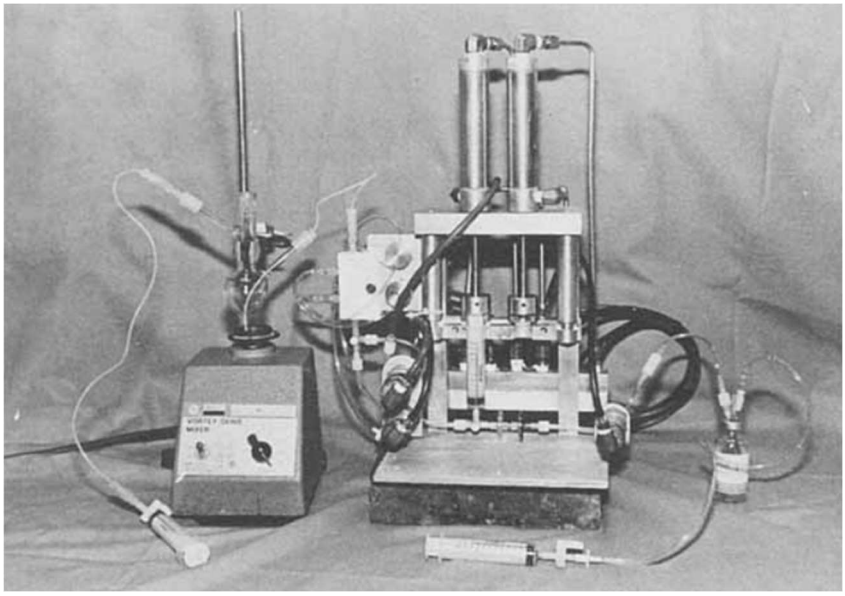


FIG. 25. Early homemade radiosynthesizer (reproduced from Ref. [32] with permission of [© 1983 John Wiley & Sons, Ltd.]).

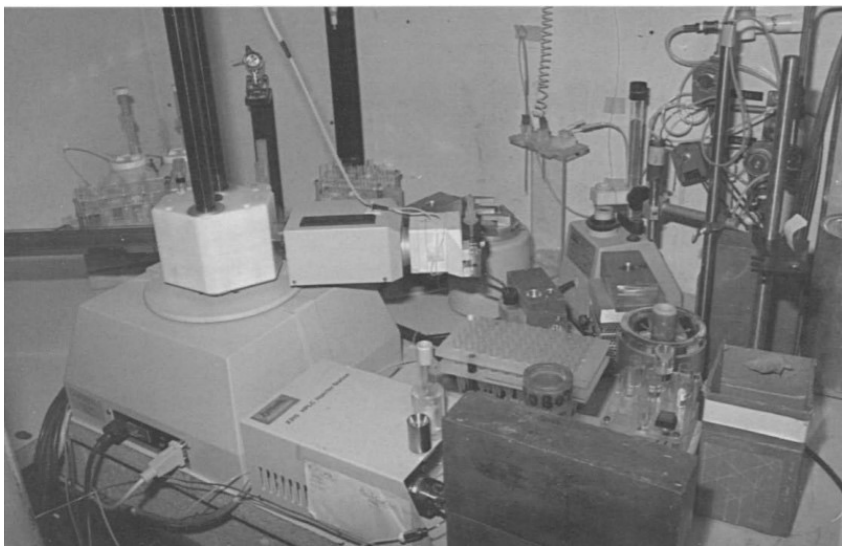


FIG. 26. Early radiochemistry automated by a laboratory robot (reproduced from Ref. [33] with permission of [© 1988 Elsevier Ltd.]).

By the 1990s, demand for [^{18}F]FDG was continuing to increase because of manufacturing and reimbursement approval. At the same time, radiopharmaceutical manufacture was becoming increasingly regulated. To address all of these demands, ASUs were introduced for its production that utilized the latest automation and computing technology [34]. For example, the chemistry process control unit (CPCU, Siemens/CTI systems) and FDG Synthesizer (later rebranded TRACERLab MX_{FDG}) (GE/Coincidence) were introduced (Fig. 27) [35]. Such modules introduced an important new concept, the use of disposable, pre-assembled (and sterilized) synthesis pathways and reagents (termed 'kits') dedicated to individual radiosyntheses.

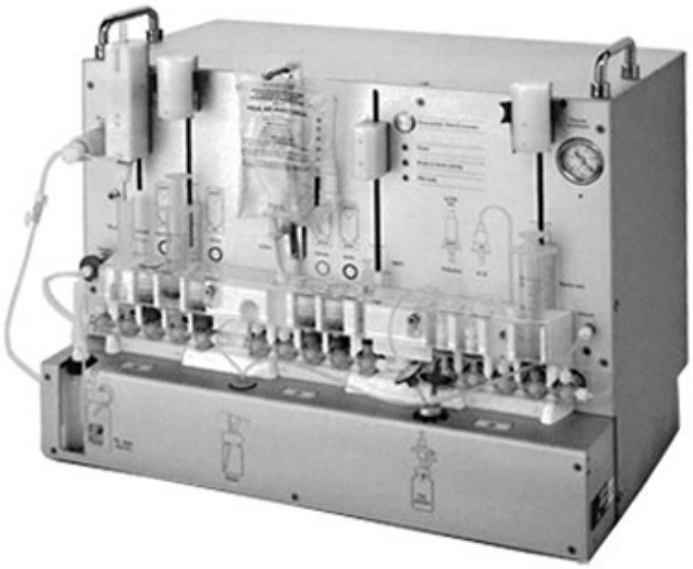


FIG. 27. Early cassette-based synthesis module (reproduced from Ref. [35] with permission).

Radiochemistry synthesis modules have continued to evolve, leading to sophisticated modules that are in routine use today. These new synthesis modules incorporate new features that benefit from modern components and computing power, including:

- Current GPM principles (e.g. access controls, audit trails);
- Incorporation of sensors and detectors that enable pre-run diagnostics, post-run troubleshooting and trend analysis;
- Features enabling real time control;
- Extensive process documentation to meet modern regulatory requirements.

The current 'state of the art' utilizes cassette based systems for routine syntheses, fixed tube systems for research syntheses, hybrid systems that can be useful for labs with limited hot cell space and, increasingly, microfluidic synthesizers. These subclasses of ASUs are described in the following sections.

4.2.2. Modern cassette based synthesis modules

Modern cassette based synthesis modules are the mainstay of ^{18}F radiopharmaceutical manufacture. These systems have evolved from the early cassette based systems described above and have been designed and built to meet the current demands for radiopharmaceutical production. PET imaging is evolving from a research technique to a powerful standard of care. Reflecting this, demand for established radiopharmaceuticals like [^{18}F]FDG continues to increase (millions of [^{18}F]FDG PET scans now occur worldwide on an annual basis), while new ^{18}F labelled radiopharmaceuticals continue to garner regulatory approval (e.g. [^{18}F]6-fluoro-L-DOPA, [^{18}F]fluoroestradiol, [^{18}F]flutemetamol, [^{18}F]florbetapir, [^{18}F]florbetaben).

The result of these developments is a more complex manufacturing paradigm for radiopharmaceutical producers. On the one hand, larger batches of established radiopharmaceuticals need to be prepared, necessitating larger amounts of starting ^{18}F. On the other hand, new radiopharmaceuticals also need to be incorporated into daily manufacturing schedules. Finally, the growing use of PET imaging as a standard of care necessitates that all

of these radiopharmaceuticals are manufactured in compliance with stringent current GMP regulations.

The synthesis modules in use today have been designed to solve these challenges. For example, to address the need for access to high activity batches, systems like the Siemens Explora, IBA Synthera, Eckert&Ziegler Pharmtracer and GE FASTLab (Fig. 28), and even earlier modules like the TRACERLAab MX_{FDG}, are compatible with very high levels of [^{18}F]fluoride. Many commercial radiopharmaceutical manufacturers routinely use such systems to prepare high activity batches, and a recent report from Eberl et all. [36] describes routine production of >500 GBq (>13 Ci) of [^{18}F]FDG. The new modules are also designed to be able to synthesize multiple batches throughout the day from a single cassette (FASTLab 2 can produce two batches of [^{18}F]FDG, while the Siemens Explora can produce four).

FIG. 28. Current cassette based of a radiopharmaceutical synthesis unit (courtesy of GE).

To address the challenge of integrating multiple different products into busy production schedules, the new modules are more compact such that multiple units can be placed in a single hot cell, and kits to produce different radiopharmaceuticals are increasingly commercially available. Although beyond the scope of this work, further flexibility is offered through cross-compatibility of single synthesis modules with different radionuclides such as ^{68}Ga [35]. The cassettes (Fig. 29) for these systems are preloaded with chemicals as well as all of the components required for synthesis (reactor) and purification (sep-paks). Cassettes are assembled in clean rooms, sterilized with gamma radiation and vacuum packed. The cassette is then simply attached to the synthesis module by the radiochemist, and a barcode on the cassette is scanned, communicating to the synthesis unit which synthesis program should be initiated for the installed cassette. Employing single use cassettes ensures straightforward switching between syntheses, reducing errors associated with reconfiguring modules. Sterile cassettes with no common components also eliminate the need for cleaning validation, and simplifies aseptic manufacturing [37].

FIG. 29. A sample of a radiopharmaceutical cassette (courtesy of GE).

4.2.3. Modern fixed tube synthesis modules

For routine production of established radiopharmaceuticals like [^{18}F]FDG, cassette based radiochemistry synthesis modules are the industry standard. In such instances, the commercial need has driven development and commercialization of the appropriate cassette. However, other synthesis strategies are needed for the production of experimental radiopharmaceuticals for which no commercial cassette exists. One approach is the use of customizable cassettes that can be designed and built for the preparation of non-commercial products, and this approach is increasingly common for ^{18}F labelled radiopharmaceuticals that can be prepared using standard ^{18}F radiochemistry. Contrastingly, since cassette based synthesis modules are somewhat limited in how much they can be reconfigured within the confines of the cassette, production of radiopharmaceuticals that involves more complex radiofluorination (e.g. new radiochemistry, multi-reactor protocols, gas-phase chemistry, high temperature and/or pressure requirements) tends to be accomplished using fully-automated fixed tube synthesis modules (Fig. 30).

FIG. 30. Fixed tube radiopharmaceutical synthesis units (left image courtesy of GE, right image courtesy of Synthra).

Fully automated fixed tube synthesis modules [38–40] have replaced the early homemade remote systems developed in the 1980s and described above [31, 32]. Fixed tube synthesis units contain a variety of modular components for conducting ^{18}F radiochemistry:

(a) A module for receiving and processing ^{18}F from a cyclotron (usually utilizing trap and release from a QMA sep-pak cartridge);

(b) A synthesis component which typically contains one or more reaction vessels, a heater and a vacuum pump. This unit is used for azeotropic drying of ^{18}F by evaporating solvents with heat and vacuum as well as the subsequent labelling reaction;
(c) A semi-preparative high performance liquid chromatography (HPLC) purification system;
(d) A module for reformulation of a purified radiopharmaceutical into an isotonic solution; and
(e) A means to pass the final formulated radiopharmaceutical through a sterilizing filter to provide a sterile formulated product suitable for intravenous injection.

Like the cassette based systems, these fixed tube modules are housed in hot cells or mini cells and remote controlled by a laptop or tablet computer. The computer runs a short program, known as a timelist that controls the valves, heaters, vacuum pumps and HPLC system to automate each step of the radiosynthesis described above. After a synthesis is complete, common components (e.g. lines, valves, vials) necessitate cleaning and disinfecting fixed tube modules before the next radiosynthesis. This is usually accomplished by running dedicated cleaning (sterile water), disinfecting (70% ethanol), and drying (acetone) sequences.

The basic module configuration is typically designed for production of ^{18}F labelled radiopharmaceuticals using standard nucleophilic fluorination and requiring HPLC purification (e.g. [^{18}F]FLT). However, the modules can easily be modified to incorporate a reformulation step when using non-injectable HPLC mobile phases (e.g. [^{18}F]FES), or switched for purification by sep-pak cartridge (e.g. [^{18}F]NaF). These modules are also compatible with gas phase radiochemistry, which is relatively rare but can be used to label [^{18}F]fluorocholine or conduct radio click chemistry. For example, in 2011the synthesis of seven different ^{18}F labelled radiotracers using multiple labelling strategies with a single TRACERLab FX_{FN} synthesis was successfully accomplished [38].

4.2.4. Hybrid synthesis modules

The need for flexible automation that can also accommodate production of routine radiotracers like [^{18}F]FDG as well as experimental products for preclinical and clinical research studies led to the introduction of new hybrid synthesis modules. The Sofie Biosciences ELIXYS FLEX/CHEM is a hybrid synthesis module that includes cassette features as well as a three reactor design (Fig. 31) [41]. Thus, it is able to be used for routine production as well as synthesis of research products that require complex multi pot ^{18}F radiochemistry. The cassettes for this module include the fluidic paths and various reagent vials, enabling a large number of probes to be produced within the same module. For example, Collins et all. [42] recently described use of the ELIXYS FLEX/CHEM to produce 24 different ^{18}F labelled radiopharmaceuticals and prosthetic groups (PG), demonstrating utility of a hybrid system to access diverse products. The ELIXYS FLEX/CHEM allows for automation up to the final purification step. The crude material then needs to be transferred to a separate ELIXYS PURE/FORM module (or other purification system available in the lab).

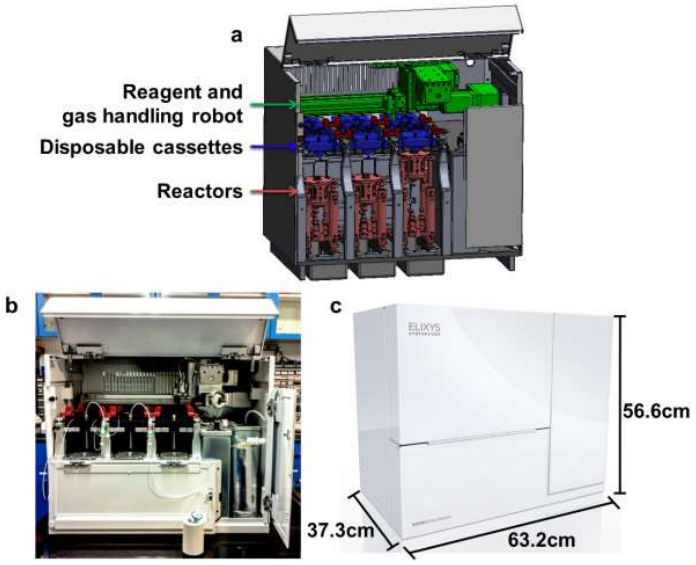


FIG. 31. Hybrid synthesis units (reproduced from Ref. [41] with permission).

4.2.5. Microfluidic synthesis modules

The need for flexible synthesis modules for producing diverse ^{18}F labelled radiopharmaceuticals has led a number of groups to investigate microfluidic devices in the context of PET radiochemistry. Many such systems remain experimental in nature, but some microfluidic devices have been used to synthesize radiopharmaceuticals for clinical research. To date, commercialization has been limited, but such systems include the GE Isar (Fig. 32) [43], the Advion Nanotek [44, 45] and the ABT BG75 [46]. The ABT and Advion systems are more traditional microfluidic systems but, although clinical production has been demonstrated [45], such devices can face challenges at the 'macro to micro' interface and incompatibility between the components and volumes used in traditional radiochemistry. The GE module attempts to overcome this issue by embracing the concept of parallel fluidics and by using chips that utilize the principles of microfluidics in conjunction with approaches and components used in the standard synthesis modules described throughout this section [43]. At the time of writing, for the most part these microfluidic systems remain experimental in nature and a new laboratory looking to make [^{18}F]FDG, other established radiotracers and/or some products for research should consider one of the cassette based, fixed tube or hybrid systems described earlier in the section. However, we expect microfluidic systems to continue to develop and think they will play an important role in the radiopharmaceutical manufacturing facilities of the future.

FIG. 32. A microfluidic synthesis unit (reproduced from Ref. [43] with permission).

4.3. PURIFICATION, ISOLATION AND QC ASPECTS OF ^{18}F COMPOUNDS

4.3.1. Purification and reformulation

Most radiopharmaceuticals require a purification step as part of their manufacturing process to remove potential radiochemical and chemical impurities in the crude product. A range of different impurities need to be considered when selecting the purification method(s), from unreacted precursor to precursor by-products (e.g. deprotected precursor), side products, other reagents, organic solvents, metal catalysts, acids and bases, and phase transfer agents.

Rapid purification methods are typically required due to the short half-life of the product. HPLC is one of the most common techniques used, particularly for research and development, and early phase clinical evaluation of the radiopharmaceuticals. A number of SPE purification techniques are also available, which tend to be preferred for commercial solutions and large scale production of radiopharmaceuticals (e.g. [^{18}F]FDG). The use of a cartridge, or combination of cartridges, is of interest for multiple aspects:

— Cartridges are typically single use items, and often mounted directly in cassette kits available for well-established processes;
— Cartridges are less expensive than semi-prep HPLC systems, especially when cost of annual maintenance and occasional repairs are added to the initial purchasing price;
— Automated systems are of reduced footprint (no need for a semi-prep pump, injection valve, UV detector, radioactivity detector), which can be critical in some settings. On the other hand, cartridge purification is not always as efficient as semi-prep HPLC purification, and level of impurities in the final product tend to be higher in general.

Where semi-prep HPLC is used, the crude reaction mixture is typically diluted with mobile phase and loaded into an HPLC injection valve at the end of the synthesis and injected onto a semi-prep HPLC column. Where required, the unreacted [^{18}F]fluoride may also be removed from the reaction mixture prior to loading onto the HPLC, using for example an Alumina cartridge. HPLC methods are developed to typically produce retention times in the region of 8 to 20 min for the product of interest. The elution of the product is monitored with

ultraviolet/visible and radioactivity detectors, and usually a valve is allowing the collection of the peak of interest by diverting the HPLC flow to a collection reservoir. The collected product peak still contains organic solvents from the mobile phase (e.g. acetonitrile or methanol), as well as buffer salts and/or acids or bases, which should be removed prior to the injection of the radiotracer to the subjects. This reformulation step is typically performed though use of SPE cartridges, typically reversed phase C18 cartridges:

(a) The collected product is first diluted with large amounts of water to reduce the organic content and allow efficient trapping of the compound of interest onto the cartridge;
(b) Similarly, the pH of that solution might also require to be adjusted based on the HPLC eluent used.
(c) This solution is then transferred to the SPE cartridge;
(d) The cart ridge is washed with water to remove the final traces of organic solvents, buffer salts, acids/bases.
(e) Finally, the purified product is recovered from the SPE cartridge via elution with typically a small amount of ethanol into the product collection vial where formulation takes place and diluted with a saline based solution.

An alternative method to the reformulation by SPE cartridge, is the use of rotary evaporators to remove solvents from the semi-prep HPLC eluent. The concentrated residue needs then only to be diluted with the formulation solution. This method is not the method of choice and use of SPE cartridges is typically preferred, as easier to automate, and a wide range of cartridges are nowadays available, to fit as best as possible the physico-chemical properties of the product.

The need for reformulation can sometimes be avoided by running HPLC conditions using eluents that are suitable for iv injections (e.g. mix of ethanol and a suitable buffer). In this case, the collected product peak can be directly used, or need only a simple dilution (for example to adjust pH or reduce ethanol content below hemolytic concerns).

One important aspect to consider when developing purification and reformulation steps is the risk of radiolysis. If the product is susceptible to radiolysis, the risk of having radio-impurities in the final product is increased during those steps. More particularly with the use of SPE reformulation, as at this stage the product is no longer in solution and is concentrated on the cartridge for a short period of time. Options are available to mitigate the impact, for example using anti-oxidants such as ascorbic acid, which can be added either in the reservoir receiving the collected product peak, in the water used to rinse the SPE cartridge, in the final formulation, or in a combination of the above.

The last step is the final sterilisation of the product is typically achieved by passing the formulated product through a sterile 0.22 μm filter. Other sterilisation methods, such as the use of autoclaves, are not very suited for PET radiopharmaceuticals due to the short half-life of the isotopes, but also the potential impact on product stability. Attention to this filtration step should be devoted early enough in the development of a new manufacturing process, as often non-specific binding on the membrane filter is a cause of trouble, and formulation may need to be adjusted to allow good recovery of the product. In such instances, use of co-solvents (e.g. ethanol, polyethylene glycols), surfactants (e.g. polysorbates), or cyclodextrins may be of interest.

4.3.2. Quality control

This section provides a brief overview of typical tests, product specifications and analytical methods that are required to be considered when performing QC of ^{18}F radiopharmaceuticals. More in depth information, including choice of techniques and instrumentation can be found in previously published IAEA-TECDOC-1856, Quality Control in the Production of Radiopharmaceuticals. It provides insights into the necessary requirements for validation of QC methods but focus mostly on chromatographic methods. A more comprehensive review is provided in the recently published EANM 'guideline on the validation of analytical methods for radiopharmaceuticals' [47].

Tests to be included in the QC process will be based on product specifications that will be defined using toxicological information for the product and some known impurities, but also derived from the manufacturing process, as use of particular reagents (e.g. metal catalysts, solvents) will trigger requirement for testing. In the case of ^{18}F radiopharmaceuticals, only a few monographs are available (EP: [^{18}F]FCH, [^{18}F]FDG, [^{18}F]FDOPA (prepared by electrophilic substitution), [^{18}F]FET, [^{18}F]FLT, [^{18}F]FMISO and [^{18}F]NaF; USP: [^{18}F]FDG and [^{18}F]NaF), and therefore specifications should be established for new products. General monographs also provide guidance on testing to be conducted, for example EP monograph 0125 'radiopharmaceutical preparations', or USP general chapter <823> 'positron emission tomography drugs for compounding, investigational, and research uses'. Typical set of product specifications for a ^{18}F radiopharmaceutical are summarised in Table 7 below.

TABLE 7. REQUIREMENTS FOR ^{18}F RADIOPHARMACEUTICALS
(courtesy of M. Huiban, Invicro LLC)

Tests	Specifications	Methods
pH	pH range of interest, e.g. 4.5–8.5	pH meter or pH indicator strip
Appearance	Clear, colourless, practically free from particles	Visual examination
Radionuclidic identity	γ photons of 511 KeV (a sum peak of 1022 KeV may be observed)	Gamma spectrometry
Radionuclidic identity	Half-life within 105–115 min	Dose Calibrator
Radionuclidic purity	> 99.9% (EP) > 99.5% (USP)	Gamma spectrometry
Identification	Retention time of the main radioactive peak is similar to the retention time of the peak obtained with the reference standard, e.g. 0.9 < RRT < 1.1	TLC or HPLC or UPLC
RCP	≥ 95% (EP) ≥ 90% (USP)	TLC or HPLC or UPLC
Enantiomeric purity (where required)	≥ 90%	Chiral TLC or chiral HPLC
Chemical purity: radiopharmaceutical assay	Based on toxicological data and/or available monograph	HPLC
Chemical purity: Phase transfer agent	K_{222} ≤ 2.2 mg/V (EP) ≤ 50 µg/mL (USP) TBA ≤ 2.6 mg/V (EP)	TLC spot test or HPLC
Molar Activity	Based on toxicological data, nature of the product (e.g. stability) and/or clinical trial requirements	HPLC/UPLC and Dose Calibrator
Chemical purity: other known and unknown impurities	Based on toxicological data and/or available monograph	HPLC
Residual solvents	According to ICH Q3C(R6) limits	GC

TABLE 8. REQUIREMENTS FOR ^{18}F RADIOPHARMACEUTICALS *('cont')*

Tests	Specifications	Methods
Residual metals	According to ICH Q3D limits	ICP-AES, ICP-MS, AA or IC
Filter integrity (sterilising filter)	≥ manufacturer Bubble Point, or ≥ validated Bubble Point	Automated or Manual Bubble Point determination
Bacterial endotoxins	≤ 175 IU/V	LAL test
Sterility	Sterile	Membrane filtration test, or direct inoculation test

4.3.2.1. Appearance and pH

The appearance test aims at identifying a potential colour change of the finished product solution and determining presence of any particulate matter in the solution. This test is conducted by visual inspection of the product vial or a sample, in accordance to pharmacopeial methods (e.g. EP monograph 20920 'particulate contamination: visible particles').

As radiopharmaceuticals are typically administered by iv route, defining a suitable pH range is allowing not to create discomfort to the subject at time of injection. A pH value close to the physiologic value of 7.4 is desired, but as most radiopharmaceuticals are injected in relatively low volumes (typically <10 mL), a wider range can usually be used. A very typical range for ^{18}F radiopharmaceuticals is 4.5 to 8.5. The pH can be measured either by using pH indicator strips or a calibrated pH meter.

4.3.2.2. Radionuclidic identity and purity

These tests are meant to confirm that the correct isotope was incorporated into the product of interest, and that no other radionuclides are present in the final product. In the case of ^{18}F, potential contaminants can be formed during irradiation, from the target water (^{3}H and ^{13}N), and from the target entrance window and target body (e.g. cobalt and manganese isotopes).

Radionuclidic identity is typically confirmed by both estimating the half-life and recording the gamma energy spectrum of the product. The half-life is calculated from typically three measurements taken from a dose calibrator. For ^{18}F, the half-life is expected to range from 105 to 115 min (half-life of ^{18}F: 109.7 min). The gamma spectrum is obtained from a gamma spectrometer and confirms the energy signature of the radionuclide of interest. For ^{18}F, a principal gamma photon peak at 511 keV, and a second sum peak at 1 022 keV (that may or may not be observed).

Radionuclidic purity is further assessed by acquiring a gamma spectrum of a decayed sample. Delaying this second examination allows ^{18}F to decay to levels that permit the detection of impurities. Recommended acceptance criteria from the EP and USP are for total radionuclidic impurities to be not more than 0.1% and 0.5%, respectively, of the total radioactivity. This last test is meant to be included in validation activities primarily.

4.3.2.3. Radiochemical identity and purity

These tests are meant to confirm that the correct radiopharmaceutical was prepared, and that the injection will administer a single radioactive molecule.

As ^{18}F radiopharmaceuticals are produced in negligible mass, standard NMR testing can't be used to confirm identity of the product. LCMS techniques may be used but are not common in radiochemistry labs. Typically, identification is inferred by comparison with a reference standard (^{19}F version of the radiopharmaceutical). These tests are usually done by TLC or HPLC/UPLC, comparing the ^{18}F radiopharmaceutical retention factor or retention time obtained from a radiodetector to those of the reference standard obtained from a UV detector. General acceptance criteria are for relative retention factor/time to be within 0.9–1.1 range.

The radiochemical purity (RCP) is assessed from the TLC or HPLC/UPLC radiochromatogram by simply calculating the ratio between the peak corresponding to the product and the sum of all peaks detectable on the radiochromatogram. Acceptance criteria may vary, but RCP above 90% are normally expected.

When the ^{18}F radiopharmaceutical is a chiral substance, the enantiomeric purity also needs to be assessed, to have complete view on the RCP of the product. This is performed by using chiral TLC or HPLC methods. As per the previous test, acceptance criteria may vary, but it is typically expected that the enantiomer of interest should represent a minimum of 90% of the sum of both enantiomers.

4.3.2.4. Radiopharmaceutical content

The radiopharmaceutical concentration needs to be evaluated to ensure that injected doses will not administer unsafe levels of the drug substance. The analysis is typically performed by HPLC/UPLC, using a suitable calibration curve obtained from injections of the reference material. The acceptance criteria will depend on available toxicological information, or can be taken from the relevant monograph, where applicable.

The radioactivity concentration at the end of synthesis, which is defining the strength of the radiopharmaceuticals, is also established during QC testing, using a dose calibrator and determining either the weight or the volume of the bulk solution. Acceptance criteria on the acceptable range for the strength will typically be dictated by the impact of the radioactive concentration on the stability of the product (radiolysis). The strength will also be used to ensure that required amount of activity will be available at time of injection, with actual dose typically between 90 and 110% of the intended injected dose at time indicated on the label. Therefore, this parameter may also be trial dependent. From the above two measurements, the molar activity can also be calculated. Similarly, the acceptance criteria will be based on the nature of the product, and the clinical trial requirements.

4.3.2.5. Chemical purity

A wide range of tests may need to be performed as part of QC release testing to confirm levels of certain impurities in the final product. Some of these tests may not be required for all ^{18}F radiopharmaceuticals, and some other tests may be necessary: requirements will be driven by the manufacturing process being used.

A first test that is typically being included is the determination of residual phase transfer agent within the finished product. These tests are primarily looking at either residual K_{222} (where [^{18}F]KF is being used as the labelling reagent) or residual tetrabutyl ammonium cations (use of [^{18}F]TBAF). Testing can be performed either by TLC or HPLC. With TLC, usually colorimetric assays (spots test) have been designed to allow testing to be fast (Fig. 23). Residual

content of K_{222} should be less than 2.2 mg per volume (EP) or less than 50 µg/mL (USP). Residual content of tetrabutyl ammonium cations should be less than 2.6 mg per volume (EP); there is currently no limit specified in the USP for this impurity.

Another test typically included in the QC process is the determination of residual solvents in the final product. Many solvents may be used in the manufacture of the ^{18}F radiopharmaceutical (e.g. labelling reaction, semi-prep HPLC purification, cleaning), and a review should be performed for each process. Presence of solvents is determined by using gas chromatography. Limits for residual solvents content are defined in ICH guideline Q3C (R6) on impurities: guideline for residual solvents.

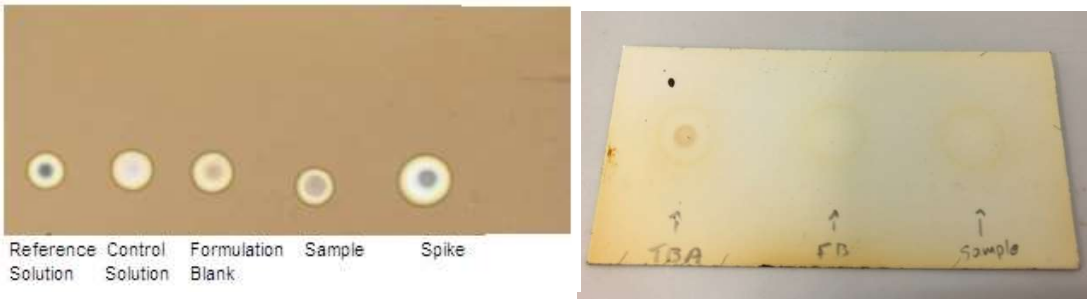


FIG. 33. Colorimetric assays for K_{222} (left) and TBA (right) testing (courtesy of M. Huiban, Invicro LLC).

Where metal catalysts are being used (copper and palladium catalysts are for example usual in ^{18}F chemistries), content of residual metals in the finished product should also be assessed. Various techniques may be used (ICP-AES, ICP-MS, AA, IC). Limits for residual solvent content are defined in ICH guideline Q3D on elemental impurities.

For other known impurities, specific testing may be required. Good examples are coming from monographs, where process impurities are typically identified (e.g. levodopa in [^{18}F]FDOPA), with corresponding limits in terms of their content in the finished product. The radiolabelling precursor may also fall in this category.

Unknown impurities also need to be taken into considerations. Usually, level of unknown impurities will be defined as part of the same HPLC assay used to determine the radiopharmaceutical identity and purity. Defining an acceptance criterion may be more complicated here, as by definition, the impurities are unknown. The EMEA 'guideline on the limits of genotoxic impurities' can be used in some cases. This guideline establishes a threshold of toxicological concern approach to define a risk level associated with the intake of genotoxic impurities. A typical value of 1.5 µg/day intake for a given impurity is associated in that guideline with an acceptable risk.

4.3.2.6. Microbiological contamination

As ^{18}F radiopharmaceuticals are prepared as solutions for iv administration, they should be sterile, and therefore a sterility assurance program should be in place, covering for example aspects such as environmental monitoring and aseptic processing. In addition, a few tests are typically being performed during release testing to confirm that risk on patient safety is low.

Firstly, the sterilising filter is normally being tested to confirm that the filter membrane is still integral post use, confirming therefore the efficiency of the filtration step. Different tests are possible, but typically a bubble point test will be used, as it is simple to perform. The test may

be done manually, or through the use of an automated device. Typically, acceptance criteria are defined by the manufacturer, but the formulation used for the product should be taken into consideration, as it is well known that ethanol for example will reduce the bubble point. Therefore, where required, a bubble point defined experimentally for the use of that specific formulation can be taken as the acceptance criterion.

Apyrogenicity should also be confirmed by assessing the presence and quantity of bacterial endotoxins in the product. For this, a LAL test is performed, and typically the use of an endotoxin testing system is preferred, as the test is fast, and results can be available prior to product release. Usual acceptance criterion is an endotoxin content less than 175 IU/injection.

Finally, sterility testing should be performed. Due to the length of the test, the results are necessarily obtained post-release. Sterility can be assessed by either direct inoculation or membrane filtration methods, and the test should be performed in accordance to relevant pharmacopeial requirements (Ph. Eur. 2.6.1. Sterility, JP 4.06 Sterility Test and USP <71> Sterility Tests). The outcome of the test is binary: 'sterile' or 'nonsterile'.

5. GENERAL PRINCIPLES AND CONCEPTS OF [^{18}F]FLUORINATIONS

In this section an overview is given on different [^{18}F]fluorination methods. These methods range from the well-known nucleophilic and electrophilic substitution reactions that have been used for decades now (e.g. FDG) to indirect [^{18}F]fluorination methods using small ^{18}F intermediates and PGs and finally to more recently developed methods that start to find their wider application in the preparation of radiopharmaceuticals. The newer methods include the so-called late stage fluorination reactions making use of transition metal based catalysts, [^{18}F]fluorination on heteroatom such as aluminium, silica and boron to establish kit type of labelling methods and finally the concept of trifluoromethylation.

In the following sections the most commonly used methods are being discussed regarding some theoretical backgrounds, a few examples are given and the current position in the development towards clinical use is being discussed.

5.1. SUBSTITUTION REACTIONS (AROMATIC AND ALIPHATIC)

5.1.1. Electrophilic substitution

[^{18}F]Fluorination of electron rich compounds (alkenes or aromatic rings) can easily be achieved using [^{18}F]Fluorine or [^{18}F]NF reagents (fluoro-pyridones, fluoro-N-sulfonamides) such as [^{18}F]Selectfluor. In particular, the selective electrophilic aromatic substitution (S_EAr) carried out by displacement of a metallic substituent (Hg, Sn) ([^{18}F]fluorodemetallation) is a method of choice to introduce a fluorine atom on a specific position (see Fig. 34), and has been for decades the only reliable route to prepare [^{18}F]FDOPA (Fig. 35) [48].

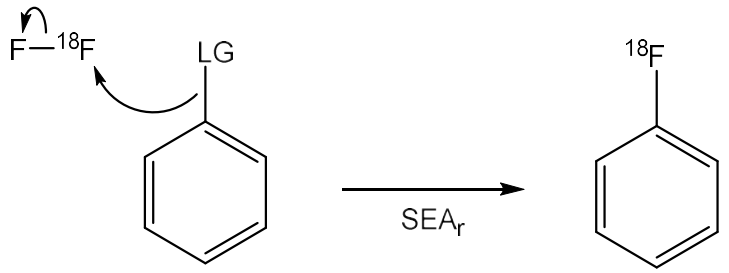


FIG. 34. General principle of electrophilic aromatic substitutions (courtesy of M. Huiban, Invicro LLC).

These reactions are not commonly used for the synthesis of radiopharmaceuticals, due to several limitations:

(a) Apart from [^{18}F]Fluorodemetallation, electrophilic fluorination of aromatic compounds using [^{18}F]F_2 is generally a poor and non-selective method;
(b) Cyclotron production of [^{18}F]F_2 commonly requires carrier fluorine, which leads to radiopharmaceuticals with poor molar activity;
(c) The use of [^{18}F]F_2 limits the theoretical RCY to 50%;
(d) Most other fluorinating reagents are prepared from [^{18}F]F_2, and therefore suffer from the same challenges, even if they allow to modulate its reactivity.

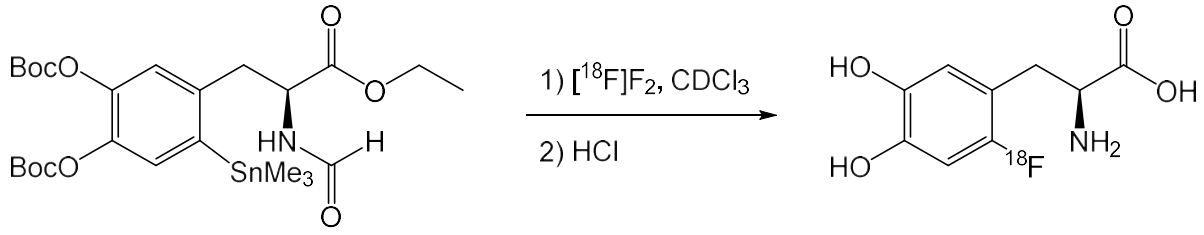


FIG. 35. Preparation of [^{18}F]FDOPA via electrophilic aromatic substitution (courtesy of M. Huiban, Invicro LLC).

Electrophilic substitution reactions are overall very limited in use, and are associated with technical challenges in particular as related to the high reactivity of reagents such as [^{18}F]fluorine, and are therefore not the first choice for [^{18}F]fluorinations nowadays despite the fact that these synthetic routes are often used in organo-fluorine chemistry.

5.1.2. Nucleophilic aliphatic substitution

Aliphatic nucleophilic substitution with [^{18}F]fluoride is a commonly used method for the labelling of molecules with ^{18}F. Reactions are typically easy to perform, can have high yielding under favourable circumstances and usually provide products with high molar activity. Labelling is achieved by displacement of a suitable leaving group (LG) by the [^{18}F]fluoride anion, following a S_N2 type reaction (see Fig. 36). The [^{18}F]F^- attacks the sp_3 hybridized centre at the opposite side relative to the LG group, resulting in substitution with inversion of the configuration of the carbon centre. Usually, the leaving groups are sulfonates (e.g. triflate, mesylate, tosylate or nosylate) or other halides (Cl, Br or I), with the leaving ability decreasing in the order -OTf > -OTs & -OMs > -I > -Br > -Cl. Ring opening reactions are also possible, including use of cyclic sulphates, epoxide or aziridine precursors, but are not always very efficient (examples include clinically relevant [^{18}F]FES [49] and [^{18}F]FLT [50]).

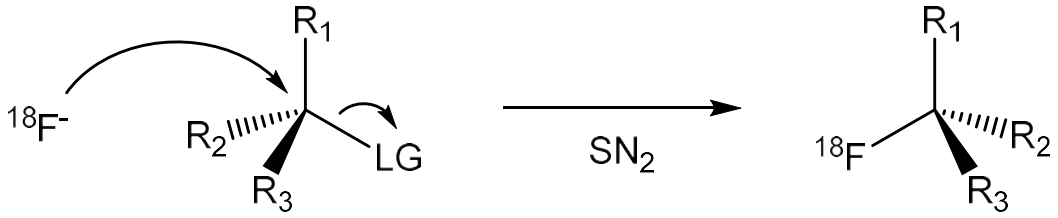


FIG. 36. General principle of nucleophilic aliphatic substitutions (courtesy of M. Huiban, Invicro LLC).

Typically, these reactions are performed in polar aprotic solvents, often in acetonitrile, DMF or DMSO. Acetonitrile is of particular interest in multistep procedures, as it can be easily removed if required. Protic solvents, such as alcohols, are generally not used for nucleophilic substitution reactions because of their ability to solvate the nucleophile and decrease its reactivity. Though, it has been reported that sterically hindered alcohols, such as tert-butyl alcohol, are suitable in some cases. The presence of small amounts of water is also proving beneficial for some reactions.

Competing sites of nucleophilic attack in the molecule need to be protected (principally acid, alcohol, or amine groups), leading to multistep radiolabelling procedures, potentially requiring intermediate purification steps, with both an impact on yields and synthesis time. With a secondary carbon, the substitution reaction may also compete with elimination from the precursor, impacting reaction yields.

Several of the most important PET radiotracers are routinely being manufactured for clinical use using this methodology. As examples, we can cite the widely used [^{18}F]FDG, [^{18}F]FMISO for imaging tissue hypoxia or [^{18}F]Florbetaben ([^{18}F]NeuraCeq) for imaging β^-amyloid plaque deposit in Alzheimer's disease (Fig. 37).

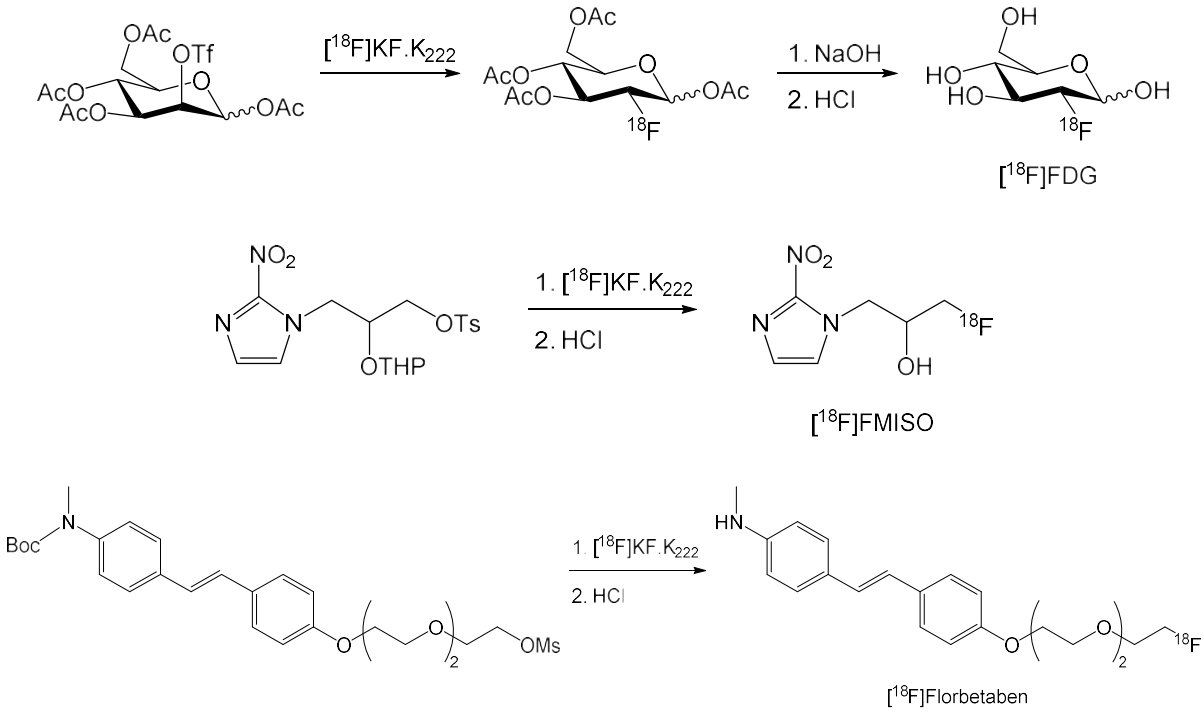


FIG. 37. Preparations of [^{18}F]FDG, [^{18}F]FMISO and [^{18}F]Florbetaben via classical S_N2 reaction mechanism (courtesy of M. Huiban, Invicro LLC).

Some other transformations have been used, for example the enzyme catalysed chemical transformations was reported by O'Hagan et all. [51]. These enzymatic reactions are attractive due to their inherent stereo, regio and chemo selectivity. They can also typically be performed in aqueous solutions, avoiding the need to dry the [^{18}F]fluoride reagent, and run at near room temperature conditions. Though the high specificity of the transformations, and considering the relatively long reaction time, these enzymatic methods have yet not led to its use for the production of radiopharmaceuticals batches for clinical use.

Overall, aliphatic nucleophilic substitution with [^{18}F]fluoride remains a method of choice. At present, this method is well established for the majority of routine ^{18}F radiotracer productions and is employed in most commercially available synthesis platforms.

5.1.3. Nucleophilic aromatic substitution

'Classical' aromatic nucleophilic substitution reactions are very suitable for electron-poor (hetero)arenes, but lack of applicability for labelling of electron-neutral or electron-rich aromatic rings. New methodologies have been emerging, particularly in the recent decade, to address this problem. The section below is providing an overview of both classical transformations and a selection of the newer technologies.

5.1.3.1. Electron poor aromatic rings

Aromatic nucleophilic substitutions with [^{18}F]fluoride, following a S_NAr type reaction, have been to date by far the most successful approach for introducing ^{18}F at aryl carbons. This reaction usually requires the presence on the aromatic ring of a good LG, and at least one activating electron withdrawing group on the ortho or para position (see Fig. 38). Radiochemical yields may vary, but typically products are obtained with high molar activity.

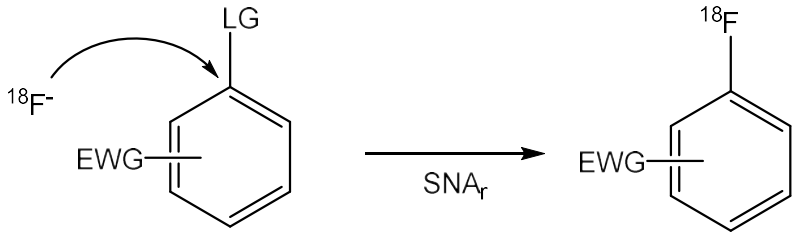


FIG. 38. General principle of nucleophilic aromatic substitutions (electron withdrawing group on ortho or para position) (courtesy of M. Huiban, Invicro LLC).

Usually, the leaving groups are the trimethylammonium group, the nitro group, or other halides (Cl, Br or I), with the leaving ability decreasing in the order -N^+Me_3 ≈ -NO2 > -Cl > -Br > -I. The trimethylammonium and nitro groups are the most widely used, and the trimethylammonium group can be of advantage as permitting easy separation of the labelled product from the precursor (charge difference). Though, the use of the trimethylammonium group can also lead to the formation of the volatile [^{18}F]fluoromethane as a possible side reaction, and therefore the use of this leaving group should be carefully considered. It can be noted that exchange reactions with [^{18}F]fluoride ion (where the leaving group is -^{19}F) have also been reported [52]. These reactions are usually avoided for clinical use due to the resulting low molar activity.

Activating groups electron withdrawing group include the nitro, the trifluoromethyl and the cyano groups, as well as the carbonyl functions (aldehyde, ketone, ester and carboxylic acids), with the activating ability decreasing in the order $-NO_2$ > $-CF_3$ > -CN > -CHO > -COR > -COOR > $-CO_2H$. These groups should be present in the ortho, and even preferably para position, with low RCYs being typically obtained when they are in the meta position. If these groups are not desired in the final product, additional steps after the [^{18}F]fluorination are required to remove or transform these activating groups. This will contribute to decrease the RCY quite significantly, due to process steps losses and increased preparation time.

Aromatic nucleophilic substitutions with [^{18}F]fluoride are performed in polar aprotic solvents, often in acetonitrile, DMF or DMSO. Acetonitrile is not always possible to use, due to the need for high temperature during the labelling step and tends to be more used with trimethylammionium salts. Reactions are also often performed under basic conditions due to the presence of the mixture of the krypt and the base from the [^{18}F]fluoride activation procedure, and usually require moderate to high temperatures (100 to 170°C) to be applied for relatively long reaction times (10 to 30 min). These relatively harsh conditions are typically not adequate for the radiolabelling of sensitive substrates such as biomolecules (peptides or proteins). In those circumstances, multi-step procedures involving indirect labelling through the use of small ^{18}F labelled intermediates (PGs) is required. See also section 5.2 and 5.3 for more details.

Despite some of the drawbacks mentioned above, S_NAr reactions have been to date the most successful routes to the preparation of multiple radiotracers used daily in clinics, with some examples presented below in Fig. 39:

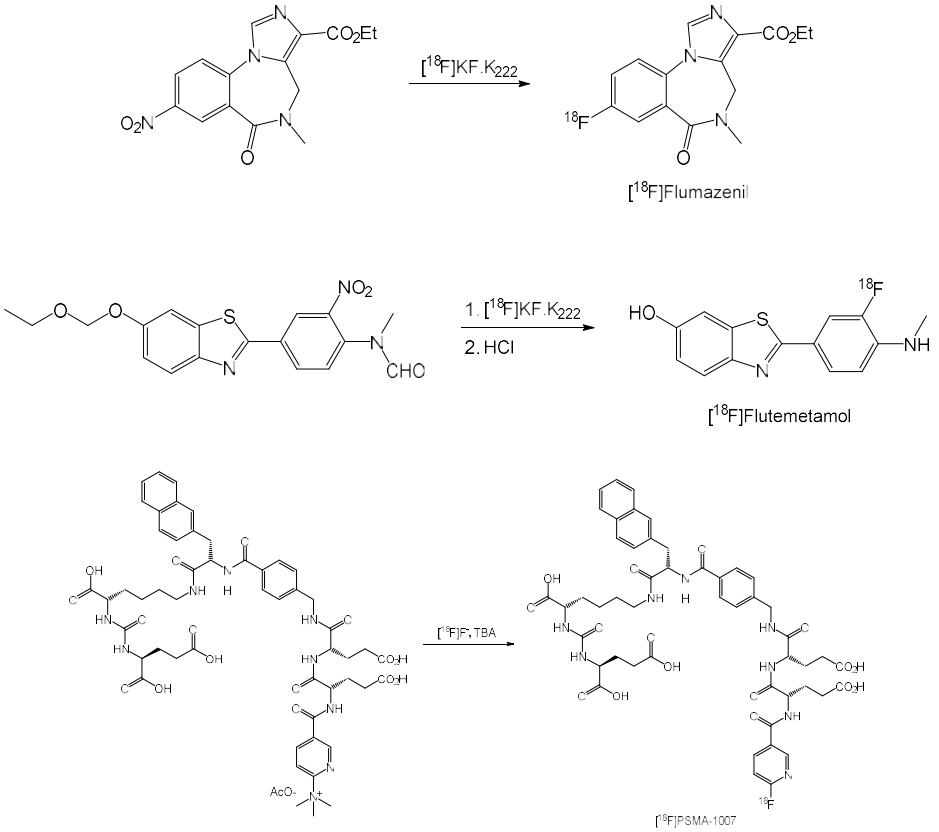


FIG. 39. Preparations of [^{18}F]Flumazenil, [^{18}F]Flutemetamol and [^{18}F]PSMA-1007 via classical S_NAr (courtesy of M. Huiban, Invicro LLC).

In particular, a step by step procedure for the preparation of the prostate specific membrane antigen (PSMA) imaging probe [^{18}F]PSMA-1007 is provided in Table 8 below. This procedure was reported for the preparation of the radiotracer on a single use cassette type synthesizer for clinical examination, more particularly the CFN-MPS200 synthesizer from Sumitomo Heavy industries (Japan). Variations on the procedure, as used with other commercially available synthesizers (e.g. GE FASTlab, NEPTIS RS, IBA Synthera+, Trasis AllinOne, Eckert & Ziegler Modular Lab), have also been reported. See also section 8.4 for additional details.

TABLE 9. STEPWISE SYNTHESIS OF [^{18}F]PSMA-1007 VIA CLASSICAL S_NAR
(courtesy of M. Huiban, Invicro LLC)

Synthetic step	Conditions
1. $^{18}F^-$ production	Production of $^{18}F^-$ (depends on the nuclear reaction and the cyclotron used).
2. $^{18}F^-$ preparation	Before the azeotropic drying, the $^{18}F^-$ is separated from the ^{18}O-water using an anion exchange cartridge (Sep-Pak® Light Waters Accell^{TM} Plus QMA cartridge) preconditioned to obtain it in its HCO_3-form.
3. $^{18}F^-$ drying	Azeotropic drying of $^{18}F^-$ using TBA hydrogen carbonate (0.6 mL, 0.075 M) aqueous solution, stabilized with ethanol, followed by one additional drying step after addition of 1 mL of MeCN.
4. $^{18}F^-$ fluorination	The quaternary ammonium acetate salt precursor in DMSO (2 mL) is added to the dry $^{18}F^-$ from step 3 and the resulting solution is heated to 95˚C for 10 min.
5. Purification	The crude mixture is diluted with a 5% ethanol solution (10 mL), and passed through 2 cartridges connected in series, first a Chromafix PS-H^+ cartridge and then a Chromafix C_{18ec} cartridge, for the SPE of [^{18}F]PSMA-1007. Both cartridges are further washed with first a 5% ethanol solution (23 mL), and then a 30% ethanol solution (3 mL).
6. Reformulation	The purified [^{18}F]PSMA-1007 is eluted from the cartridges with a 30% ethanol solution (4 mL) and diluted with a solution of sodium ascorbate (100 mg) in normal saline (11 mL). The resulting solution is filtered through a sterile 0.22 μm Millex GV filter.
7. Quality control	Radiochemical and chemical purity data of [^{18}F]PSMA-1007 are obtained via HPLC, using a Merck Chromolith performance RP-18e 100 mm × 4.6 mm column and a UV detector (254 nm) coupled in series with a radioactivity detector. Gradient elution is performed at a flow rate of 3 mL/min with a mixture of MeCN (solvent A) and 0.1% TFA (solvent B). Gradient conditions are: A 5% to 15% from 0 to 1.5 min; A 15% to 35% from 1.5 to 10.5 min; A 35% to 95% from 10.5 to 13 min; A 95% to 5% from 13 to 19 min). The column temperature is set to 30°C.

5.1.3.2. Electron-neutral and electron-rich aromatic rings

For aromatic rings that are not bearing electron withdrawing substituents, the SNAr method described above is inefficient, and therefore many strategies have been developed over the years to overcome that challenge.

The use of diazonium salts and triazenes as precursors, in the Balz–Schiemann and Wallach reactions, respectively, have not seen widespread use in the preparation of ^{18}F radiotracers, despite being long known transformations (Fig. 40). Several difficulties (low RCYs, low molar activities, side reactions, difficult preparation of precursors) are encountered when employing these reactions, hence the reason to focus in emerging alternatives.

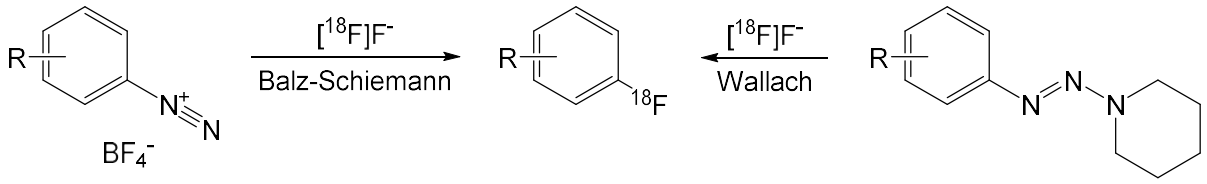


FIG. 40. Balz–Schiemann and Wallach reactions (courtesy of M. Huiban, Invicro LLC).

Diaryliodonium salts have to the contrary been of particular interest in the past two decades (Fig. 41). Like in the Balz-Schiemann and Wallach reactions, there is no need for an electron withdrawing group on the aromatic ring, and the reaction works with both electron-rich and electron-deficient arenes. These transformations are believed to operate by initial coordination of fluoride to the iodine centre, followed by reductive elimination to form an ^{18}F–C sp_2 bond. In contrast to the classical S_NAr, the synthesis of meta-substituted [^{18}F]fluoroarenes is possible from iodonium precursors. The nature of the anion was also noted to influence the reaction rate and RCYs, with RCYs decreasing in the order -Br > -OTf > -I > -OTs.

The regioselectivity of this reaction depends on the electron density of the aryl rings, with the fluorination occurring preferentially at the less electron-rich arene. The regioselectivity was also found to be controlled by the bulky ortho substituents hinderance (so called 'ortho effect') that can influence the preferential nucleophilic attack in which the electron-rich rings are fluorinated. Unsymmetrical diaryl iodonium salts containing arenes such as anisole and thiophene as directing groups are therefore typically used, but the combined electronic and steric effects mean caution should be exercised when selecting a directing group. More interestingly, recent work has showed that in the presence of a copper catalyst the regioselectivity of the radiofluorination reaction can be controlled with high selectivity. Refer to section 5.4 for additional details.

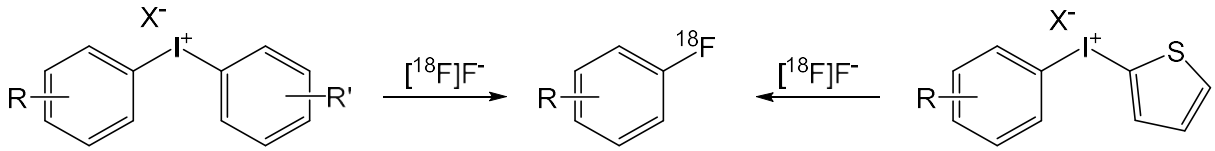


FIG. 41. Use of iodonium salts in radiofluorination reactions (courtesy of M. Huiban, Invicro LLC).

Often with iodonium salts, radiofluorination reactions require very high temperatures and long reaction times. These harsh conditions provide low to modest RCYs and demonstrate limited tolerance to common functional groups. One proposed solution has been the use of radical scavengers, such as TEMPO, which appear to be effective in avoiding decomposition of the precursor salt before reaction with [^{18}F]fluoride ion is complete. Another limitation of this methodology is the preparation of the starting materials, which can be challenging. These salts tend also to have modest shelf lives.

So far, this ^{18}F labelling approach has mainly been investigated for simple readily prepared diaryliodonium salts. In particular, diaryliodonium salts have been used to prepare intermediates such as 4-[^{18}F]fluoroiodobenzene or 1-bromo-4-[^{18}F]fluorobenzene that can then be engaged in follow up cross-coupling reactions. Only a few applications have emerged for the preparation of specific radiotracers of relevance for clinical and preclinical evaluations, of which [^{18}F]DAA1106 [53], [^{18}F]Flumazenil [54] and [^{18}F]UCB-H [55] can be cited as successful examples (Fig. 42).

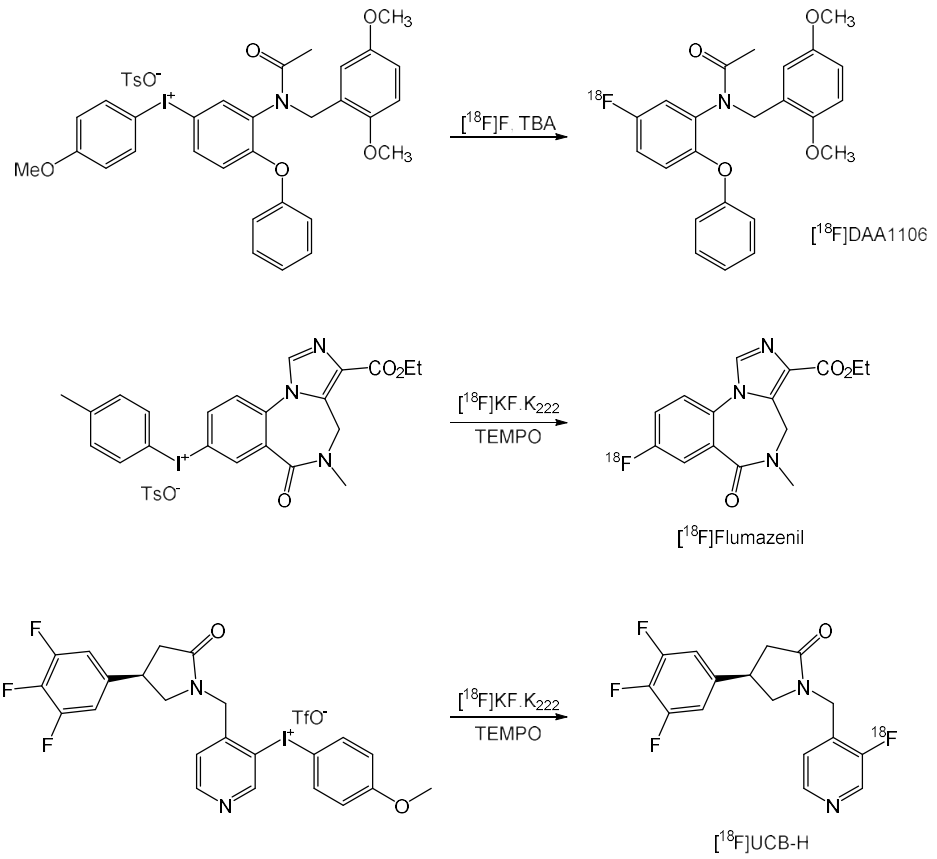


FIG. 42. Preparations of [^{18}F]DAA1106, [^{18}F]Flumazenil and [^{18}F]UCB-H from iodonium salts (courtesy of M. Huiban, Invicro LLC).

Iodonium ylides are another class of hypervalent iodine (III) reagents that have also been investigated as precursors for aromatic radiofluorination to synthesize PET radiotracers (Fig. 43). As with the reactions of diaryliodonium salts, aryliodonium ylides react with nucleophilic [^{18}F]fluoride anion to form [^{18}F]fluoroarenes, following the same mechanistic principles (addition of fluoride onto the iodine centre, followed by reductive elimination of the auxiliary) [56]. Iodonium ylides derived from Meldrum's acid were firstly reported, but their use was leading to formation of regioisomers in certain cases. Further optimization studies on the dicarbonyl auxiliary, to design more stable iodonium ylide precursors, led to the preparation of spirocyclic iodonium ylides [57].

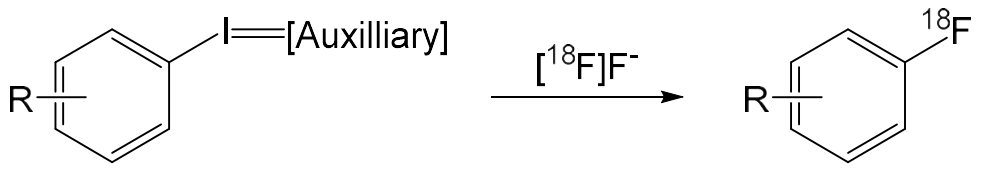


FIG. 43. Use of iodonium ylides in radiofluorination reactions (courtesy of M. Huiban, Invicro LLC).

Iodonium ylides offer several advantages over diaryliodonium salts:

(a) Easier methods are available for their synthesis. For example, the corresponding (diacetoxyiodo)arenes or directly from the aryl iodides;
(b) The lack of counterion also avoids difficult purification procedures;

(c) The spirocyclic iodonium ylides are crystalline solids, with improved chemical stability;
(d) The presence of the spirocyclic auxiliary enhance the regioselectivity of the radiofluorination reaction, as from a mechanistic point of view, an unfavourable anionic intermediate would result from the reductive elimination of the auxiliary;
(e) Mechanistic studies are also showing that iodonium ylides are comparatively more reactive than diaryliodonium salts.

Iodonium ylides have already been applied to the preparation of radiopharmaceuticals of clinical relevance. Best examples are the preparation of [^{18}F]FMT and [^{18}F]FPEB (Fig. 44).

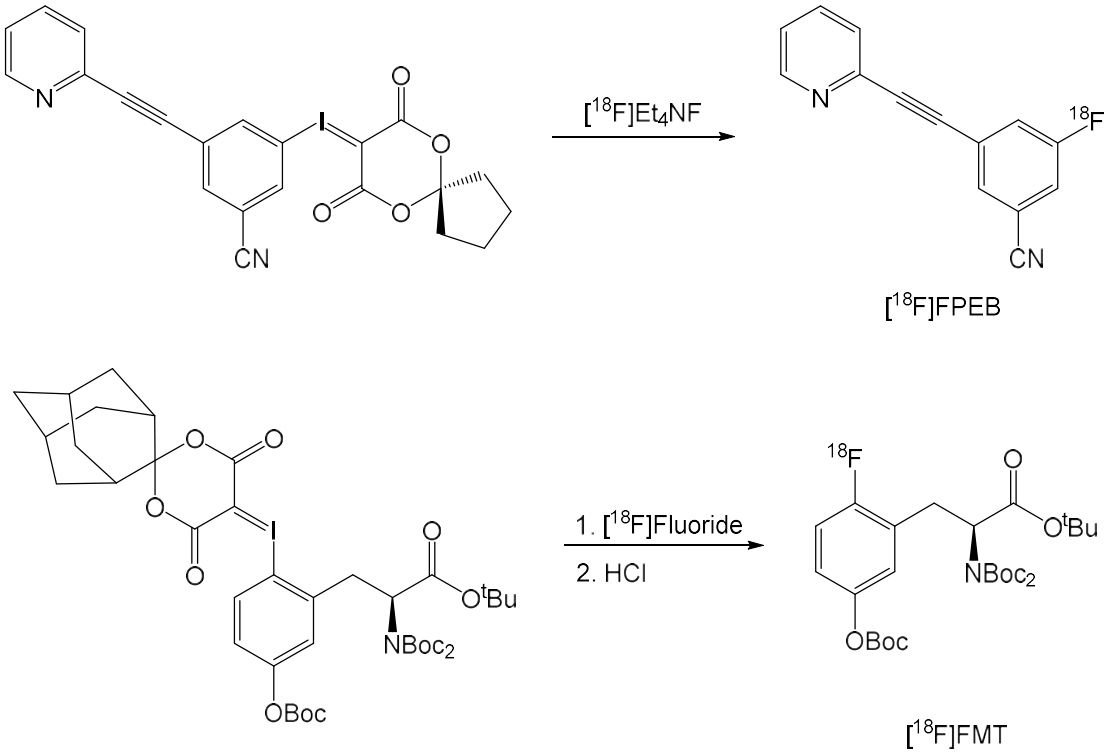


FIG. 44. Preparations of [^{18}F]FPEB and [^{18}F]FMT from spirocyclic iodonium ylides (courtesy of M. Huiban, Invicro LLC).

While more attention was given to iodonium salts, sulfonium salts have also been investigated as potential useful precursors. Despite a first report in the late 80s, demonstrating the suitability of the dimethylsulfonium moity as a LG [58], most of this work occurred in the recent decade. First triaryl sulfonium salts were investigated, moving rapidly towards the use of dibenzothiophene sulfonium salts [59] (Fig. 45). Similarly, to the iodonium salts, the reaction is believed to operate by initial formation of a fluoride adduct to the sulphur centre, followed by reductive elimination to form the C-^{18}F bond. Using dibenzothiophene sulfonium salts, these reactions were showed to work with electron-poor, non-activated and electron-rich substrates, when selecting the correct substituents R' and R'' on the dibenzothiophene moity. Use of acetonitrile and DMSO was reported, as well as overall milder reaction conditions as compared to classical nucleophilic aromatic substitutions, with temperatures in range 50 to 110°C, and reaction times in range 5 to 20 min.

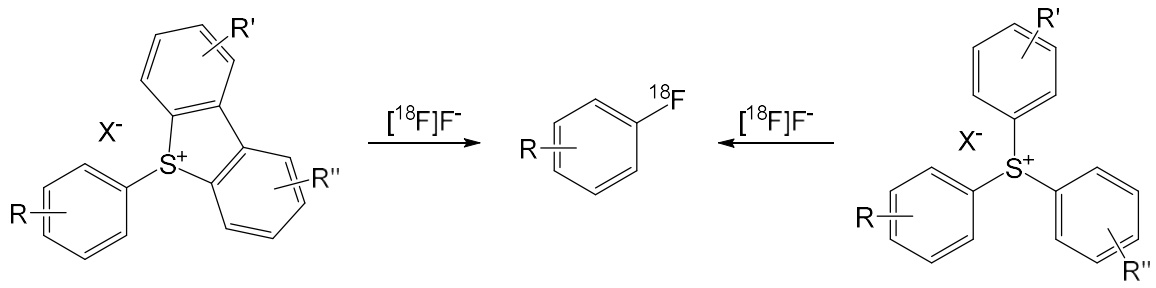


FIG. 45. Use of sulfonium salts in radiofluorination reactions (courtesy of M. Huiban, Invicro LLC).

The most relevant example, in terms of clinical use, is probably here again the preparation of [^{18}F]FPEB [60] (Fig. 46).

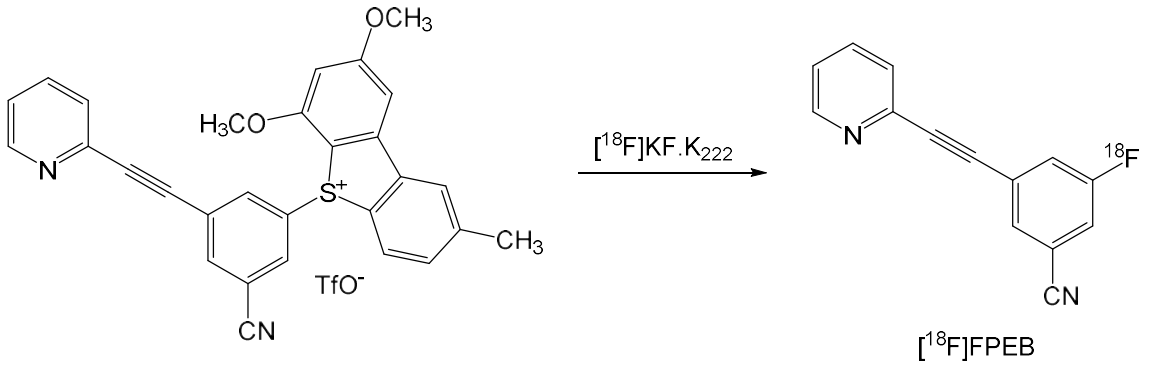


FIG. 46. Preparation of [^{18}F]FPEB from a sulfonium salt (courtesy of M. Huiban, Invicro LLC).

To summarise, aromatic nucleophilic substitutions with [^{18}F]fluoride are an important class of reactions available to the radiochemist, that are used daily worldwide to prepare a wide range of different radiopharmaceuticals in clinical settings. The limitations of the classical S_NAr reaction led to the development of new types of precursors such as iodonium ylides or sulfonium salts as described above, but also the more recently published N-aryl sydnones and uronium salts. Some of these new technologies are still fairly recent and it is therefore difficult to evaluate their full impact yet, but they are likely to contribute to the preparation of novel radiopharmaceuticals in the future.

5.1.4. Nucleophilic heteroaromatic substitution

Classical nucleophilic substitution reactions with [^{18}F]fluoride ion have been expanded to heteroarenes, in particular in the pyridine series [61]. Unlike with homoarenes, activating groups are not usually required for the introduction of [^{18}F]fluoride ion into a pyridinyl 2- or 4-position. If electron withdrawing substituents (such as cyano or amido groups) are present at the ortho or para positions to activate the meta leaving groups, [^{18}F]fluoride ion introduction into the pyridinyl 3-position is possible. Either trimethylammonium, nitro, bromo or chloro can serve as the leaving group. The labelling conditions are similar to the S_NAr conditions used for the homoarenes discussed above: both high temperatures and high boiling solvents like DMSO or DMF are needed. The RCYs are generally moderate to good, with highest yields typically obtained when labelling the second position of the pyridinyl ring. Besides the pyridine series,

further heteroarenes have also proved to be amenable to nucleophilic radiofluorination, but examples are scarce.

Other transformations cited above, using 'onium' salts as precursors, also reported to allow labelling of heteroarenes, pyridines and quinolines in particular, but limited examples are available in terms of use for labelling of radiopharmaceuticals of interest.

The ease of labelling at the second position of the pyridinyl ring has found widespread use for preparation of PGs such as [^{18}F]FPyBrA, [^{18}F]FPyMe, [^{18}F]FPyKYNE or [^{18}F]FPy-TFP for the indirect labelling of peptides, proteins, and oligonucleotides (Fig. 47). Examples of radiopharmaceuticals used in clinical trials include both enantiomers of [^{18}F]flubatine [62] for imaging of cerebral $\alpha_4\beta_2$ nicotinic acetylcholine receptors, and [^{18}F]LSN3316612 for imaging the O-GlcNAcase enzyme.

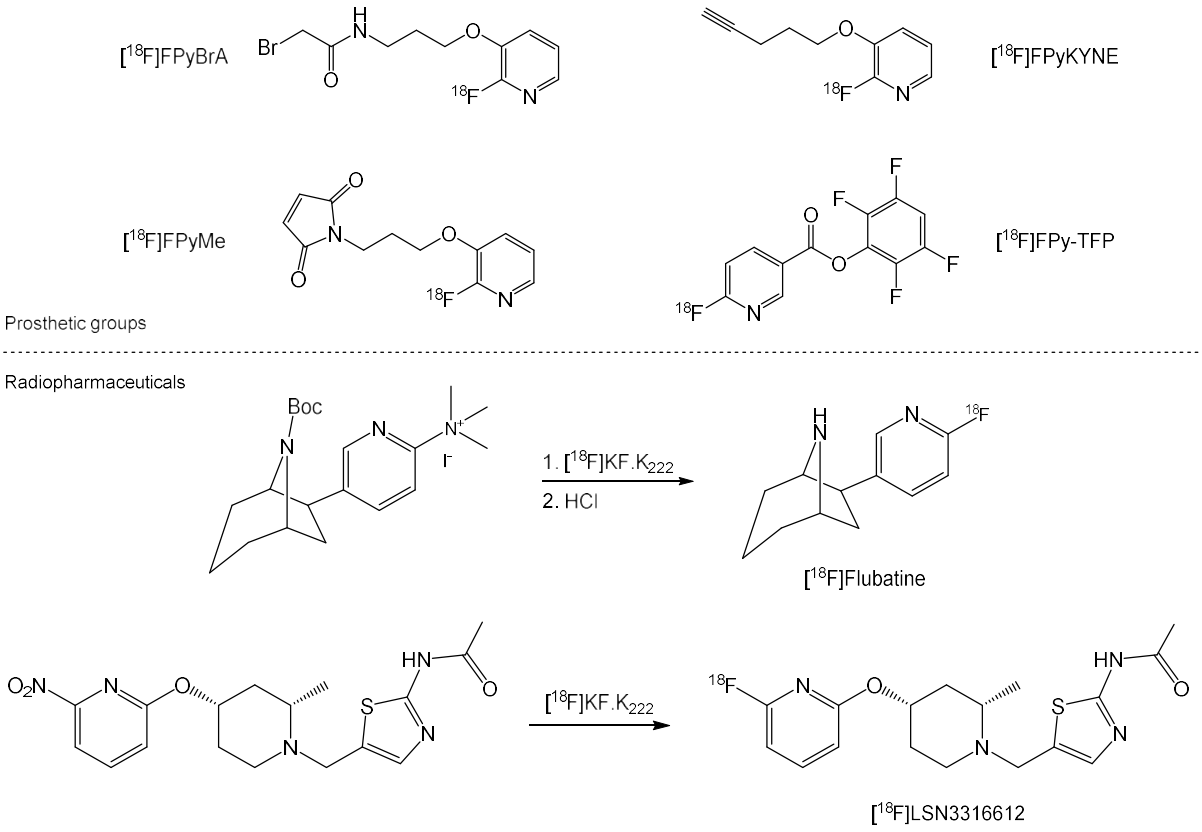


FIG. 47. Examples of heteroarenes prepared via classical S_NAr.

5.2. [^{18}F]FLUORINATION VIA SMALL ^{18}F INTERMEDIATES

When radiolabelling small molecules with ^{18}F, in an ideal world radiolabelling is done as late in the process as possible. As such, direct [^{18}F]fluorination is preferred with perhaps straightforward removal of any protecting groups after labelling, if needed. This thinking has prompted research into new methods for the late stage [^{18}F]fluorination of complex molecules and there have been significant advances in recent years that are detailed throughout this publication (section 5.5) [63]. However, in certain cases direct [^{18}F]fluorination is not feasible and necessitates alternative labelling approaches. One such approach is to first label a building block with ^{18}F [64], and then couple the ^{18}F building block with another molecule to produce the radiotracer. Such building blocks are prepared using standard ^{18}F radiochemistry, and then

reacted with other molecules under milder conditions to generate radiotracers. Mild conditions are particularly important when radiotracers contain sensitive functionality. Challenges when working with ^{18}F building blocks include issues with selectively (protecting groups might be needed) and purity of the building block due to the presence of unreacted precursor that might complicate and compete in subsequent reactions. The latter challenge can be addressed by intermediate purification of the building block, accomplished via SPE, distillation, GC or HPLC, but does introduce operational complexities as it mandates a two-pot labelling procedure. The building block approach is similar in concept to the preparation of PGs for labelling biomolecules that are too sensitive to ^{18}F-fluorination conditions for direct labelling (see 5.3). This section will describe four building block approaches to assembling ^{18}F-labeled molecules: 1. building blocks for ^{18}F-fluoroalkylation, 2. ^{18}F-labeled aromatic building blocks, 3. ^{18}F-building blocks for click chemistry, and 4. use of radiotracers as building blocks.

5.2.1. Building Blocks for [^{18}F]Fluoroalkylation

The classical ^{18}F building blocks are designed for [^{18}F]fluoroalkylation reactions such as [^{18}F]fluoromethylation and [^{18}F]fluoroethylation, and this labelling approach has recently been reviewed [64]. These building blocks are also attractive because they can take the place of a [^{11}C]methyl group. As such, precursors previously labelled via ^{11}C-methylation (with [^{11}C]methyl iodide or [^{11}C]methyl triflate) can oftentimes also be readily [^{18}F]fluoroalkylated since the ^{18}F building blocks are frequently compatible with the same functionality. This facilitates rapid development of [^{18}F]fluorinated versions of promising ^{11}C labelled radiotracers by simply switching out the alkylating agent.

The simplest of such ^{18}F building blocks is the [^{18}F]fluoromethyl group. Probably the best known use of this chemistry is in the production of [^{18}F]fluoromethylcholine for imaging prostate cancer. [^{18}F]Fluoromethyl groups are installed using [^{18}F]fluoromethyl halides such as [^{18}F]fluoromethyl bromide and [^{18}F]fluoromethyl iodide, accessed by radiofluorination of dibromomethane or diiodomethane, respectively [64]. [^{18}F]Fluoromethyl bromide and [^{18}F]fluoromethyl iodide are both volatile and working with them can be somewhat challenging because nucleophilic ^{18}F chemistry is predominantly liquid phase. Nevertheless, innovative labelling procedures have been developed over the years where, for example, [^{18}F]fluoromethylbromide (B.P. = 19ºC) and [^{18}F]fluoromethyliodide (B.P. = 53ºC) can be readily purified from dibromomethane (B.P. = 97ºC) and diiodomethane (B.P. = 181ºC) precursors (as well as other non-volatile reaction components including unreacted [^{18}F]fluoride, K_2CO_3 and kryptofix) by simple distillation, or using gas chromatography. The volatile ^{18}F building block is then reacted with the molecule to be labelled that is either in solution, or pre-loaded on a Sep-Pak. For example, [^{18}F]fluoromethylation of dimethylamino ethanol (DMAE) is utilized in the classical production of [^{18}F]fluorocholine (Fig. 48) [65].

An alternative solution to the volatility issue is to use sulfonate ester-based [^{18}F]fluoromethylating agents such as [^{18}F]fluoromethyl tosylate and [^{18}F]fluoromethyl triflate instead. These agents are not volatile making them more compatible with standard liquid phase ^{18}F radiochemistry. They can be used in one-pot processes, such as the synthesis of [^{18}F]fluoromethylcholine reported by the Scott lab described in Table 9, or purified in between like the dihalomethane precursors.

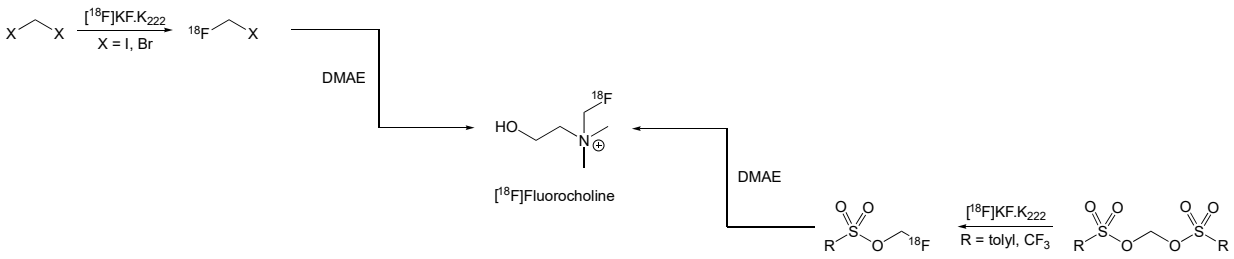


FIG. 48. Strategies for [^{18}F]Fluoromethylation (courtesy of P. Scott, University of Michigan).

Another common ^{18}F building block is the [^{18}F]fluoroethyl group [64]. The chemistry is similar to that of the [^{18}F]fluoromethyl building block, with both 2-[^{18}F]fluoroethyl halides and 2-[^{18}F]fluoroethyl sulfonate esters commonly employed to install it. For example, 2-[^{18}F]fluoroethyl tosylate ([^{18}F]FETs) is one of the most commonly used reagents for [^{18}F]fluoroethylation and the chemistry of this reagent has been extensively reviewed [64]. 2-[^{18}F]Fluoroethyl tosylate is non-volatile, mostly stable (except with certain solvents and bases at high temperatures), and easy to prepare from ethylene ditosylate (Fig. 49). Reflecting this it has been widely used to synthesize many different ^{18}F labelled radiotracers such as [^{18}F]fluoroethyltyrosine (via O-[^{18}F]fluoroethylation), [^{18}F]fluoroethylcholine and dopamine transporter imaging agents (via N-[^{18}F]fluoroethylation), and [^{18}F]fluoroethyl methionine (via S-[^{18}F]fluoroethylation).

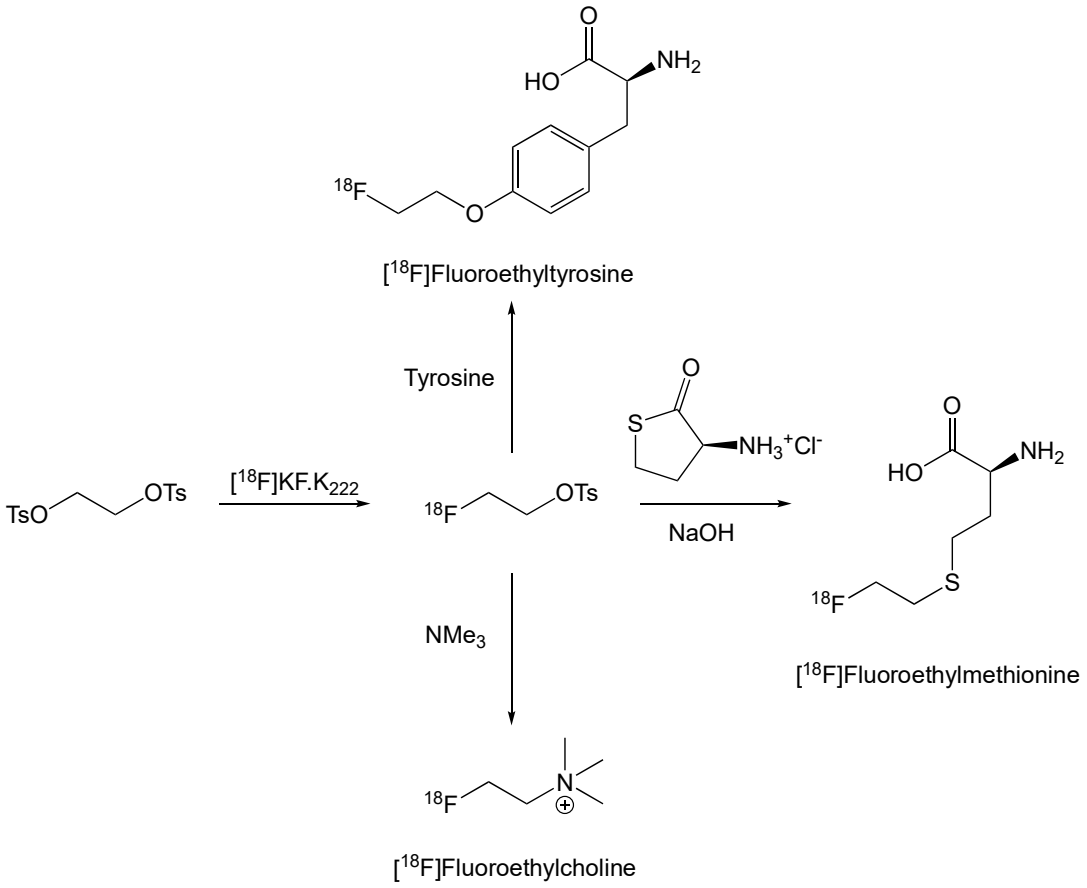


FIG. 49. Strategies for [^{18}F]Fluoroethylation (courtesy of P. Scott, University of Michigan).

Beyond [^{18}F]fluoromethyl and [^{18}F]fluoroethyl alkylating agents, longer chain alkyl and cycloalkyl derivatives have also been utilized as building blocks. Since the chemistry is identical to that already described and, in the interests of brevity, a comprehensive overview of these other agents is beyond the scope of this section.

TABLE 10. SYNTHESIS OF [^{18}F]FLUOROCHOLINE
(Courtesy of P. Scott, University of Michigan)

Synthetic step	Conditions
1. $^{18}F^-$ production	Production of $^{18}F^-$ via $^{18}O(p,n)^{18}F$ nuclear reaction.
2. $^{18}F^-$ preparation	$^{18}F^-$ is separated from the ^{18}O-water using an anion exchange cartridge (Sep-Pak® Light Waters Accell^{TM} Plus QMA cartridge) preconditioned to obtain it in its HCO_3 form.
3. $^{18}F^-$ drying	Azeotropic drying of $^{18}F^-$ using K_{222} (15 mg), K_2CO_3 (3.5 mg), in aqueous MeCN (1.5 mL, 66% MeCN).
4. [^{18}F]fluoromethyl tosylate synthesis	Ditosylmethane (7–8 mg) in MeCN (0.75 mL) and water (10 µL) is added to the dry $^{18}F^-$ from step 3 and heated at 120˚C for 10 min. The reaction mixture is cooled to 50°C.
5. Synthesis of [^{18}F]fluorocholine	DMAE (40 µL in 350 µL MeCN) is added to the reactor containing [^{18}F]methyl tosylate, and the reactor is heated to 120°C with stirring for further 10 min. The reaction mixture is then cooled to 60°C, and the reaction solvent is evaporated by maintaining 60°C and subjecting the reaction to Ar stream and vacuum for 5 min.
6. Purification of [^{18}F]fluorocholine	Sterile water (5.5 mL) is added to the dried crude reaction mixture from Step 5, and the resulting solution is passed through a C18-Plus Sep-Pak into a round bottom flask containing ethanol (10 mL). Unreacted ditosylmethane and [^{18}F]fluoromethyltosylate, as well as any tosylmethylcholine generated as a by-product, are removed by C18. This is repeated with an additional lot of sterile water (5.5 mL).
7. Formulation of [^{18}F]fluorocholine	The mixture from the round-bottomed flask is transferred through a CM-Light Sep-Pak to trap [^{18}F]fluorocholine. The Sep-Pak is washed with ethanol (15 mL) to remove unreacted DMAE, and water (20 mL) to remove residual ethanol. [^{18}F]Fluorocholine is eluted into a collection vial with saline, USP (3 mL) and diluted with an addition 7 mL saline. The formulated product (10 mL) is passed through a sterile filter (0.22 µm) into a sterile dose vial.
8. QC of [^{18}F]fluorocholine	QC of [^{18}F]fluorocholine is carried out in accordance with appropriate local regulations and using standard procedures [66]. RCP and concentration of fluorocholine and DMAE in a batch are determined using HPLC (Column: Waters SCX column, IC-PakTM Cation M/D, 3.9 × 150 mm, pn: WAT036570; mobile phase: 5 mM HCl (Fisher Scientific); flow rate: 1.25 mL/min; t_R~6.5 min; RCP should be > 95%, and residual DMAE should be <20 µg/mL.

5.2.2. ^{18}F labelled aromatic building blocks

A large number of ^{18}F labelled aromatic building blocks have been reported [64]. They benefit from the metabolic stability of fluorinated (hetero)arenes and most commonly involve incorporation of [^{18}F]fluoride into a phenyl or pyridyl ring, typically via S_NAr. Reactivity of the building block can be selected through functionality on that aromatic ring that is compatible with downstream reactions to be conducted. Three sub-types will be described: (i) [^{18}F]arenes bearing a carbonyl group, (ii) [^{18}F]fluoroaryl halides, (iii) [^{18}F]aromatic amines and alcohols. [^{18}F]Fluorophenyl azides and alkynes will be discussed in section 5.3 in the context of radioclick chemistry.

5.2.3. [^{18}F]arenes bearing a carbonyl group

Perhaps the best known example of an [^{18}F]arene bearing a carbonyl group is the [^{18}F]fluorobenzaldehyde building block (Fig. 50) [64]. Given that the aldehyde unit is strongly electron withdrawing, 2-[^{18}F]- and 4-[^{18}F]-fluorobenzaldehydes can be readily synthesized via S_NAr from the corresponding nitro-, trimethylammonium triflate or iodonium salt precursors,

while 6-[^{18}F]fluoronictotinaldehyde can be prepared from 6-chloronicotinaldehyde. They can also be synthesized using newer copper mediated radiofluorination reactions. The labelled aldehyde building blocks can then be employed in subsequent labelling reactions. For example, they can be coupled with amines to form imines or, under reductive amination conditions, secondary amines. In related chemistry, they can also be used to form oximes and hydrazones, or employed in aldol or multicomponent reactions.

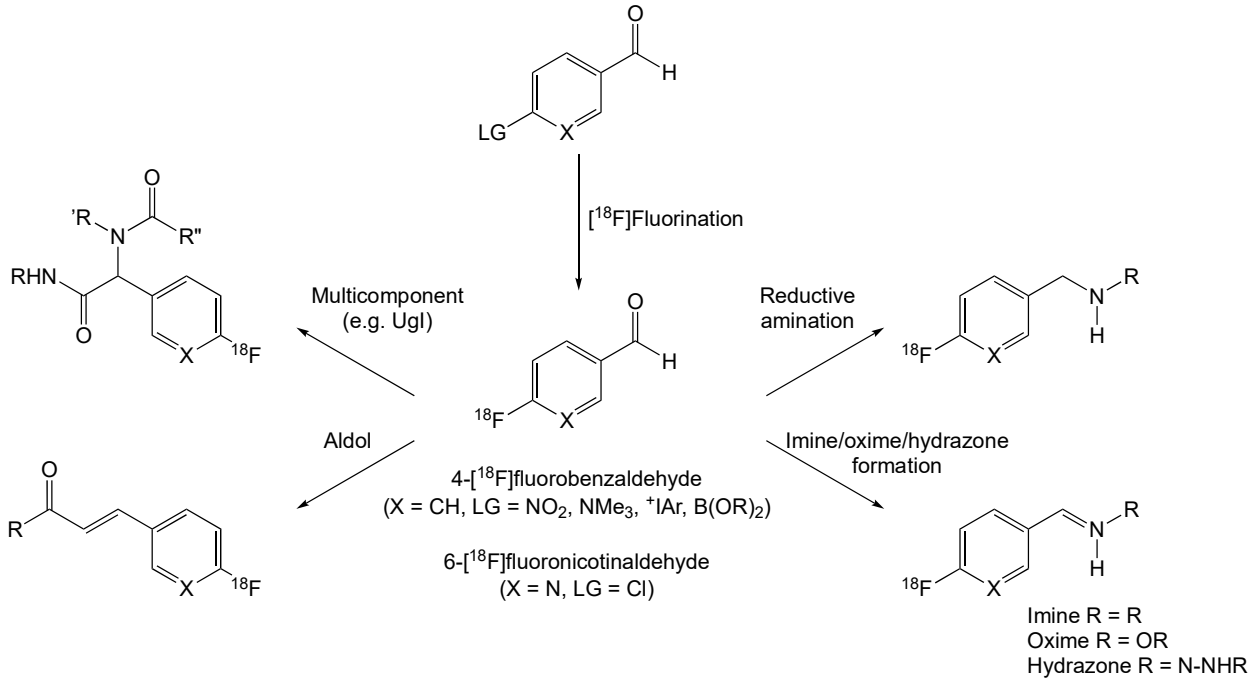


FIG. 50. [^{18}F]fluorobenzaldehyde building block (courtesy of P. Scott, University of Michigan).

[^{18}F]Fluorobenzoic acid can be synthesized by S_NAr using an ethyl benzoate precursor (usually -NO_2 or -N^+Me_3), followed by hydrolysis with a base (e.g. NaOH) [64]. [^{18}F]Fluorobenzoic acid can then be employed in coupling reactions directly or, more frequently, converted to an activated form such as N-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB). [^{18}F]SFB and related activated esters are discussed in section 5.3 in the context of their use as PGs for labelling peptides and proteins. However, activated esters of [^{18}F]fluorobenzoic acid can also be used to label sensitive small molecules [64].

5.2.4. [^{18}F]Fluoroaryl halides

Another important class of ^{18}F labelled aromatic building blocks are [^{18}F]fluoroaryl halides. For example, 4-[^{18}F]fluoroiodobenzene can be synthesized via S_NAr (Fig. 51). Historically this was accomplished using classical precursors (-NO_2, -N^+Me_3, -Br, -I, -IO_2, -$N_2^+BF_4$) with mixed results (since the aromatic ring is not activated for fluorination, RCYs tend to be low). Newer late stage fluorination of aryl iodonium salt, iodonium ylide and arylsulfonium salt precursors provide superior yields. Related building blocks include 1-bromo-3-[^{18}F]fluorobenzene and 2-bromo-[^{18}F]fluoropyridine. The building blocks can then be employed in a variety of different labelling reactions, including Pd-catalyzed Suzuki, Sonogashira and Stille reactions with organo-borons, alkynes and stannanes, respectively. Alternatively, they can be coupled with amines using Pd- (Buchwald-Hartwig) or Cu mediated reactions [2].

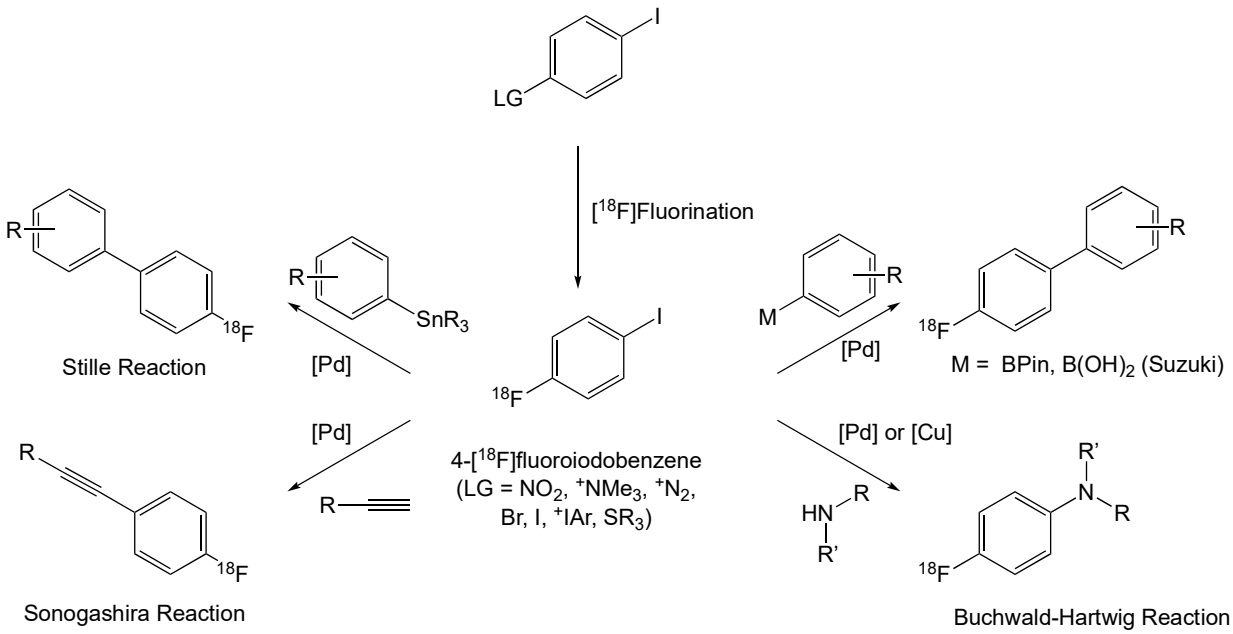


FIG. 51. 4-[^{18}F]Fluoroiodobenzene building block (courtesy of P. Scott, University of Michigan).

[^{18}F]Fluorobenzyl halides can be accessed from the corresponding [^{18}F]fluorobenzaldehyde building blocks. Reduction to the benzyl alcohol and subsequent halogenation provides the [^{18}F]fluorobenzyl halide. Alterantively, they can be synthesized via an appropriate precursor via S_NAr. Examples include 4-[^{18}F]fluorobenzyl bromide (Fig. 52) and they can be used to [^{18}F]fluoroalkylate a variety of molecules, including alcohols, phosphines and amines. Extending the alkyl chain to intermediates such as [^{18}F]fluorophenethyl bromide is also possible.

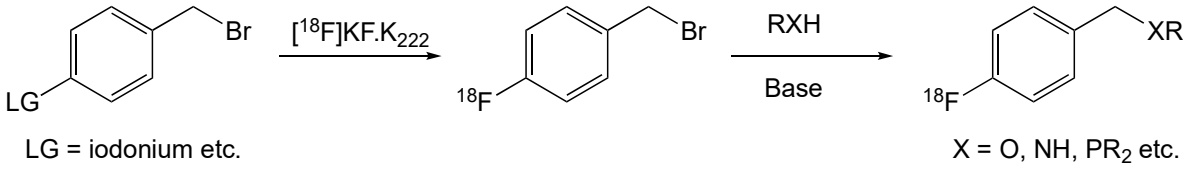


FIG. 52. 4-[^{18}F]fluorobenzyl bromide building block (courtesy of P. Scott, University of Michigan).

5.2.5. [^{18}F]Aromatic amines and alcohols

Aromatic amino building blocks based upon [^{18}F]fluorophenyl amines have been developed including: [^{18}F]fluoroanilines, [^{18}F]fluorobenzylamines, [^{18}F]fluorophenethyl amines and [^{18}F]fluorobenzohydrazides [64]. Similarly, [^{18}F]fluorophenols and [^{18}F]fluorobenzyl alcohols can be synthesized (Fig. 53) [64]. These building blocks contain a nucleophilic moiety, making them complimentary to the electrophilic halides described above. Thus, the [^{18}F]aromatic amines can be reacted with a variety of electrophiles (E^+) such as acid chlorides (amide coupling), sulfonyl chlorides (sulfonamide coupling), aryl chlorides (S_NAr), α,β-unsaturated carbonyl compounds (Michael reactions) or to synthesize guanidines.

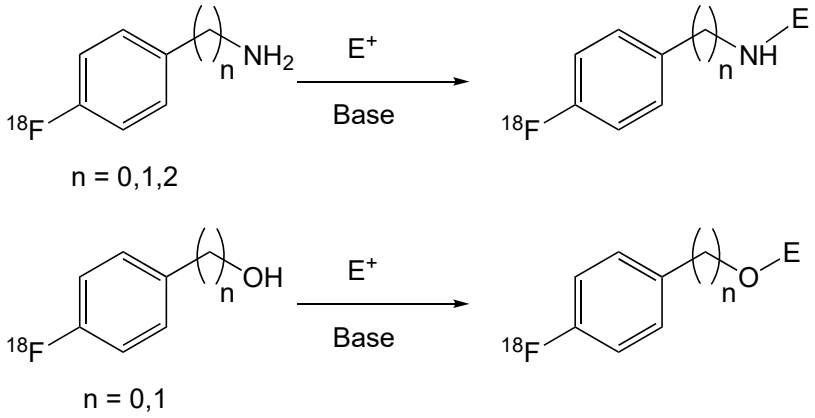


FIG. 53. [^{18}F]Aromatic amine and alcohol building blocks (courtesy of P. Scott, University of Michigan).

5.2.6. ^{18}F building blocks for click chemistry

'Click chemistry' is a term that was introduced by Sharpless in 2001 to describe reactions that include certain characteristics such as high yields and broad substrate scope, tolerance of air and moisture (water can even be a co-solvent), and which are quick and straightforward to conduct. Since these characteristics are particularly well tuned to the needs of radiochemists working with ^{18}F, there have been considerable efforts to develop radio-click chemistry [67]. For the purposes of this section, we will focus on use of radio-click chemistry to label small molecules.

A quintessential example of a click reaction is the copper-mediated 1,3-Huisgen cycloaddition reaction between an azide and an alkyne to generate a 1,2,3-triazole, and the first adaptation of this reaction for ^{18}F radiochemistry was reported in 2006 by Marik and Sutcliffe who synthesized ^{18}F-labeled alkynes, and used them to label azide functionalized peptides [68]. [^{18}F]alkynes can be prepared from the corresponding tosylate precursors using standard fluorination conditions ([^{18}F]fluoride, K_2CO_3, K_{222}, MeCN, 100ºC, 15 min), and the volatility of alkynes such as [^{18}F]fluoropentyne allows straightforward distillation (usually co-distillation with MeCN reaction solvent) from the labelling reaction into a second reactor containing the click reaction cocktail (e.g. azide functionalized component, CuI, sodium ascorbate, DIPEA, solvent). The click reaction is then stirred (at rt for 5 to 10 min) to generate the triazole product (Table 10, Fig. 54), which can then be purified and formulated for injection using standard approaches (HPLC and/or SPE).

An alternative strategy is to radiolabel the azide component (Fig. 53). In 2007, Glaser and Årstad reported the synthesis of [^{18}F]fluoroethylazide ([^{18}F]FEA) from 2-azidoethyl-4-toluenesulfonate ([^{18}F]fluoride, K_2CO_3, K_{222}, MeCN, 80ºC, 15 min) [7]. This volatile building block can also be co-distilled (usually with acetonitrile) into a second reactor containing the click reaction mixture (e.g. alkyne functionalized component, CuI, sodium ascorbate, DIPEA). Distillation of [^{18}F]FEA needs to occur ≤90ºC, otherwise 2-azidoethyl-4-toluenesulfonate co-distils and can participate in the click reaction to generate unwanted side products.

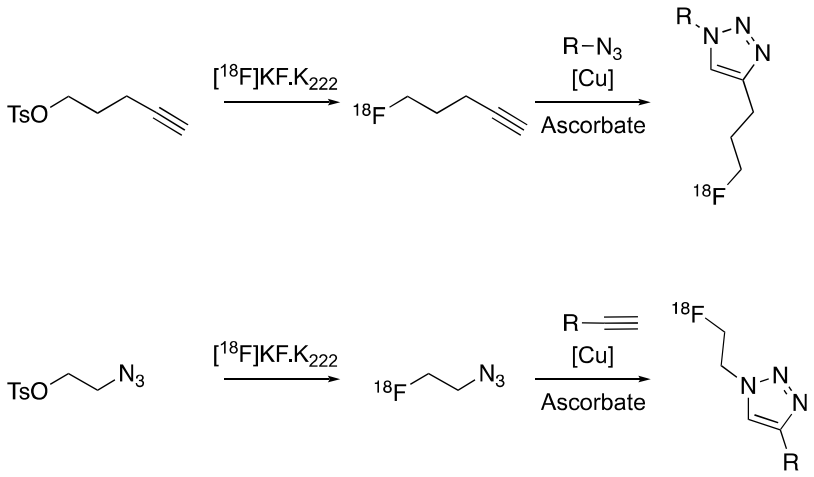


FIG. 54. Strategies for radio-click chemistry (courtesy of P. Scott, University of Michigan).

[^{18}F]FEA is likely the most popular building block for conducting radio-click chemistry using the copper mediated 1,3 Huisgen cycloaddition, and has been used to prepare hundreds of different PET radiotracers that would be difficult to access using direct [^{18}F]fluorination. For example, a variety of RGD peptides targeting the integrin $\alpha_v\beta_3$ receptor have been synthesized in this fashion. Besides these early building blocks, a variety of ^{18}F labelled azides and alkynes have been prepared for use in radio-click chemistry. Other notable examples include [^{18}F]aryl click building blocks such as 2-[^{18}F]fluoro-3-(hex-5-ynyloxy)pyridine ([^{18}F]FPy5yne) and (azidomethyl)-[^{18}F]fluorobenzenes that can be prepared using the S_NAr and late stage fluorination strategies described elsewhere in this publication. Radio-click chemistry with these other reagents proceed as described above.

TABLE 11. RADIO-CLICK CHEMISTRY
(courtesy of P. Scott, University of Michigan)

	Synthetic step	Conditions
1.	^{18}F-production	Production of $^{18}F^-$ via $^{18}O(p,n)^{18}F$ nuclear reaction.
2.	^{18}F-preparation	$^{18}F^-$ is separated from the ^{18}O-water using an anion exchange cartridge (Sep-Pak® Light Waters Accell™ Plus QMA cartridge) preconditioned to obtain it in its HCO_3^- form.
3.	^{18}F-drying	Azeotropic drying of $^{18}F^-$ using K_{222} (15 mg), K_2CO_3 (3.5 mg), in aqueous MeCN (1.5 mL, 66% MeCN).
4.	5-[^{18}F]fluoro-1-pentyne synthesis	Pent-4-ynyl-4-methylbenzenesulfonate (20–25 mg) in MeCN (1 mL) is added to the dry $^{18}F^-$ from step 3 and heated at 10˚C for 10 min to generate 5-[^{18}F]fluoro-1-pentyne which is gently distilled into a second reactor containing the click reaction mixture
5.	Synthesis of ^{18}F-triazoles via click chemistry	A typical click reaction mixture contains 0.1 mg of azide precursor, 0.2 mg phosphoramidite Monophos (N,N-dimethyldinaphto[2,1-d: 1,2-f][1,3,2]dioxaphosphepin-4-amine) in 0.1 mL DMSO, 1 mol% (0.05 mg) $CuSO_4.5H_2O$, 5 mol% (0.25 mg) sodium ascorbate (to reduce Cu(II) to Cu(I)), in 0.25 mL EtOH and 0.25 mL MeCN. The click reaction is stirred at room temperature for 10 min.
6.	Purification of ^{18}F-triazole	The crude reaction mixture is diluted and purified by semi-preparative HPLC.
7.	Formulation of ^{18}F-triazole	The HPLC fraction corresponding to the [^{18}F]triazole is collected, reformulated (e.g. C18 sep-pak) if needed and the final product is passed through a sterile filter (0.22 μm) into a sterile dose vial.
8.	QC of ^{18}F-triazole	QC of ^{18}F-triazoles is carried out in accordance with appropriate local regulations and using standard procedures [66].

Other variants of the classical Huisgen cycloaddition include strain promoted and copper free versions. Many of the strain promoted reactions have utilized ^{18}F labelled cyclooctynes that can react with azide functionalized molecules [69](Fig. 55). Alternatively, [^{18}F]FEA and other labelled azides are also able to react with different cyclooctyne scaffolds. The Staudinger ligation is a reaction between phosphane derivatives and azide functionalized molecules. Like the Huisgen cycloaddition reaction, either the azide (e.g. [^{18}F]FEA) or the phosphane derivative can be ^{18}F labelled. The approach has been used to prepare radiotracers where use of other approaches (direct labelling or other building blocks) have proven problematic.

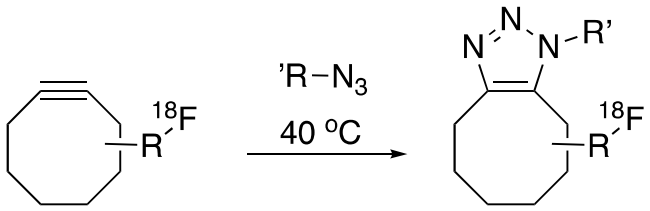


FIG. 55. Strain promoted radio-click chemistry (courtesy of P. Scott, University of Michigan).

A growing area of research involves tetrazine click reactions. The tetrazine unit is able to undergo an inverse-electron-demand Diels−Alder reaction without the need for a catalyst. Elemental nitrogen is lost meaning the reaction proceeds rapidly at room temperature and is non-reversible. Trans-cyclooctenes (TCO) are used as coupling partners and a selection of both [^{18}F]TCOs and [^{18}F]tetrazines have been prepared for such reactions [70] (Fig. 56). Eliminating the need for copper (and other reaction components) from these newer click reactions offers the enticing opportunity to conduct biorthogonal click reactions in vivo [71]. Such pretargeting approaches are attractive when an imaging agent with slow kinetics incompatible with the physical half-life of ^{18}F is needed (e.g. a labelled mAb for immunoimaging). In such cases, a TCO modified mAb is injected and given time (hours to days) to accumulate at (pretarget) the imaging site (e.g. tumour). Subsequently, an [^{18}F]tetrazine is injected and due to rapid distribution of the small molecule as well as the fast kinetics and high specificity of the click reaction, it reacts with the TCO on the mAb in vivo and enables mAb PET imaging with ^{18}F.

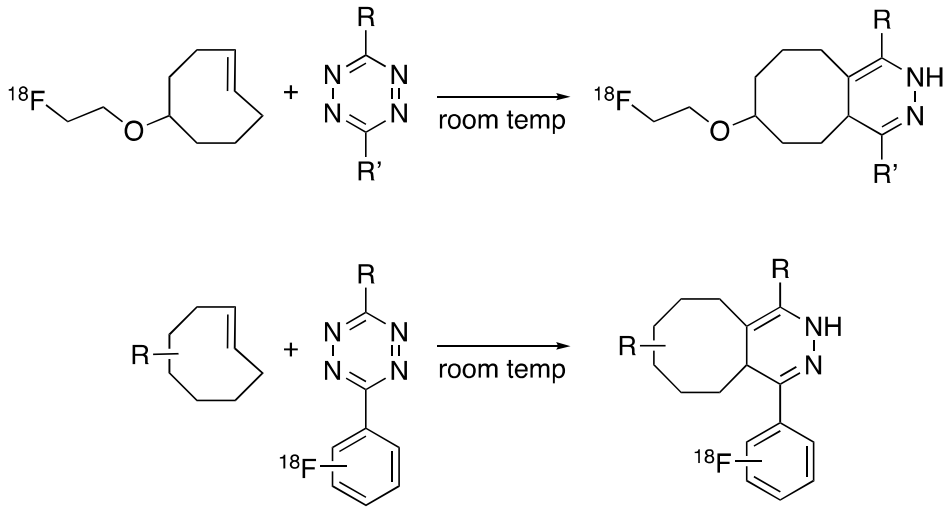


FIG. 56. Schematic representation Tetrazine / TCO Click ^{18}F radiolabelling (courtesy of P. Scott, University of Michigan).

5.2.7. Use of radiotracers as building blocks

A number of groups have investigated using commonly available small molecule PET radiotracers (and related molecules) as building blocks. For example, [^{18}F]FDG is the most widely used PET radiotracer around the world, and its daily availability in most PET centres makes it an appealing option for use as a building block. [^{18}F]FDG can either be used in its formulation, or azeotropically dried with acetonitrile if a different solvent is required. By way of example, [^{18}F]FDG can be reacted with amines and oxoamines to generate new radiotracers (Fig. 57). These radiotracers then need to be purified using the standard techniques described throughout this section (SPE, HPLC). Related work has been reported using 5-[^{18}F]fluoro-5-deoxyribose and 2-deoxy-2-[^{18}F]fluoroarabinofuranose, although these radiotracers are much less available than [^{18}F]FDG.

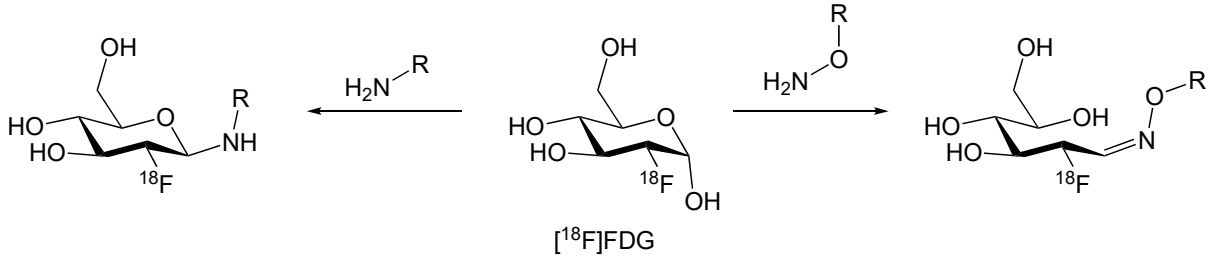


FIG. 57. Use of [^{18}F]FDG as a building block (courtesy of P. Scott, University of Michigan).

[^{18}F]FLT is another common radiotracer (vide infra) that is made routinely in many PET centres using it for multicentre oncology imaging trials. Reflecting this, it has also been employed as a building block by using the free hydroxyl group to couple it to bioactive molecules of interest (Fig. 58).

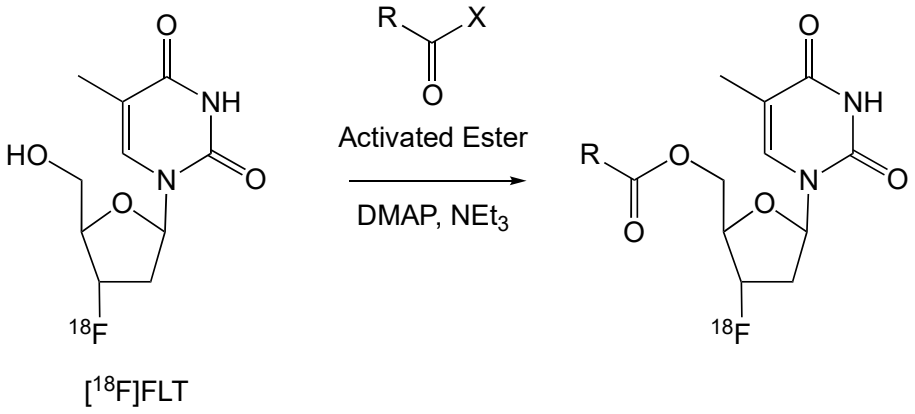


FIG. 58. Use of [^{18}F]FLT as a building block (courtesy of P. Scott, University of Michigan).

5.3. PGs FOR ^{18}F RADIOLABELLED BIOMOLECULES: FOCUS ON *N*-SUCCINIMIDYL-4-[^{18}F]FLUOROBENZOATE ([^{18}F]SFB) AND [^{18}F]MALEIMIDES

Prosthetic group labelling in ^{18}F radiochemistry constitutes a viable alternative to the direct one-step introduction of ^{18}F into radiotracers for PET. The development of small ^{18}F containing reactive building blocks, called PGs, stretches over a considerable period of time starting in the 80s. PG chemistry introduced a large variety of different molecular labelling tools such as [^{18}F]alkyltosylates (section 5.2), ^{18}F activated esters or [^{18}F]maleimides among many others

that provide access to ^{18}F radiotracers that are not easily obtainable by a simple one-step reaction with an [^{18}F]anion. In many cases, the targeted ^{18}F radiotracer is structurally too complex to enable a direct nucleophilic aliphatic (S_N2) or aromatic (S_NAr) reaction of $^{18}F^-$ with an eligible leaving group present in the precursor molecule. The most recent introduction of so called 'late stage fluorination' techniques for the ^{18}F introduction into de-activated aromatic systems has somewhat alleviated the situation at least for PET radiotracers labelled at an aromatic structural part of the molecule, but they are unable to provide radiotracers labelled at aliphatic structures or more complex molecular structures such as peptides and proteins (section 5.4). For this reason, PG radiochemistry continues to play an important role in contemporary tracer development as well as in established clinical PET tracer applications. This section will focus on two clinically relevant PGs, ^{18}F-activated esters and [^{18}F]maleimides, primarily used in peptide ^{18}F-labeling. It will provide insight into fundamental basics of PG utilization and will illustrate the underlying concepts by referring to biologically relevant examples. Other reviews covering the subject of PG radiolabelling will provide the interested reader with a more thorough insight into the applications of PGs in ^{18}F radiochemistry, covering common as well as exotic ^{18}F-PGs for PET tracer development [64, 72].

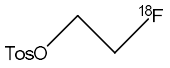
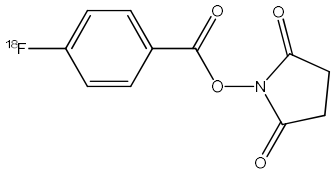
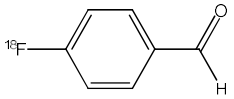
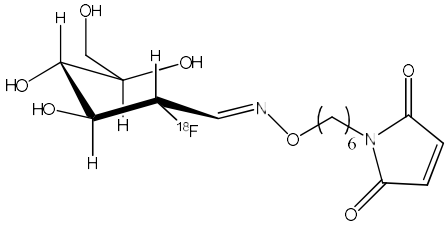
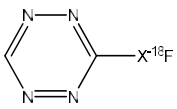
5.3.1. General considerations, scope and limitations

The synthesis of most [^{18}F]PGs relies on the introduction of nucleophilic $^{18}F^-$ into small molecular building blocks containing a leaving group (e.g. tosylate or triflate) that is replaced by ^{18}F and a reactive part that is intended to react with a chemical group (e.g. NH_2, OH or SH) present in the molecule to be radiolabelled. To ensure a high quality of the resulting PET tracer from PG labelling, the PG synthesis should be short, efficient, automated and yield the PG in high RCY and RCP. This will ensure a high molar activity of the radiotracer which is important for its clinical application [73]. Additionally, the PG should be highly reactive towards the intended group labelling and if possible be orthogonal, meaning that the PG exclusively or preferably reacts with just one chemical group in the radiotracer precursor molecule. Click chemistry (see section 5.2.5), a term coined by Sharpless in 2001 [74], has found its entry into radiochemistry and impressively introduced a great variety of novel orthogonal PGs omitting the commonly required need for protecting group chemistry alongside the PG radiolabelling procedure. Today, radiochemists can choose from a rich catalogue of diverse PGs varying in structure, reactivity and scope (Table 10). Some are less orthogonal but useful to quickly radiolabel large molecules such as proteins in one step without chemically modifying the protein (e.g. active ester PGs). Others such as [^{18}F]maleimides or [^{18}F]thiols for peptide labelling are very specific in their reaction pattern but potentially necessitate the chemical modification of the precursor molecule by chemically introducing the complementary clickable group (either a maleimide for thiols or a thiol for maleimides). The most prominent PG is without doubt 2-[^{18}F]fluoroethyl tosylate ([^{18}F]FETOs). This PG was introduced in 1987 by Block and Coenen and over decades became the 'work-horse' in PG labelling of complex radiotracers for neurological and oncological PET imaging [75]. As this particular PG is covered in-depth in Section 5.2, this section focuses in two frequently employed PGs: (a) reactive but unspecific ^{18}F activated esters for peptide and protein labelling and (b) [^{18}F]maleimides/thiols as a powerful example for a click chemistry application in radiotracer syntheses.

The most prominent active ester for ^{18}F labelling is without doubt *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) first described by Vaidyanathan and co-workers [76]. Over many years, the [^{18}F]SFB synthesis has evolved into a reliable and clinically applicable method to radiolabel complex organic molecules (peptides and proteins) by targeting nucleophilic

groups present in the precursor molecule. The previously complex and lengthy radiosynthesis of [^{18}F]SFB posed initially some obstacles for its general dissemination as a popular PG but more efficient procedures enabling the automated and HPLC free synthesis of [^{18}F]SFB elevated this PG into one of the most frequently applied labelling tools. [^{18}F]SFB's perfect balance between reactivity and stability (hydrolysis to [^{18}F]fluoro benzoic acid) in an ^{18}F labelling environment has stimulated the introduction of many derivatives featuring various structural modifications (Table 11, entry 2). In addition, efficient click chemistry entries in radiolabelling have evolved such as the reaction between amines/thiols and maleimides that perfectly convey the general principles of a contemporary orthogonal PG application. The underlying click chemistry is based on a Michael addition yielding either ^{18}F-amino- or thiopyrrolidine-2,5-diones. Some degree of selectivity between amines and thiols, common groups which can be both present in complex molecules such as peptides, stems from the faster kinetics observed for the reaction of maleimides with thiols in comparison to amines. Besides this possible competition for chemoselectivity, the Michael addition is orthogonal, highly efficient and fulfils all criteria of a click chemistry type reaction. In the following table, [^{18}F]SFB and [^{18}F]maleimides will be highlighted as to their position in modern ^{18}F labelling chemistry. Typical examples for their clinical applicability will be given and radiosynthesis as well as QC procedures will be explained to guarantee procedural reproducibility.

TABLE 12. SOME EXAMPLES OF FREQUENTLY USED PGs IN TRACER DEVELOPMENT AND PRE-CLINICAL PET IMAGING
(*courtesy of R. Schirrmacher, University of Alberta*)

Entry	PG structure	PG use in ^{18}F-labeling
1) 2-[^{18}F]FETos		Compounds of low molecular weight e.g. brain receptor ligand.
2) [^{18}F]SFB		Peptides and proteins; building block for other PGs
3) 4-[^{18}F]fluorobenzaldehyde		Peptides
4) [^{18}F]FDG-MHO		Peptides
5) 2-[^{18}F]fluoroethyl azide		Peptides
6) [^{18}F]tetrazines		Peptides

Even though 2-[^{18}F]fluoroethyl tosylate is the most frequently applied PG in ^{18}F labelling of radiotracers, it has its limits preventing its general use. Especially when temperature sensitive compounds such as peptides and proteins are the subject of radiolabelling, [^{18}F]FETs clearly reaches its limits. The problem is rooted in [^{18}F]FETs' relatively low reactivity towards

nucleophilic groups (NH_2, OH etc). This chemical behaviour stems from the fact that the tosyl leaving group is categorized as a medium reactive nucleofuge only and necessitates high temperatures which can be incompatible with some peptides and almost all proteins. Peptides, even though they would probably react with [^{18}F]FETs, would result in a multitude of different [^{18}F]fluoroethylated peptides due to multiple nucleophilic groups present on side chains, requiring a laborious purification procedure not compatible with clinical applicability. Activated esters in contrast are well suited for polyfunctional biomolecular labelling of peptides and proteins because of their higher reactivity towards nucleophiles. Most activated esters react at room temperature with nucleophilic precursors.

5.3.2. [^{18}F]SFB: a versatile PG for radiolabelling

[^{18}F]SFB is mostly used as a PG reacting with terminal NH_2 groups of Lys side chains. The first reported and rather cumbersome multistep synthesis of [^{18}F]SFB has fortunately been replaced by improved automated production procedures that can easily be implemented into any common ASU. Convenient and straightforward syntheses of [^{18}F]SFB have been reported by Wüst et al. [77], As presented in Fig. 59, where (1) N,N,N′ ,N′ -Tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (**1**) reacts with ^{18}F$^-$to yield 4-[^{18}F]fluorobenzoic t-Bu ester as an intermediate product (not isolated). (2) the t-Bu ester is cleaved by HCl treatment to yield 4-[^{18}F]fluorobenzoic acid (**2**). (3) in situ synthesis of [^{18}F]SFB (**3**) using TSTU to introduce the active ester moiety. They utilized the highly reactive precursor ethyl 4-(*N,N,N*-trimethylamonium)benzoate triflate for the introduction of ^{18}F$^-$ in high RCYs. The subsequent hydrolysis of the resulting radiolabelled ester to the corresponding carboxylic acid and the final formation of the succinimidyl ester using N,N,N′ ,N′ -tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU) yields [^{18}F]SFB in satisfactory RCYs. The reaction sequence can be done in a two reactor ASU. The automation was realized in 2005 by the same group who reported on a fully automated [^{18}F]SFB synthesis which stably and reproducibly yields the PG in good RCYs (Table 12). This automated microwave assisted procedure provides [^{18}F]SFB with 34–38% RCY and RCP of 93–96% within one hour. A TRACERlab Fx_{FDG} (GE Medical Systems) was employed for the synthesis, however any ASU capable of nucleophilic [^{18}F]fluorination can be used. The synthetic steps for a 2 reactor ASU setup are displayed in Table 12.

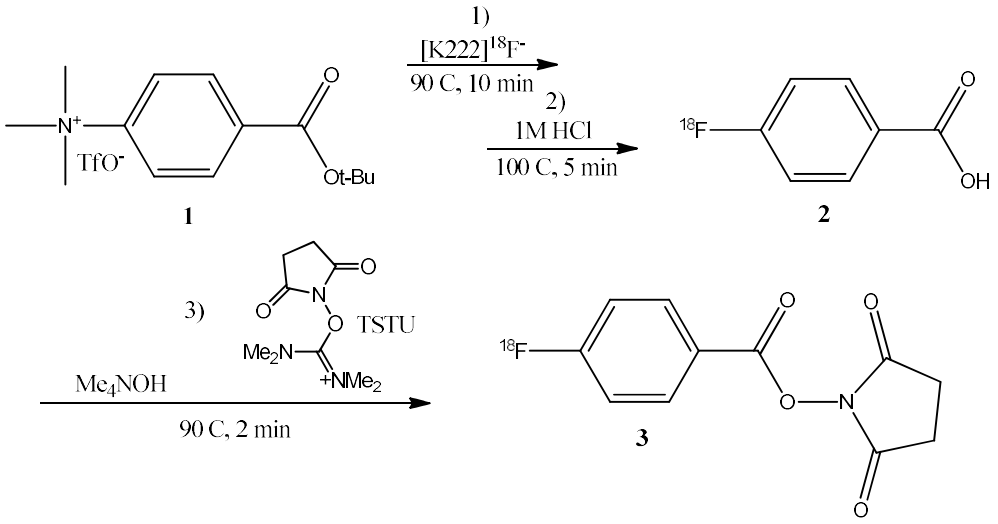
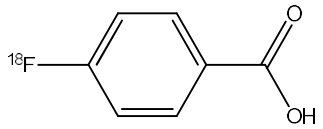
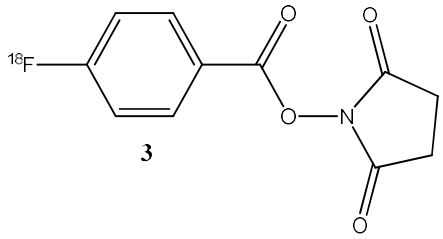


FIG. 59. Synthesis scheme of the automated [^{18}F]SFB synthesis (courtesy of R. Schirrmacher, University of Alberta).

TABLE 13. STEPWISE SYNTHESIS OF [^{18}F]SFB USING A NUCLEOPHILIC AUP (2 REACTOR SETUP)
(courtesy of R. Schirrmacher, University of Alberta)

Synthetic step	Conditions
1. ^{18}F-production	Production of $^{18}F^-$ (depends on the nuclear reaction and the cyclotron used)
2. ^{18}F-preparation, reactor 1	Before the azeotropic drying, the $^{18}F^-$ is separated from the ^{18}O-water using an anion exchange cartridge (Sep-Pak® Light Waters Accell^{TM} Plus QMA cartridge) preconditioned to obtain it in its HCO_3-form.
3. ^{18}F-drying, reactor 1	Azeotropic drying of $^{18}F^-$ using K_{222} (15 mg, 40 µmol), K_2CO_3 (2.77 mg, 20 µmol), in aqueous MeCN (1.5 mL, 66% MeCN)*.
4. 4-[^{18}F]fluorobenzoic acid (**2**) synthesis, reactor 1  **2**	N,N,N′ ,N′ -tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (**1**) (5 mg, 13 µmol) in MeCN (1 mL) is added to the dry $^{18}F^-$ from step 3 and heated at 90˚C for 10 min (introduction of ^{18}F). 1M HCL (500 µL) is added and the mixture is heated to 100˚C for 5 min (removal of the t-Bu protecting group). After cooling down, water (12 mL) is added to the reactor and the mixture is passed through a Chromafix 250-HR-P cartridge (Macherey-Nagel, Germany) for the SPE of 4-[^{18}F]fluorobenzoic acid. The compound is eluted from the cartridge into a second reactor with MeCN (3 mL).
5. Synthesis of [^{18}F]SFB (**3**), reactor 2  **3**	To the eluate from reactor 1, 25% methanolic Me_4NOH (20 µL) in MeCN (500 µL) is added. The mixture is dried in a stream of nitrogen under reduced pressure at 90˚C. The drying step is repeated by adding MeCN (3mL) under the same conditions. A solution of TSTU (15 mg, 50µmol) in anhydrous MeCN (500 µL) is added and the mixture is heated at 90˚C for 2 min (formation of [^{18}F]SFB). After cooling, 5% aqueous acetic acid (5%, 10 mL) is added and the crude [^{18}F]SFB is passed through a second Chromafix 250-HR-P cartridge to extract the [^{18}F]SFB.
6. Final formulation of **3** for subsequent radiolabelling	The Chromafix 250-HR-P cartridge loaded with [^{18}F]SFB is washed with a water:MeCN mixture (65:35) (10 mL). The purified [^{18}F]SFB is eluted from the cartridge with MeCN (3 mL).
7. QC of **3**	Radiochemical and chemical purity data of [^{18}F]SFB is obtained via HPLC, a LiChrospher WP300 RP-18 column (5 mm, 250 mm × 3 mm + precolumn 4 mm × 4 mm, Merck) and a UV detector (UV-975, 230 nm) coupled in series with a radioactivity detector can be used. HPLC runs are carried out at a flow rate of 0.5 ml/min with the following gradient of eluents (A ¼ MeCN with 0.2% TFA; B ¼ water with 0.2% TFA): 0 min – 10% A/ 90% B; 10 min – 60% A/ 40% B; 14 min – 100% A/ 0% B; 20 min –100% A/ 0% B to guarantee good separation#.

*other drying methods can be employed which remove the ^{18}F-water before the azeotropic drying step (e.g. Munich method);
other HPLC setups might yield the same analytical accuracy.

A one-pot synthesis of [^{18}F]SFB following the Wüst protocol, including the same synthesis scheme, further decreasing the synthetic effort is possible. This protocol utilizes an ASU with only one reactor which might make it more vulnerable to complications. Instead of a t-Bu ester, and 4-(trimethyl-ammonium triflate)benzoate was used as a precursor for ^{18}F introduction. The ester deprotection was achieved using tetrapropylammonium hydroxide instead of HCl. The intermittent step of removing the 4-[^{18}F]fluorobenzoic acid from the [^{18}F]fluorination cocktail was omitted by azeotropically drying the reaction mixture before adding the TSTU for active ester formation. The final workup was achieved by solid-phase extraction similar as described in the Wüst protocol. In 2014, a microwave assisted one-pot [^{18}F]SFB synthesis which slightly differs from the protocols discussed above has shown to be successful. Furthermore, a simplified protocol for the automated production of [^{18}F]SFB on an IBA Synthera module has been published. By introducing a combined hydrolysis and water removal step, the production was achieved within 58 minutes with a RCY of 75-85 %.

[^{18}F]SFB has been used for example for the synthesis of clinical grade N-(4-[^{18}F]fluoro-benzoyl)-interleukin-2 for imaging of activated T-cells in humans. The synthesis of [^{18}F]SFB and the subsequent synthesis of ^{18}F labelled interleukin-2 followed a fully GMP compliant protocol [78].

5.3.3. Maleimide derived [^{18}F]PGs

The reaction between thiols (mostly on Cys side chains) and maleimides is a powerful and versatile click chemistry method which has found multiple applications in modern ^{18}F radiolabelling for PET tracer development. A great variety of different ^{18}F labelled maleimides have been reported in the literature dating back to 1989 when the first multi-step synthesis of two different N-(p-[^{18}F]fluorophenyl)maleimides for the labelling of a monoclonal antibody was reported [72]. These first [^{18}F]maleimides were difficult to prepare and did not raise a lot of interest. Most published [^{18}F]maleimide syntheses necessitate the preparation of another PG. 4-[^{18}F]Fluorobenzaldehyde [79], an easy to synthesize building block and PG, [^{18}F]SFB and even [^{18}F]FDG have been frequently employed as integral tools for [^{18}F]maleimide synthesis and further radiolabelling of peptides and proteins. Especially the use of [^{18}F]FDG yields a maleimide-based PG that takes advantage of a commercially available radiopharmaceutical for advanced PG synthesis. The [^{18}F]FDG-maleimide PG, [^{18}F]FDG-MHO (Fig. 60 (5)), is hydrophilic and therefore might reduce the unspecific in vivo binding of labelled peptides that is often observed when PGs impart a substantial degree of lipophilicity to the tracer molecule. The conjugation of [^{18}F]FDG with aminooxymaleimide hydrochloride (Fig. 60 (4)) yields [^{18}F]FDG-MHO (Fig. 60 (5)) in high RCYs of 42% based on the starting activity of [^{18}F]FDG (Fig. 60). [^{18}F]FDG in its tautomeric open-ring acyl form reacts with an aminooxy derivatized maleimide precursor via formation of an oxime bond. The applicability of [^{18}F]FDG-MHO (Fig. 60 (5)) was demonstrated through the labelling of glutathione and Annexin A5 for imaging apoptosis. Even at a protein concentration of just 1 ng/mL, 38% RCY of ^{18}F labelled GSH were observed by yielding good molar activities. The convenient radiosynthesis of [^{18}F]FDG-MHO which still requires a final HPLC purification is further explained stepwise in Table 13.

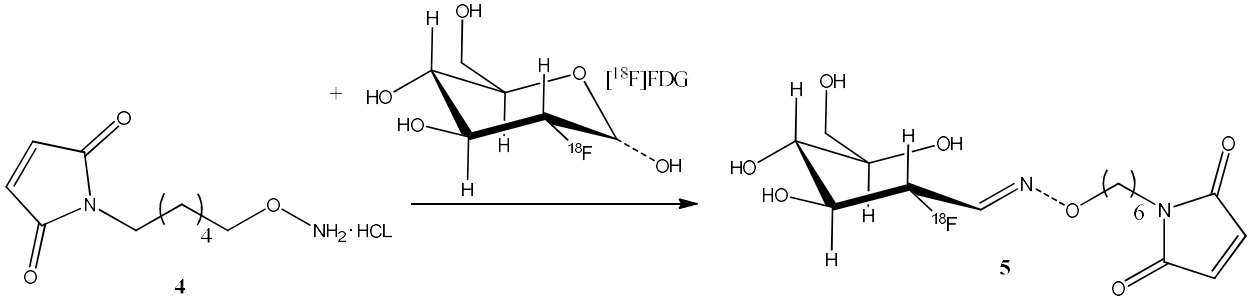
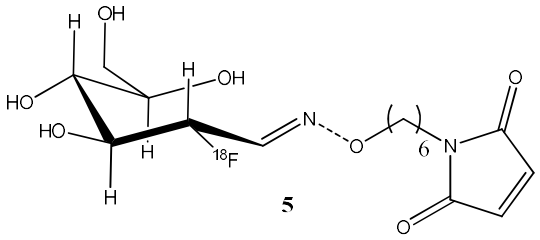


*FIG. 60. Radiochemical synthesis of [^{18}F]FDG-MHO (**5**) (courtesy of R. Schirrmacher, University of Alberta).*

TABLE 14. SYNTHESIS OF [^{18}F]FDG-MHO USING [^{18}F]FDG
(courtesy of R. Schirrmacher, University of Alberta).

Synthetic step	Conditions
1. Coupling of [^{18}F]FDG to aminooxymaleimide hydrochloride (Figure 5.25)	[^{18}F]FDG solution (1 mL) is added to aminooxymaleimide hydrochloride (5 mg, 13.3 μmol) in ethanol (4 mL) and heated to 100°C for 15 min (sealed reaction vial).
2. Solid phase cartridge extraction of crude [^{18}F]FDG-MHO	The solvent is evaporated under reduced pressure and water (20 mL) is added. The aqueous solution is passed through a SepPak cartridge to extract the crude [^{18}F]FDG-MHO. The crude, un-purified [^{18}F]FDG-MHO is eluted from the cartridge with MeCN (1 mL).
3. HPLC purification of crude [^{18}F]FDG-MHO	The MeCN solution containing the [^{18}F]FDG-MHO (1 mL) is subjected to an HPLC system equipped with a Merck LiChroCart 250-10 column at a flow rate of 4 mL/min. The peak corresponding to [^{18}F]FDG-MHO is collected, diluted with water and passed through a second SepPak cartridge to extract the product and remove the HPLC solvent.
4. Elution of purified [^{18}F]FDG-MHO from solid phase cartridge for subsequent radiolabelling.  **5**	[^{18}F]FDG-MHO is eluted from the cartridge using ethanol (1 mL) and is either used in this solution or reconstituted in another solvent for further use.

Unfortunately, many PG syntheses are not easily transferred to a commercially available ASU. Only [^{18}F]FETs and [^{18}F]SFB are prominent examples of routinely available PGs for ^{18}F labelling. Multistep syntheses that require more than two reactors in an ASU are probably not well suited for automation. The failure rate of the radiosynthesis is directly proportional to the complexity of the automated procedure. It should be noted that [^{18}F]FETs and [^{18}F]SFB cover a wide range of possible tracer syntheses. [^{18}F]FETs gives access to tracers of low molecular weight via [^{18}F]fluoroethylation of nucleophilic groups and [^{18}F]SFB covers molecules of higher molecular weight. Whereas [^{18}F]FETs have been applied in clinically used radiopharmaceuticals such as [^{18}F]fluoroethyl tyrosine, [^{18}F]SFB is still sparsely used. Besides the application of [^{18}F]SFB in N-(4-[^{18}F]fluoro-benzoyl)-interleukin-2 [79], a first in man study was published with [^{18}F]fluoro-PEG-folate [80].

5.3.4. Concluding remarks

The large available plethora of established, more experimental and even exotic PGs gives radiochemists an extensive modular toolbox at hand to synthesize almost any conceivable radiotracer. The limits to the PG approach are usually not related to the accessibility of the used PG chemistry but rather stemming from the structural alteration of the target molecule by the PG amendment. The extra molecular moiety, not being a genuine part of the molecule, sometimes changes the binding affinity of the tracer to its target or negatively influences the in vivo biodistribution. All these characteristic features of PG labelling have to be taken into consideration before engaging into a complex radiosynthesis. For most clinically applied radiotracers however, resourceful ASU manufacturing companies offer tailor made cassette based ASUs that deliver the radiotracer on demand by simply using the cassette and kit provided and feeding the ^{18}F aqueous solution from the cyclotron target into the ASU. The

synthesis and purification are done by the ASU and the radiochemist is only required to ensure an acceptable quality of the tracer. These cassette based ASUs are fully GMP compliant and deliver the radiotracer on demand. Their dissemination is becoming more widespread with the availability and introduction of more clinically useful radiotracers. Hence, PGs will continue to play an important role in radiochemistry and PET tracer manufacturing and its full potential to access clinically relevant and accepted radiopharmaceuticals should be further pursued.

5.4. LATE STAGE FLUORINATIONS THROUGH METAL CATALYSIS

5.4.1. General considerations

Traditional aromatic substitution reactions involving [^{18}F]fluoride require activating electron withdrawing groups at the ortho and/or para position of the leaving group. In the absence of such activating groups, RCYs are either extremely low or electrophilic ^{18}F could be used which results in low molar activities (see Section 5.1).

To have access to ^{18}F radiopharmaceuticals requiring high molar activity and containing aromatic scaffolds without activating groups, late stage fluorination reactions are very useful. These reaction types do not require activating groups and developments have concentrated in two areas: (i) new activated precursors (e.g. iodonium ylids, sulfonium salts, sydnones) and (ii) reactions catalysed by transition metal based catalysis. The former is described in Section 5.1 on nucleophilic aromatic substitution, while this section describes new transition metal mediated [^{18}F]fluorination reactions. In Fig. 61 several examples of such reactions are shown. Ritter and Hooker [81] reported one of the first attempts to circumvent this problem by generating a high molar activity electrophilic [^{18}F]fluorinating compound based on palladium form [^{18}F]fluoride (see Fig. 61). Because of several shortcomings of this first method regarding functional group tolerability, synthesis of Pd-starting materials and automation, the same groups also explored alternative metal catalysts such as Ni(II)-aryl complexes. However, RCYs became low when radiosyntheses were scaled up.

Currently, most of the late stage strategies for the radiofluorination of (hetero)arenes are still in the exploratory phase. However, the promising new possibilities of these strategies made radiochemists to pursue these metal catalysed reactions.

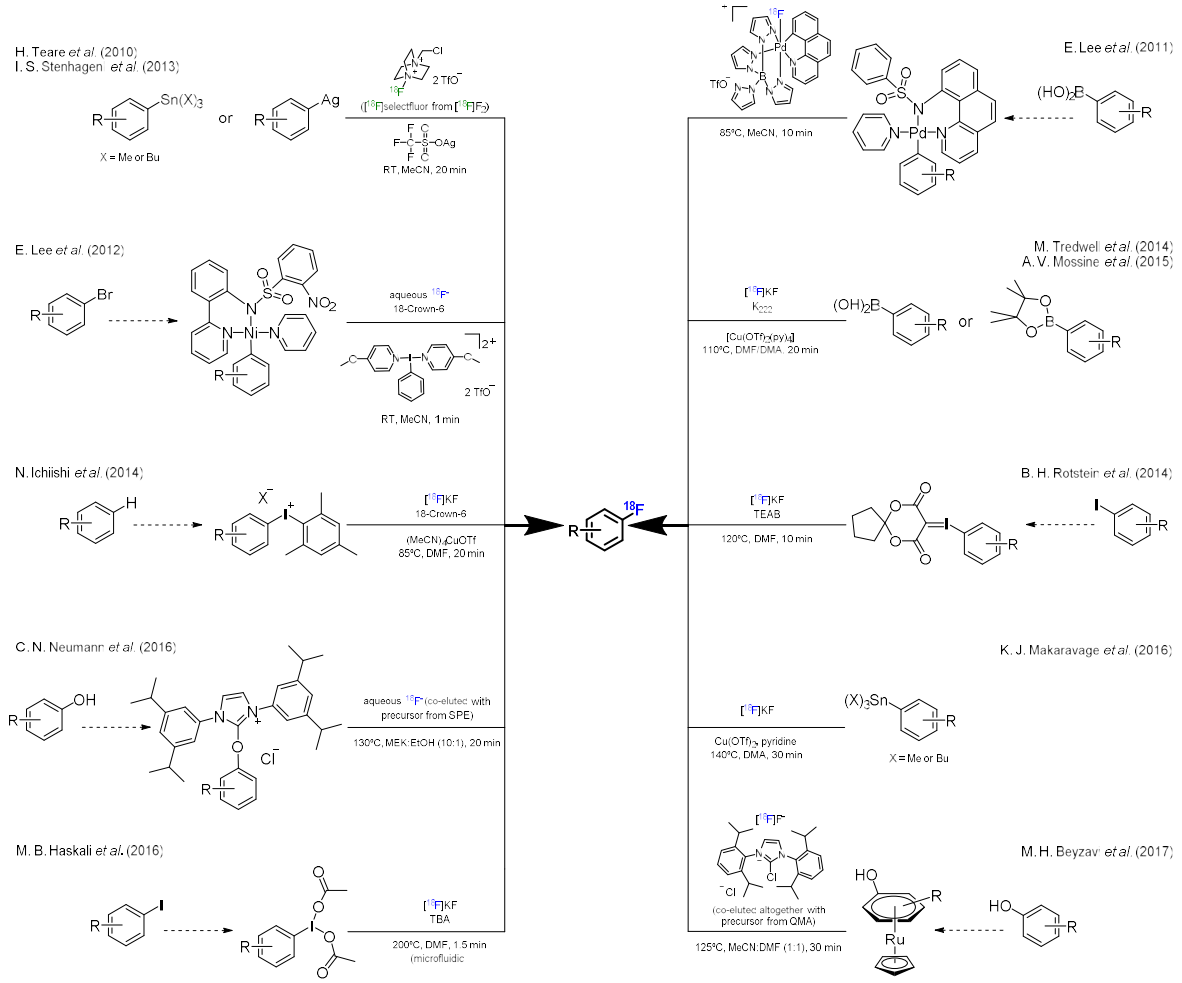


FIG. 61. Overview of some late stage strategies for the [^{18}F]fluorination of (hetero)arenes (courtesy of G. dos Santos Clemente, PhD thesis, University of Groningen).

Of the new transition metal mediated [^{18}F]fluorination reactions reported to date, Cu mediated fluorination has emerged as a useful radiofluorination strategy that has been widely utilized in the PET radiochemistry community. The organometallic chemistry was originally developed by the Sanford lab for standard fluorination of iodonium salts and organoborons, and the first report utilizing such an approach for [^{18}F]fluorination was the Cu-mediated [^{18}F]fluorination of (mesityl)(aryl)iodonium salts reported by Sanford and Scott [82]. Subsequently, a Cu mediated method for the [^{18}F]fluorination of pinacol boronate esters was described by the Gouverneur group, while Sanford and Scott have also reported [^{18}F]fluorination of organoborons [83], organostannanes [84], aryl halides [85] and aromatic C-H bonds [86]. Of these approaches, three methods displayed in Fig. 61 have been most widely used: (a) The method using iodonium salts, (b) Copper mediated oxidative radiofluorination of aryl boronic esters (aryl pinacol boronate) and (c) Cu mediated fluorination of aryl stannanes. In addition, [^{18}F]deoxyfluorination of Ru mediated phenol complexes has also found applications and will therefore be discussed in section 5.4.6 as well.

5.4.2. [^{18}F]Radiofluorination of aryl boronic pinacol esters

Radiofluorination of aryl boronic esters has been increasingly used during the last years, showing appropriate tolerance to electron-poor, neutral and rich arenes, as well as to various functional groups which sometimes require protection [87]. The boronic pinacol ester

precursors can be relatively easy prepared from the bromo aryl intermediate. By using the commercially available catalyst $Cu(OTf)_2(py)_4$, the Gouverneur lab described the synthesis of several ^{18}F labelled compounds, some of which have been used in the clinic ([^{18}F]FMTEB, [^{18}F]FPEB, [^{18}F]flumazenil, [^{18}F]DAA1106, [^{18}F]MFBG, [^{18}F]FDOPA, [^{18}F]FMT and [^{18}F]FDA). The general reaction scheme is depicted in Fig. 62.

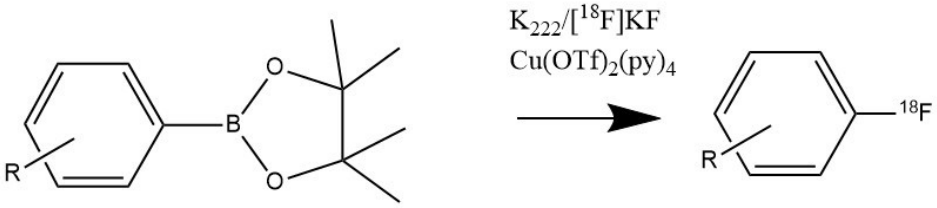


FIG. 62. General reaction scheme for Cu mediated [^{18}F]fluorination of boronic pinacol ester precursors (courtesy of P. Elsinga, University of Groningen).

The Cu complex goes through three oxidation states to enable the transmetalation between $[Cu(OTf)_2(py)_4]$ and the aryl pinacol boronate precursor, followed by [^{18}F]fluorine coordination and fast reductive elimination from an intermediate aryl-copper(III)-fluoride complex. This mechanism leads to the formation of a new covalent bond between the aryl ring and [^{18}F]fluorine, while the residual Cu(I) complex can reoxidize and be continuously involved in a transmetalation-coordination-reduction cycle (Fig. 63)[88].

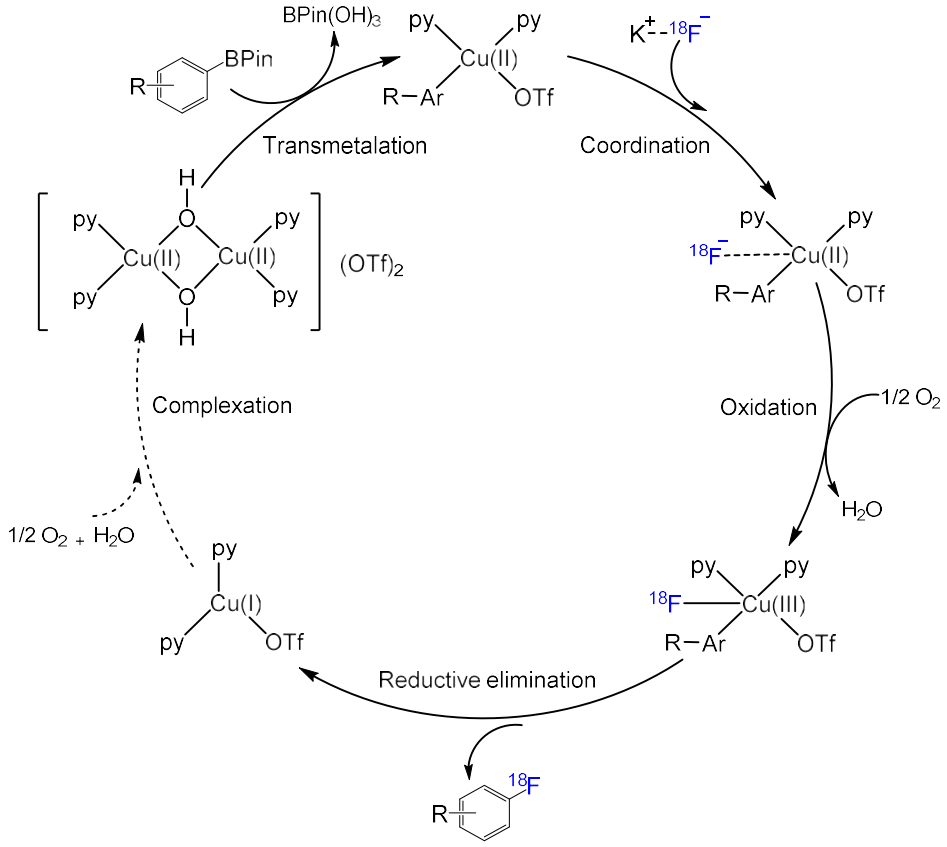


FIG. 63. Proposed mechanism for Cu mediated oxidative [^{18}F]fluorination of arylboronic esters (reproduced from Ref. [88] with permission).

The reaction is known to be base sensitive, therefore often the use $K_2C_2O_4$ is recommended to elute [^{18}F]fluoride from anion exchange cartridges instead of K_2CO_3. Furthermore, oxygen is required to maintain the catalytic cycle. [^{18}F]Fluorinations are typically performed in DMF or DMA at 110–140˚C for 10–20 min yielding ^{18}F product with high molar activities. DMA often shows to be superior to DMF with respect to RCYs. Radiochemical yields strongly vary depending on the precursor usually ranging between 15% and 70%. Functional groups containing NH, OH and SH require protection during the fluorination step. Also, the presence of some heterocycles can result in deactivation of the Cu catalyst. The main side product is the unreacted hydrolysed precursor being the H-analog of the fluoro-compounds. HPLC separation of both H and ^{18}F compounds can be challenging [89].The synthetic steps for this method are displayed in Table 14.

TABLE 15. STEPWISE SYNTHETIC PROCEDURE FOR Cu MEDIATED OXIDATIVE [^{18}F]FLUORINATION OF ARYLBORONIC ESTERS
(courtesy of P. Elsinga, University of Groningen)

Synthetic step	Conditions
1. ^{18}F-production	Production of $^{18}F^-$ (depends on the nuclear reaction and the cyclotron used)
2. ^{18}F-preparation, reactor 1	Before the azeotropic drying, the $^{18}F^-$ is separated from the ^{18}O-water using an anion exchange cartridge (PS-HCO3 anion exchange)
3. ^{18}F-drying, reactor 1	PS-HCO3 cartridge is dried by purging with air followed by washing with 2 ml of 2-propanol and purged with air.
4. ^{18}F-fluorination	[^{18}F]fluoride is eluted with a solution of 3 mg of tetraethylammonium bicarbonate in 0.5 ml of 2-propanol into reaction vial containing a mixture of 13.8 mg of tetrakis(pyridine)copper(II) triflate and 4 mg of boronic pinacol ester precursor in 0.6 ml of dimethylacetamide. The reaction mixture is heated at 140 degrees for 20 minutes under stirring. Then the reaction is cooled and potentially a deprotection step needs to be performed.
5. Purification and reformulation	The final reaction mixture is diluted with an amount of mobile phase and is purified by HPLC followed by a reformulation step.

A variation on the method described above uses $Cu(OTf)_2$ as the copper source, in conjunction with pyridine [83]. This method is compatible with both boronic acid and BP in precursors and, in contrast to the Gouverneur's approach, it can be carried out in an inert atmosphere which simplifies automation. The approach has been used to synthesize a range of ^{18}F labelled compounds including [^{18}F]TRACK and [^{18}F]FDOPA for clinical use [90].

Scaling up and automating these syntheses has presented challenges, usually associated with decreased RCYs, because of differences in reaction volume, radioactivity concentration and amount of base. To this end, significant efforts have been undertaken to optimize automation [91] and to develop minimalist and low base versions [92]. These efforts, in combination with broad substrate scope and ready availability of the precursors, have led to [^{18}F]fluorination of organoborons emerging as the most popular transition metal mediated reaction to date.

5.4.3. [^{18}F]Radiofluorination of arylstannanes

Another variation of Cu mediated fluorination is the use of arylstannanes as a precursor that, in several cases gives superior performance when compared to [^{18}F]fluorination of the analogous organoboron precursors (Fig. 64) [84]. Arylstannanes are attractive precursors because of their bench top stability, and there are a number that are commercially available because of their historical use as substrates for electrophilic [^{18}F]fluorination (see section 5.1).

They are readily accessed from aryl halides and are often intermediates 'en route' to aryl iodonium salts (see below). Thus, in some cases, [^{18}F]fluorination of the arylstannane can be a more direct route than the corresponding aryliodonium salt to a given radiopharmaceutical.

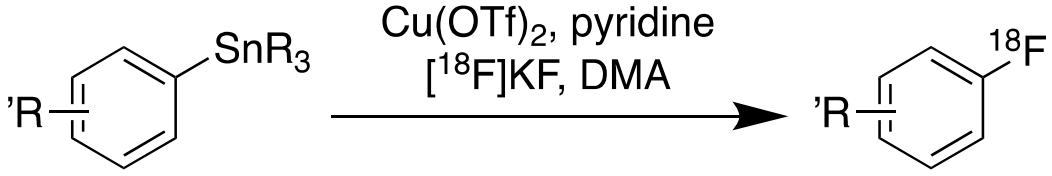


FIG. 64. General reaction scheme for Cu-catalyzed [^{18}F]radiofluorination of arylstannanes (courtesy of P. Elsinga, University of Groningen).

The [^{18}F]fluorination of clinically relevant organostannane precursors has been demonstrated, including [^{18}F]FPEB for the mGluR5 , and serotonin radioligand 2′ -methoxyphenyl-(*N*-2′ -pyridinyl)-*p*-18F-fluorobenzamidoethylpiperazine ([^{18}F]MPPF). The latter synthesis was found superior to an automated SNAr strategy using the classical nitro-precursor. The most high profile use of the method is by Li and co-workers [93], who are employing Cu mediated fluorination of an arylstannane precursor to synthesize [^{18}F]SDM-8 for clinical imaging of SV2a to quantify synaptic density.

5.4.4. Metal catalysed [^{18}F]fluorination of aryliodonium salts.

The use of aryliodonium and diarylsulphonium leaving groups enable synthesis of electron-neutral or electron-rich aromatic compounds. Also, iodonium ylides can be used to achieve the same purpose. This radiochemistry is discussed elsewhere in section 5.1. In this context, aryliodonium salts undergo nucleophilic aromatic [^{18}F]fluorination with selectivity for the more sterically congested carbon fragment. However, the Cu mediated radiofluorination of aryliodonium salts is selective for the smaller substituent (as depicted in Fig. 65). In the original report, it was applied to the synthesis of 4-[^{18}F]L-fluoro-phenylalanine and [^{18}F]FDOPA. Subsequently, 'minimalist' and low base protocols were used these to produce [^{18}F]fluorophenylalanines, such as 6-[^{18}F]fluorodopamine, [^{18}F]DAA1106 and 4-[^{18}F]L-fluoro-phenylalanine. Other groups have utilized this approach to prepare [^{18}F]4-fluoro-3-iodobenzyl)guanidine ([^{18}F]FIBG) as well as imaging agents for cyclooxygenase-2.

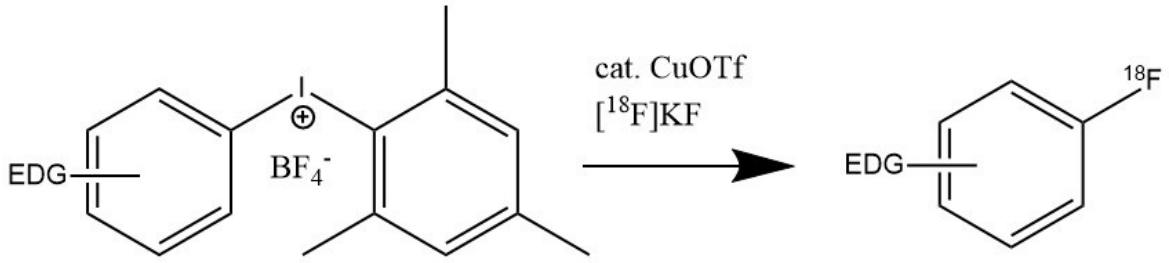


FIG. 65. General reaction scheme for Cu-catalyzed [^{18}F]radiofluorination of iodonium salts (courtesy of P. Scott, University of Michigan).

Typical [^{18}F]fluorination conditions react in DMF or acetonitrile at 125–150^{o}C for 5–10 min. Radiochemical yields vary from 14% to 63%, and high molar activities are obtained. In some cases, the precursor shows to be unstable under basic conditions. Also, the counter ion is crucial

as it can act as a nucleophile to cleave the precursor. Triflates and iodides exhibit the highest RCYs withno racemization. The synthetic steps are displayed in Table 15.

TABLE 16. STEPWISE PROCEDURE FOR Cu-CATALYZED [^{18}F]RADIOFLUORINATION OF IODONIUM SALTS
(courtesy of P. Scott, University of Michigan)

	Synthetic step	Conditions
1.	Setup	A synthesis module is pre-charged with a solution of the [Mes-I-Ar]X precursor (18 μmol) and tetrakisacetonitrile copper(I) triflate (8.0 mg, 20 μmol) in DMF (0.75 mL).
2.	^{18}F- production	Production of $^{18}F^-$ (depends on the nuclear reaction and the cyclotron used)
3.	^{18}F- preparation	Before the azeotropic drying, the $^{18}F^-$ is separated from the ^{18}O-water using an anion exchange cartridge (Sep-Pak® Light Waters Accell^{TM}Plus QMA cartridge) preconditioned to obtain it in its HCO_3-form.
4.	^{18}F- drying	Azeotropic drying of $^{18}F^-$ using 18-crown-6 (5 mg) and K_2CO_3 (3 mg), in aqueous MeCN (1.5 mL, 66% MeCN). After drying, the reaction vessel is cooled to 50°C, DMF (0.75 mL) is added, and the resulting mixture is stirred for 1 min.
5.	Cu mediated radiofluorination	The preloaded solution of iodonium salt and copper (0.75 mL volume) is added to the reaction vessel, and the vessel is sealed, heated to 85°C, and held at that temperature for 20 min to conduct radiofluorination.
6.	Post-synthesis processing	After radiolabelling, additional processing (e.g. deprotection), purification (HPLC) and/or reformulation (SPE) can be conducted using the standard methods outlined in this publication.
7.	QC	QC of products can be completed using the standard techniques (e.g. radio-TLC, radio-HPLC) described in this publication.

5.4.5. [^{18}F]deoxyfluorination of Ru mediated phenol complexes

The Ritter group has also developed ^{18}F radiochemistry to synthesize [^{18}F]arenes from the corresponding phenol precursors as shown in Fig. 66 [94]. By coordination of the aromatic ring to Ru(II) the electron density is decreased and therefore it is activated towards nucleophilic substitution. In the synthetic procedure [^{18}F]fluoride is eluted from the anion exchange cartridge with a solution of Ru precursor, N.N-bis(2,6-diisopropylphenyl)-1-chloroimidazolium chloride. The reaction has been further optimized by applying several solvent mixtures (ethanol, pivalonitril, veratrole, acetonitrile) and salt additives including bis(trimethylneopentylammonium) oxalate. The eluate is heated under vigorous stirring at 130 to 150°C for about 30 min. It was demonstrated that the reaction tolerated water in the reaction mixture contributing to the fact that this procedure is robust [95].

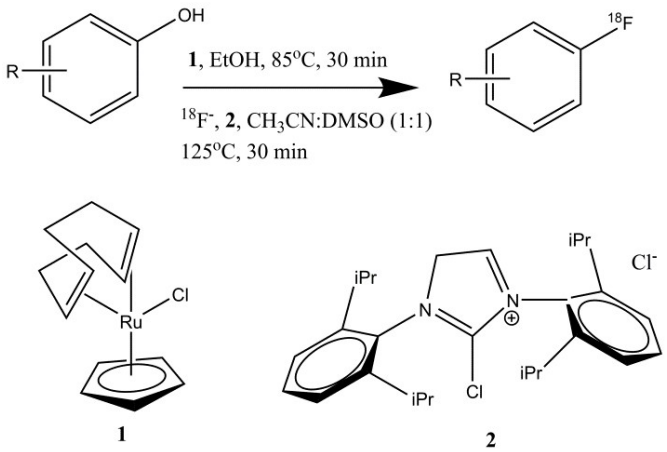


FIG. 66. General reaction scheme for [^{18}F]deoxyfluorination of Ru mediated phenol complexes (courtesy of P. Elsinga, University of Groningen).

In conclusion, metal catalysed late stage [^{18}F]fluorinations made a great impact on PET radiochemistry providing ^{18}F labelled compounds that could not be synthesized before with high molar activities. The main challenge for the late stage [^{18}F]fluorinations is the translation from small scale manual syntheses to large scale automated productions. Besides previously mentioned chemical reasons, also radiolytic processes play an important role.

5.5. GENERAL PRINCIPLES AND CONCEPTS OF [^{18}F]FLUORINATIONS USING SILICON, BORON, AND ALUMINUM

Direct incorporation of ^{18}F as the final step in radiotracer syntheses is highly desirable. The moderately short half-life of ^{18}F limits the amount of time available for additional synthetic steps after radiolabelling. Unlike other halogens, fluorine is more challenging to incorporate through carbon-fluorine bond formation. Fluorine is not easily oxidized to an activated species that can react with electron-rich moieties. Alternatively, it reacts as nucleophile in substitution reactions, with precursor molecules often containing a preinstalled leaving group, or a sufficiently electron-deficient aromatic ring. These reactions generally require elevated temperatures and drying of the aqueous solution in which ^{18}F is generated, with a swap to non-aqueous solvent. By-products are also typically formed, necessitating HPLC purification of the radiotracer mixture.

These limitations have led to the major development of methods based on fluorine-heteroatom bond formation, namely boron, silicon, and aluminium. The heteroatoms are advantageous due to the particularly strong bonds formed with the fluoride anion. Despite early work from the 1960's, these approaches have only received major research interest in the last fifteen years [96]. Two general approaches are used for boron and silicon radiofluorination: (a) fluorine displacement of a LG from the heteroatom; and (b) isotopic exchange (IE) of a ^{18}F with a ^{19}F. Distinctly, aluminium methodologies are based on binding of [^{18}F]fluoride by aluminium, and chelation of this complex by the radiotracer target. Together, these methods offer a versatile approach to ^{18}F radiolabelling of complex organic compounds, with numerous examples of recent clinical applications.

5.5.1. Silicon ^{18}F labelling: general considerations

In 1985, Rosenthal et al. [97] demonstrated that [^{18}F]fluorotrimethylsilane could be formed from chlorotrimethylsilane with high efficiency (80% RCY). However, in vivo studies showed rapid bone-uptake of radioactivity, indicating fast hydrolysis of the fluorine from the silicon moiety. In 2005, the ^{18}F labelling of a biotin-linked alkyl triethoxysilane (Fig. 67 (1)), using aqueous conditions, with carrier-added fluoride (KHF_2) was reported. They demonstrated the formation of [^{18}F]tetrafluorosilicate (Fig. 67 (([^{18}F])2)), in near quantitative RCY.

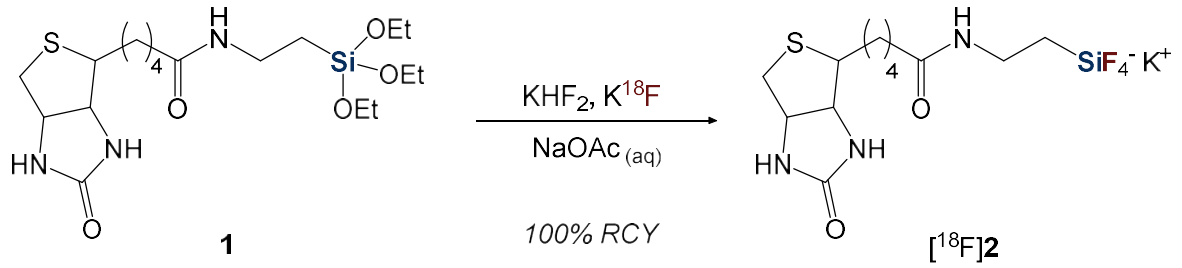


FIG. 67. Radiofluorination of a biotin-linked trialkoxysilane (courtesy of P. Elsinga, University of Groningen).

Shortly after, Schirrmacher et al. [98] showed that [^{18}F]dialkylfluorosilane (Fig. 68 ([^{18}F]3)) could be generated through an IE from the corresponding ^{19}F compound (Fig. 68 (3)), coined a silicon-fluoride acceptor (SiFA) (Fig. 68). An alternative LG approach using rapid substitution of tert-butyldiphenylmethoxysilane to form [^{18}F]tert-butyldiphenylfluorosilane with aqueous fluoride at room temperature was introduced. The two tert-butyl groups on the silicon were found impart the necessary stability towards hydrolysis. However, this comes at the cost of a significant increase in lipophilicity, which often has to be countered by the addition of polar auxiliaries to clinical SiFA candidates.

FIG. 68. Radiofluorination of a dialkylfluorosilane SiFA compound (courtesy of R. Schirrmacher, University of Alberta).

Since these pioneering reports, there have been numerous innovations in SiFA radiolabelling methodology (Fig. 69), both within the IE strategy, and the LG strategy, which has seen the exploration of various functionalities as silicon leaving groups. Both approaches are able to deliver labelled compounds in high RCY, with molar activities of 1–6 Ci (37–222 GBq) µmol^{-1}. However, one advantage of the SiFA IE is that it typically proceeds at room temperature, whereas the LG methods require elevated temperatures, potentially limiting its application towards temperature insensitive biomolecules. Another benefit of IE is that the [^{18}F]SiFA product is chemically identical to the ^{19}F starting material, and the mild conditions tend not to produce side products. Thus, purification can be performed rapidly using a solid phase cartridge (SPE technique), whereas LG methods require time consuming HPLC purification to separate the ^{18}F product from the chemically distinct starting material.

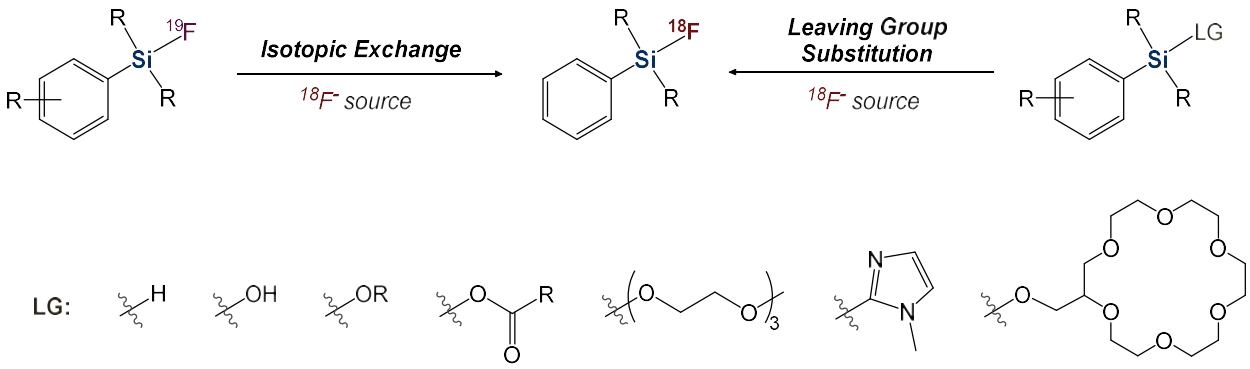


FIG. 69. General scheme for radiofluorination of SiFA compounds (courtesy of R. Schirrmacher, University of Alberta).

Much of the SiFA labelling work has focused on peptides, which are typically large enough to tolerate addition of the SiFA moiety and retain their biological activity. Conversely, addition of SiFA to a small molecule has a major effect on its physiochemical properties, typically adversely impacting biodistribution and other pharmacological properties. Since various peptide receptors are upregulated in many cancers, there has been major focus on radiolabelling tumour homing peptides for PET imaging.

5.5.2. Boron ^{18}F labelling: general considerations

Boron forms one of the strongest bonds with fluorine (732 kJ.mol^{-1}). In the early 1960's, initial attempts were made to incorporate ^{18}F into small molecules via boron binding. This strategy received a major revival in 2005, with the work published by Ting and Perrin [99]. These authors demonstrated that a pinacol phenylboronate ester biotin conjugate (Fig. 70 (4)) could be efficiently radiolabelled with aqueous $^{18}F^-$ and carrier added KHF_2, resulting in an easily isolable [^{18}F]aryltrifluoroborate precipitate (Fig. 70 ([^{18}F]5)). For in vivo applications, they also studied the hydrolytic stability of the aryltrifluoroborates at a physiological pH, and found that they were reasonably stable after 60 min without any significant release of ^{18}F anions. This work countered the common belief that radiofluorination does not occur in aqueous media, due to the attenuated reactivity of highly solvated $^{18}F^-$ [100].

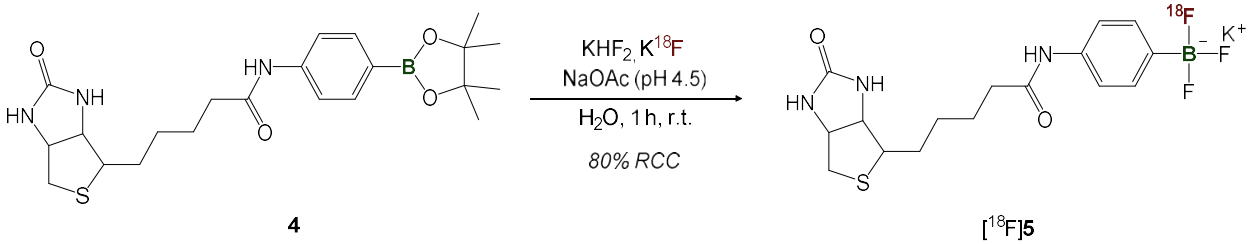


FIG. 70. Radiofluorination of a biotin-linked phenylboronate (courtesy of R. Schirrmacher, University of Alberta).

As with silicon and following the SiFA IE strategy, isotopic exchange strategies have emerged a viable approach to form ^{18}F labelled trifluoroborates. The milder reaction conditions used for isotopic exchange (pH~2, room temperature), with minimal side product formation, are favourable preconditions that lead to facile purification, without the need for HPLC [100]. The weakness of this methodology lies in the problematic, pH independent hydrolysis of the trifluoroborate (RBF_3^-) to the analogous boronic acid ($RB(OH)_2$). Hydrolysis results in the release of the [^{18}F]F^- anion, which can accumulate in calcium rich fluorophilic bones in vivo.

This increases background noise in the PET images and is therefore important to prevent. Perrin et al. [99] discovered in 2015 that the hydrolysis rate follows a "simple linear correlation between the hydrolysis rate of RBF_3^- and the pK_a of a corresponding carboxylic acid of the trifluoroborate"[101], allowing for predictable design of trifluoroborate precursors.

A major improvement in the hydrolytic stability came in 2014, with the development of a zwitterionic alkylammoniomethyltrifluoroborate ($AmBF_3$). Perrin et al. [99] synthesized rhodamine containing $AmBF_3$ (Fig. 71 ([^{18}F]6)), using non-predried ^{18}F solution, and producing the desired product with a molar activity of 150 GBq μmol^{-1} (Fig. 71). Compound (Fig. 71 ([^{18}F]6)) showed minimal hydrolysis in serum at 37°C after 150 min, as well as excellent in vivo stability in mice models, as well as minimal bone uptake.

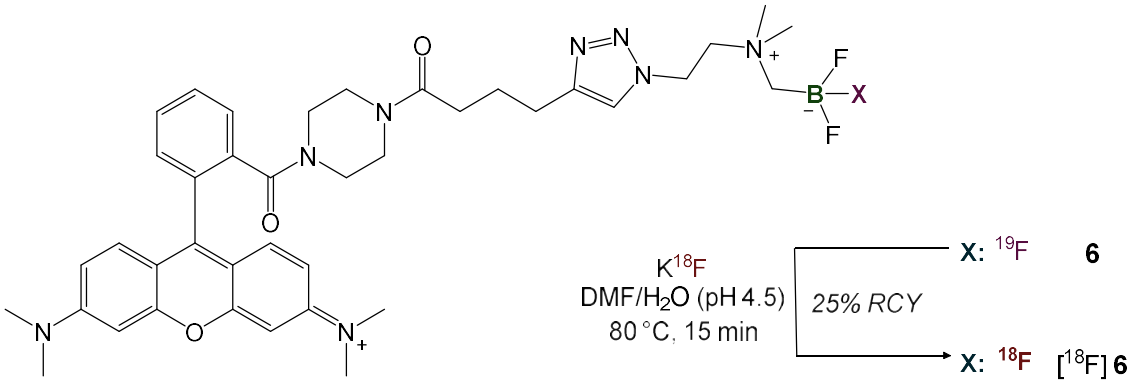


FIG. 71. Direct radiofluorination of a zwitterionic $AmBF_3$-conjugate through ^{19}F-^{18}F isotopic exchange (courtesy of R. Schirrmacher, University of Alberta).

5.5.3. Aluminium ^{18}F labelling: general considerations

As with ^{68}Ga, aluminium ^{18}F radiochemistry is based on chelation, which has many benefits over covalent bond formation. As such, it began gaining more interest in the late 2000's for use in PET probe development. The general mechanism is based on the formation of a complex between a 9-membered cyclic chelator and a $[^{18}F][AlF]^{2+}$ cation generated in situ (Fig. 72). Advantages of this approach include the applicability in aqueous medium, as well as a straightforward one-step fluorination procedure [102].

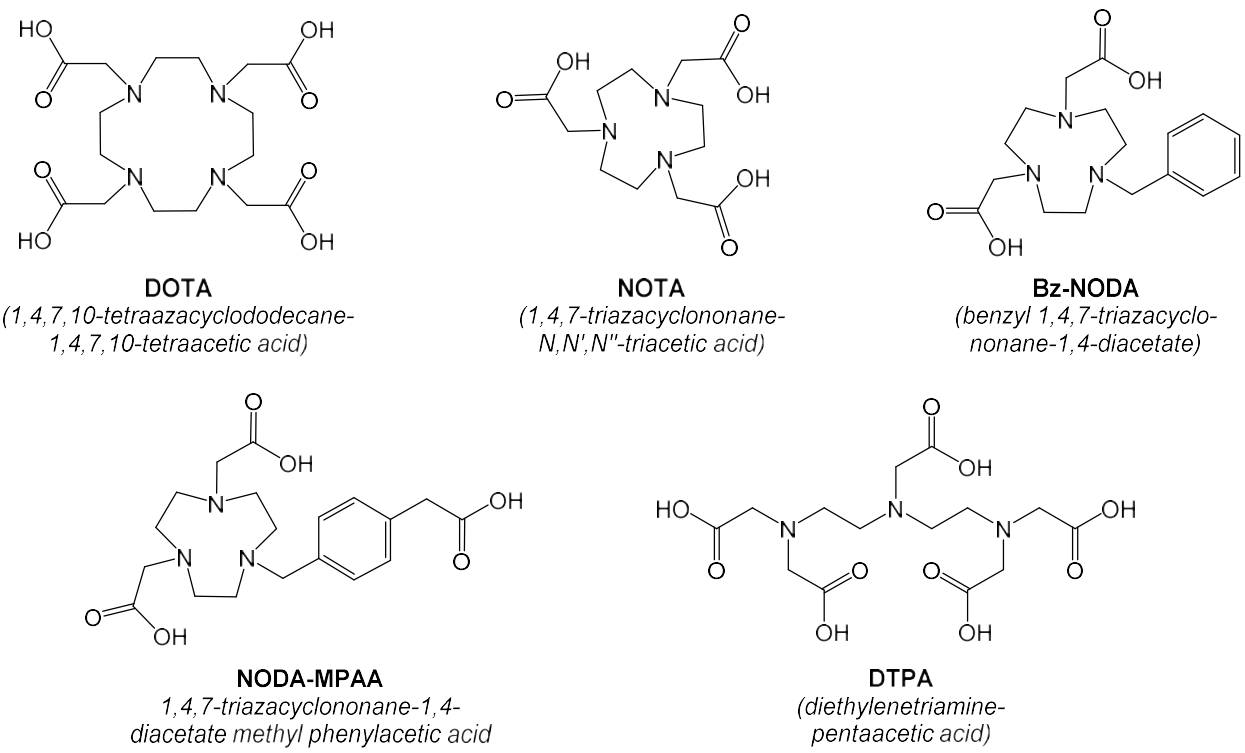


FIG. 72. Chelators for the radiofluorination using [^{18}F]aluminium fluoride cation (courtesy of R. Schirrmacher, University of Alberta).

The negative charge on oxygen donor sites (e.g. alkoxides and carboxylates) is crucial for the formation of stable aluminium (III) complexes with multidentate ligands. The ionic character of these interactions is essential and affinity to the aluminium shows a linear dependence on the basicity of the ligand's donor sites. The strength of an aluminium fluoride bond is approximately 670 kJ/mol, which leads to high stability in vivo, and subsequent compatibility with biological systems.

DTPA has long been known to form stable complexes with other group thirteen atoms, such as indium, which served as an inspiration for the first chelation studies with Al[^{18}F]F [103]. These initial studies showed that pH level is crucial in the formation of the $(AlF)^{2+}$ cation, with an optimal value of roughly 4, for this specific type of reaction. However, it was found that the peptide DTPA complex with $[AlF^{18}]^{2+}$ lacked sufficient stability in water and serum [104]. Attempts with other chelators such as NOTA and DOTA lead to disappointing yields (5–20% for NOTA peptides). The labelling efficiency was improved by using ligands with a '3N, 2O' configuration, such as in NODA-MPAA, Bz-NODA, or pentavalent NOTA analogs ($NOTA_{(5)}$). This configuration bears a free coordination site for fluoride binding, and leads to the formation of a stable octahedral complex [105].

An illustrative radiolabelling example is shown in Fig. 73. The reaction utilizes non-purified ^{18}F, meaning that metal impurities are not removed before usage. An elevated temperature is required to ensure a rapid reaction, with the optimal pH value of 4 being obtained using a small amount of acetate solution. In general, the highest RCYs are obtained by keeping the final reaction volume small and buffer solutions as concentrated as possible. A slight excess of the macrocycle-conjugate over Al^{3+} is also beneficial, as is the addition of organic co-solvents such as ethanol or acetonitrile.

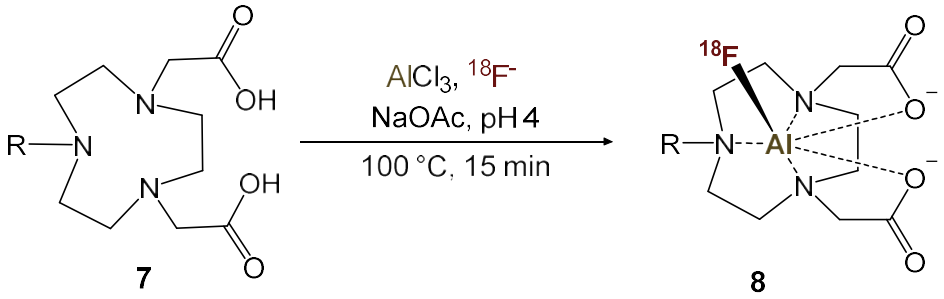


FIG. 73. General scheme for Al^{18}F radiofluorination (courtesy of R. Schirrmacher, University of Alberta).

5.5.4. Conclusion

Since the breakthrough developments in the mid 2000's, the heteroatom based ^{18}F methodologies as well as the Al-based radiochemistry have grown substantially. Both, the leaving group and isotopic exchange approaches, continue to be developed for the boron and silicon methods, whereas the aluminium strategy has benefited from the application of new chelating moieties. The use of these methods to label peptide probes is becoming more common, with many performing well in clinical trials. The simplicity and practicality of these methods represent a major advantage over the classical carbon-fluorine bond formation reactions, and they may soon be used to create compounds to replace some standard clinical tracers used for PET imaging.

5.6. [^{18}F]TRIFLUOROMETHYLATIONS

The trifluoromethyl (CF_3) group is a common motif in diverse classes of bioactive organic molecules, especially in medicinal chemistry, and is therefore an attractive target in ^{18}F chemistry. Numerous methods for labelling this functional group have been developed over the years, with some of these methods being presented below.

5.6.1. Halex reactions

Historically, [^{18}F]trifluoromethyl groups have been prepared via halogen exchange, through nucleophilic substitution of an halogen leaving group by [^{18}F]fluoride [106]. Reactions can occur in aliphatic or aromatic series, providing the substrate bears a halo-difluoromethyl moiety. First attempts focused on isotopic exchange (leaving group = ^{19}F), but further studies showed that bromide was easier to use (milder conditions required, in comparison with fluoride or chloride). It can be noted that more recent work has also reported the use of iodide leaving groups (refer to section 5.6.4 for additional details).

These reactions suffer from various limitations. In case of isotopic exchange, reactions were reported to be irreproducible, and products are obtained with modest yields and extremely low molar activities (carrier added conditions). With use of brominated precursors, slightly better yields could be obtained, but molar activity was still found to be very low. In those non-carrier added conditions, a competing isotopic exchange reaction is still occurring, and it was hypothesised that the decomposition of the precursor may be the source of [^{19}F]fluoride in that case. In addition, the typical conditions used for the preparation of bromodifluoro precursors (e.g. radical bromination) may prevent preparation of more functionalized targets required for late stage [^{18}F]fluorination, and therefore may lead to multistep synthesis, which is undesirable with short lived isotopes.

Nonetheless, these reactions allowed preparation of molecules of interest, in particular [^{18}F]Fluoxetine [107], which was studied as a potential serotonin reuptake marker, and [^{18}F]Celecoxib, a PET probe for COX-2 expression (Fig. 74).

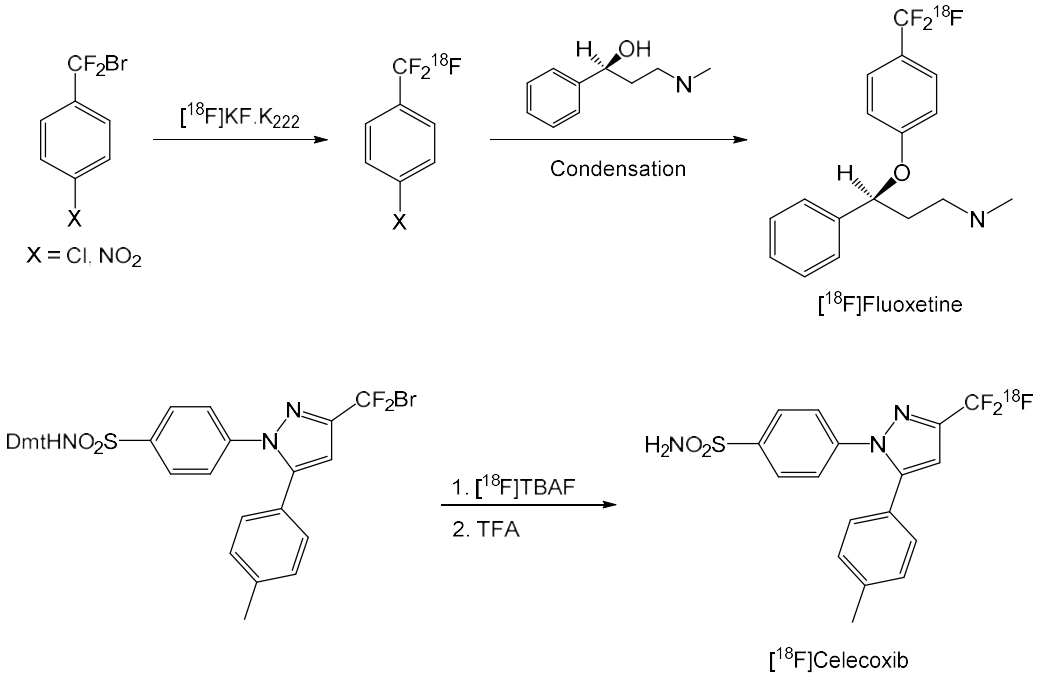


FIG. 74. [^{18}F]Trifluoromethylations via Halex reactions (courtesy of M. Huiban, Invicro LLC).

5.6.2. Electrophilic fluorinating agents

Electrophilic reactions have also been considered for the preparation of [^{18}F]trifluoromethylated products. A first example used the addition of [^{18}F]F_2 to a fluorinated double bond, as reported in the preparation of the hypoxia imaging agent [^{18}F]EF5 [108] (Fig. 75). The process could be automated on a commercially available radiosynthesis module, leading to the compound of interest in sufficient amounts and purity for clinical PET studies. The second example involved a silver-catalyzed decarboxylative fluorination, utilizing [^{18}F]Selectfluor bis(triflate), which afforded a broad range of [^{18}F]trifluoromethyl arenes in moderate to good yields, but no examples of more complex substrate were included in the study.

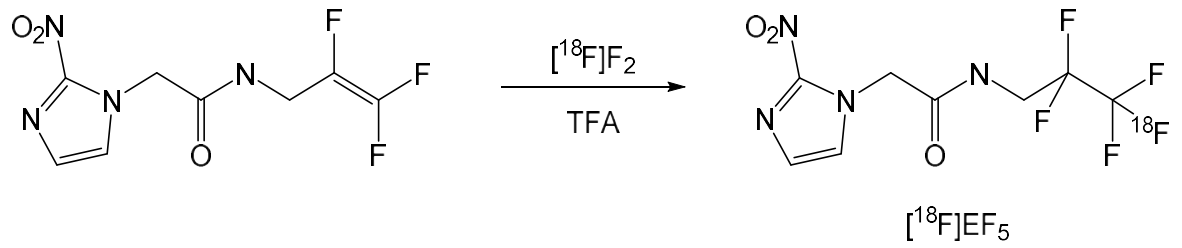


FIG. 75. [^{18}F]Trifluoromethylation via addition of [^{18}F]F_2 (courtesy of M. Huiban, Invicro LLC).

Similarly, to electrophilic substitution reactions (see also section 5.1), these reactions are not the most used transformations, due to the use of reagents such as [^{18}F]F_2 or [^{18}F]Selectfluor

(technical challenge for production, low yields, poor molar activity, side reactions). Nonetheless, it is reported to allow clinical use of [^{18}F]EF5 to image hypoxia in head and neck cancer, lung cancer and ovarian tumours.

5.6.3. Radiolabelling of aliphatic trifluoromethyl groups

The radiosynthesis of aliphatic [^{18}F]trifluoromethyl groups has focused mainly on the preparation of [^{18}F]trifluoroethylated compounds, for which various methods have been described. A first method involves the reaction of 1,1-difluorovinyl precursors with [^{18}F]fluoride ion, which results in the equivalent of a direct nucleophilic addition of H[^{18}F]F. The initially reported method provided a simple and efficient procedure for the preparation of 2-[^{18}F]fluoro-2,2-difluoroethyltosylate, that could be used as a PG for radiolabelling bioactive molecules [109]. Further developments extended the concept to prepare complex radiopharmaceuticals directly in a one-step late-stage fluorination, as reported for the preparation of [^{18}F]-N-Methyl lansoprazole. It can be noted that this method affords products with high molar activity, which is not very common with [^{18}F]trifluoromethylation reactions. A second approach also looked at the preparation of 2-[^{18}F]fluoro-2,2-difluoroethyl alkylating agents, but this time via halex reactions. This method allowed preparation of 2-[^{18}F]fluoro-2,2-difluoroethyltriflate via [^{18}F]Br substitution, but also [^{18}F]TFMISO in a multi-step synthesis procedure starting with an ^{18}F-^{19}F exchange [110]. In both cases, products were obtained in poor molar activity, as previously noted in section 5.6.1.

Apart from [^{18}F]trifluoroethylated compounds, the labelling of the trifluoropropyl group has also been reported, through the preparation of [^{18}F]trifluoropropylamine as a building block. Here an oxidative [^{18}F]fluorodesulfurization was developed as a new approach, and applied to the synthesis of the hypoxia marker [^{18}F]EF3 [111]. The product was obtained in a four step procedure, and in low molar activity due to the involvement of a stoichiometric fluoride source to allow complete desulfurization of the precursor.

Overall, the PGs mentioned above have seen very little use. In addition to the need for multi-step radiolabelling procedures, reported molar activities are very low. Competing elimination reactions may also occur, impacting yields, and even formation of the desired product in some cases, but this can be mitigated upon addition of proton sources such as trace amount of water or preferably isopropyl alcohol or even ammonium chloride. The formation of undesired products also means robust purification methods are required.

The approach remains of interest, in particular as exemplified by the preparation of [^{18}F]-N-Methyl lansoprazole ([^{18}F]NML) (Fig. 76), which was investigated clinically as an imaging agent for quantifying Tau aggregates in neurodegenerative disorders such as Alzheimer's disease.

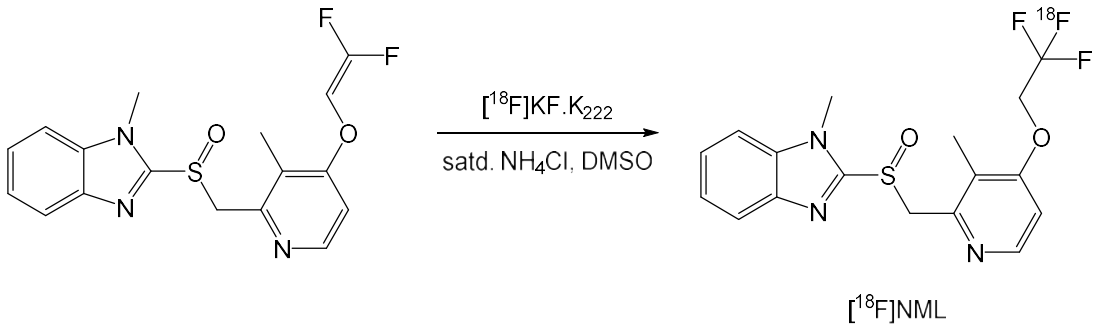


FIG. 76. [^{18}F]Frifluoromethylation via addition of [^{18}F]fluoride on gem-difluoro enol ethers (courtesy of M. Huiban, Invicro LLC).

5.6.4. Metal mediated cross coupling reactions of (hetero)arenes

In non-radioactive chemistry, the most synthetically useful method for introduction of a trifluoromethyl group onto aromatic and heteroaromatic ring systems is the coupling of the corresponding aryl and heteroaryl halides with a trifluoromethylcopper species. Following the same principles, more recent methods for the labelling of CF_3 groups with ^{18}F have therefore been based on generation of [^{18}F]$CuCF_3$ from [^{18}F]fluoride ion, either directly or via synthesis of [^{18}F]fluoroform.

(a) Trifluoromethylation using methyl chlorodifluoroacetate ($ClCF_2CO_2Me$)

In this method, chlorodifluoroacetate is used to generate a difluorocarbene that can be intercepted by [^{18}F]fluoride, which in presence of a copper catalyst leads to the generation in situ of '[^{18}F]$CuCF_3$' (Fig. 77) [112]. Aryl and heteroaryl iodides were reported to be labelled in moderate to high RCYs, with the method tolerating a variety of substrates. The reaction is extremely simple, can be performed with commercially available reagents and do not require special care, all of which allow easy automation and provide robustness to the method.

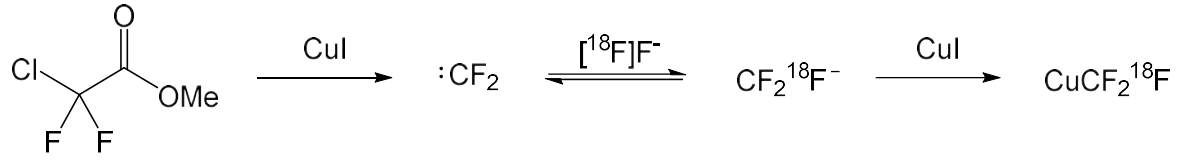


FIG. 77. Preparation of [^{18}F]$CuCF_3$ from difluorocarbene intermediate (courtesy of M. Huiban, Invicro LLC).

Due to the generation of the highly reactive carbene intermediate, side reactions are unavoidable, including release of [^{19}F]fluoride ion, which leads to isotopic dilution and therefore poor molar activity, despite being performed in non-carrier-added conditions. Nevertheless, the scope and ease of this reaction remains of interest, and can be of use for the study of non-saturable biological systems, for example in supporting clinical biodistribution studies in drug development programs, or labelling of nucleoside and amino acid analogues for applications in oncology studies (Fig. 78). The stepwise synthesis of [^{18}F]TFT is also presented in Fig. 78 and Table 16, to further highlight the simplicity of the reaction and the ease of its automation.

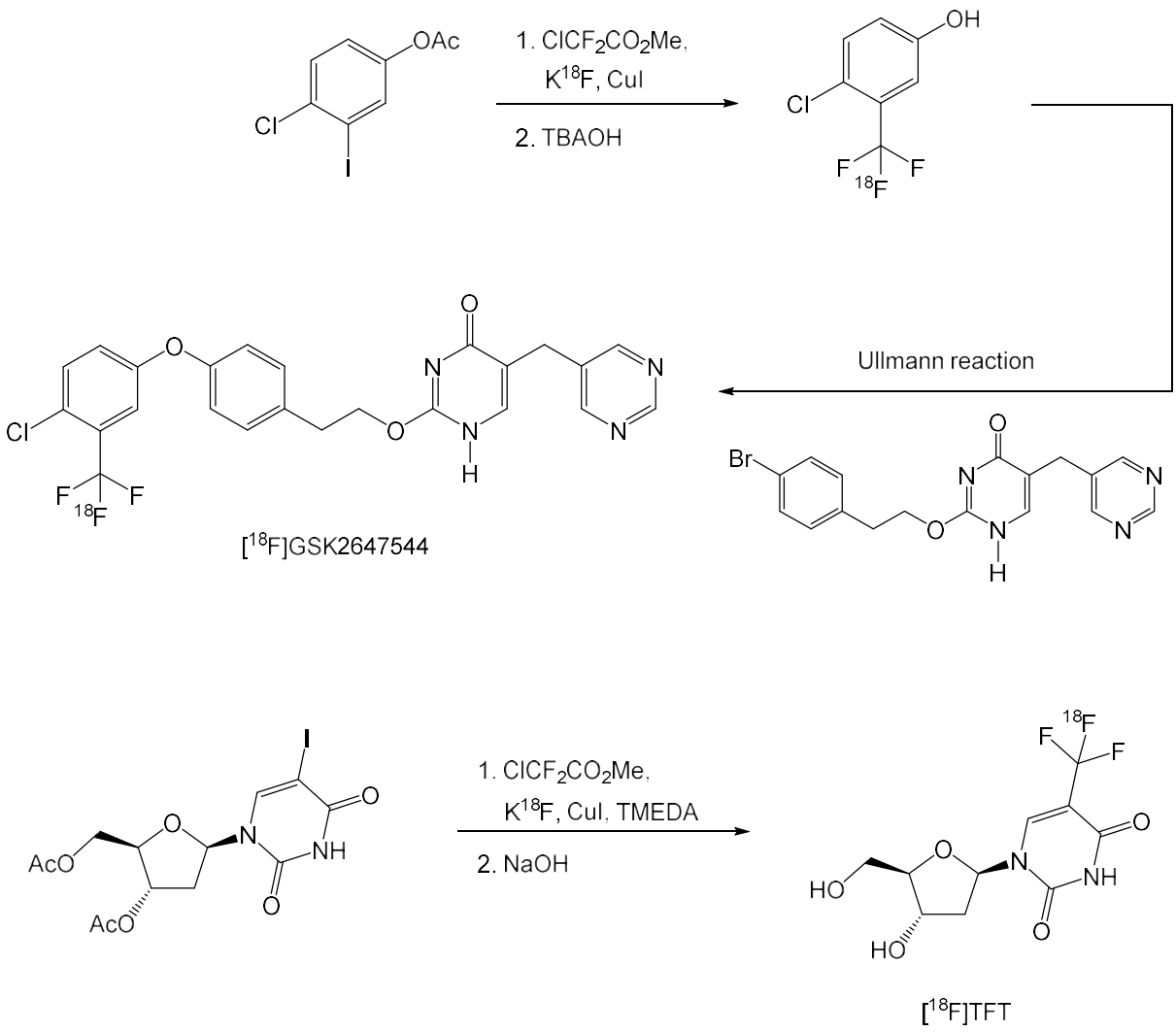


FIG. 78. [^{18}F]Trifluoromethylation via [^{18}F]CuCF$_3$ generated in situ (courtesy of M. Huiban, Invicro LLC).

TABLE 17. STEPWISE SYNTHESIS OF [^{18}F]TFT ON A TRASIS ALLINONE SYNTHESIS PLATFORM. *(courtesy of M. Huiban, Invicro LLC).*

Synthetic step	Conditions
1. $^{18}F^-$ production	Production of $^{18}F^-$ (depends on the nuclear reaction and the cyclotron used).
2. $^{18}F^-$ preparation	Before the azeotropic drying, the $^{18}F^-$ is separated from the ^{18}O-water using an anion exchange cartridge (Sep-Pak® Light Waters Accell^{TM} Plus QMA cartridge) preconditioned to obtain it in its HCO_3^-form.
3. $^{18}F^-$ drying	Azeotropic drying of $^{18}F^-$ using K_{222} (2.75 mg) and $KHCO_3$ (0.55 mg) in aqueous MeCN (90% MeCN).
4. ^{18}F-fluorination	After cooling to room temperature, a mixture of CuI (17.5 mg, 0.092 mmol), TMEDA (14 µL, 0.092 mmol), $ClCF_2CO_2Me$ (10 µL, 0.092 mmol) and the precursor (10 mg, 0.023 mmol) in DMF (500 µL) is added to the dry $^{18}F^-$ from step 3 and the resulting solution is heated to 150˚C for 15 min.
5. Intermediate Purification	After cooling to room temperature, the crude mixture is diluted with aqueous MeCN (11 mL, 9% MeCN) and passed through a hydrophilic lipophilic balanced (HLB)-SPE cartridge. The cartridge is dried under nitrogen to remove excess of water. The ^{18}F- labelled intermediate is eluted off the cartridge with MeCN and transferred back into the reaction vessel. MeCN is evaporated.

TABLE 18. STEPWISE SYNTHESIS OF [^{18}F]TFT ON A TRASIS ALLINONE SYNTHESIS PLATFORM ('cont'). *(courtesy of M. Huiban, Invicro LLC).*

Synthetic step	Conditions
6. Deprotection	After cooling to room temperature, 0.1 M NaOH (1 mL) is added to the reactor. Hydrolysis of the acetate protecting groups is carried out at room temperature with nitrogen bubbling through for 5 minutes.
7. Purification	The crude mixture is neutralised with NaH_2PO_4 buffer (1 mL) and diluted with water (10 mL), and the resulting solution is loaded onto a semi-prep HPLC system for purification. The peak corresponding to [^{18}F]TFT is collected and passed through 2 HLB cartridges connected in series to extract the product and remove the HPLC solvent.
8. Reformulation	The purified [^{18}F]TFT is eluted from the cartridges with ethanol (1 mL) and reconstituted in another solvent for further use.

(b) Trifluoromethylation using [^{18}F]Fluoroform

An alternative strategy for generation of [^{18}F]trifluoromethide, and subsequently [^{18}F]$CuCF_3$, is based on the deprotonation of [^{18}F]trifluoromethane. Multiple procedures have been reported to do so (Fig. 79): a first method reported a one-pot procedure allowing the preparation in situ of [^{18}F]trifluoromethane from difluoroiodomethane and [^{18}F]KF/K_{222}, which in presence of CuBr, DIPEA, $KHCO_3$, and the desired aryl iodide precursor, allowed preparation of the corresponding products in good yields, but with poor molar activities. A second method looked at first generating [^{18}F]trifluoromethane, which was subsequently quenched in a solution containing the copper source and a base (KOtBu) [113]. The formed [^{18}F]$CuCF_3$ species was stabilised by addition of trimethylamine trihydrofluoride and further reacted with either aryl iodides or aryl boronic acids. Good functional group tolerance was demonstrated, and products were obtained in good yields, but low molar activities. Though the molar activity could be raised very significantly by reducing the amount of starting difluoroiodomethane, however yields would be reduced significantly. A third method, following the same approach as the previous method, used a difluoromethylsulfonium salt to generate [^{18}F]trifluoromethane, and trimethylborate to stabilise the formed [^{18}F]$CuCF_3$ species [114]. A range of different model iodides or boronic acids could here also be converted into their [^{18}F]trifluoromethylated analogues in generally high yields, but with poor molar activities. A last method was reported recently, looking at the preparation of [^{18}F]trifluoromethane in gas phase. After treatment of methylmesylate with [^{18}F]KF/K_{222}, the formed [^{18}F]fluoromethane gas was passed through a column loaded with cobalt(III) fluoride and heated at high temperature for online conversion into [^{18}F]trifluoromethane. The formed [^{18}F]trifluoromethane could be used directly for reactions with carbonyl functions, or converted into [^{18}F]$CuCF_3$ (stabilised with trimethylamine trihydrofluoride as previously reported) to be reacted with a range of aryl iodides, arylboronic acids and aryldiazonium salts. Moderate to very high yields were obtained, with moderate to high molar activities, for this method.

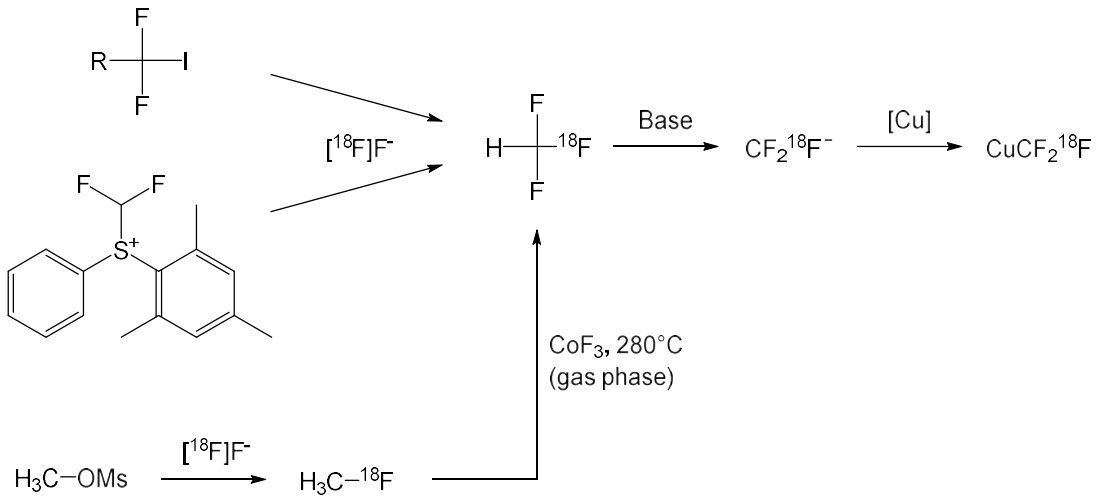


FIG. 79. Preparation of [^{18}F]CuCF$_3$ via [^{18}F]Fluoroform (courtesy of M. Huiban, Invicro LLC).

Whichever procedure is used for the generation of [^{18}F]fluoroform, its use is not simple, from an equipment and automation point of view, and require tight control on the handling and purification of the gaseous intermediate. In most of the procedures presented above, [^{18}F]fluoroform is also obtained in only a very low molar activity, with the hypothesis that the precursor (difluoroiodomethane, difluoromethylsulfonium) decomposes under the basic radiolabelling conditions to release [^{19}F]fluoride. There are some promising results though, with the newly developed gas phase method, or also the very recently published method to prepare [^{18}F]fluoroform from [^{18}F]triflyl fluoride [115]. Finally, the use of [^{18}F]fluoroform was tested with model test compounds, and also allowed preparation of bioactive compounds of possible interest to the PET imaging community, but has not been reported to date to support actual clinical work. Therefore, it is still unclear how this method will compare with the ones presented above (for example as compared to the use of methyl chlorodifluoroacetate, or use of gem-difluoro enol ethers).

(c) Other metal mediated cross coupling reactions

A few other methods have been reported over the years, involving metal mediated labelling of trifluoromethylaryl compounds: (i) Silver mediated halex [^{18}F]trifluoromethylations showed that bromodifluoromethylaryl precursors in particular could lead to desired products in much milder reaction conditions (room temperature) as opposed to standard halex reaction. Moderate yields were obtained on a series of model substrates, and with low molar activity. (ii) Manganese–salen complex catalysed oxidative benzylic fluorination was also reported, using [^{18}F]fluoride and difluoromethylaryl precursors. The reaction allows ^{18}F-fluorination of non-activated C-H bonds, providing the desired products in low to good yields, and with low molar activity. (iii) Finally, proof of concept of radiosynthesis via C-F reductive elimination from Au(III) complexes was established, enabling preparation of aliphatic [^{18}F]trifluoromethyl groups in low molar activity. To date, none of the above methods have been reported for the preparation of radiopharmaceuticals for clinical use.

5.6.5. Other trifluoromethylation reactions

More recently, radiosynthetic routes have also appeared towards the ^{18}F labelling of aryl−SCF$_3$ and aryl−OCF$_3$ groups. These routes have been established mainly using methods described

above, in particular the halex reactions with bromodifluoromethyl precursors [116], or through the use of [^{18}F]trifluoromethide (generated from a carbene intermediate or from [^{18}F]fluoroform). One different route looked more particularly at the labelling of free thiol functions of cysteine residues, using an ^{18}F-labelled version of the Umemoto reagent, offering therefore a new approach to direct labelling of unmodified peptides (no need for amino acid derivatisation, or use of PGs).

Lastly, it is worth highlighting a new approach to [^{18}F]trifluoromethylation that has been reported very recently, using selective radical chemistry, to allow labelling of native aromatic residues in peptides [117]. The method reports the preparation of [^{18}F]Trifluoromethanesulfinate, and its application to direct [^{18}F]CF_3 incorporation at tryptophan or tyrosine residues using unmodified peptides.

All the above transformations are more recent developments in the area of [^{18}F]trifluoromethylation reactions, and therefore have not translated to clinical applications yet. Though the two methods allowing direct labelling of unmodified peptides are very interesting and hold great promise for ^{18}F labelling of biomolecules.

6. UPTAKE MECHANISMS OF ^{18}F RADIOPHARMACEUTICALS

6.1. UPTAKE MECHANISM OF [^{18}F]FDG

[^{18}F]FDG is taken up by living cells because it mimics glucose in the initial mechanism of glycolysis. Glucose molecules are taken up from the blood by cells with the help of glucose transporter (GLUT) proteins and then converted to glucose-6-phosphate with the help of the enzyme hexokinase. The glycolysis step goes further by conserving glucose-6-phosphate as glycogen in the liver or converting glucose into pyruvic acid and release of energy. The glucose molecules taken up by the cells are not trapped in the cell but metabolized and either stored or used up.

On the other hand, [^{18}F]FDG is also transported by GLUT inside the cell and converted to [^{18}F]FDG-6-phosphate by the enzyme hexokinase. However, beyond this step [^{18}F]FDG does not get metabolized as the in vivo biochemical mechanisms are highly specific and the difference in the molecular structure is recognized by the enzymes responsible for further steps in the glycolyis. Hence, [^{18}F]FDG molecules get trapped inside the cells thereby helping the imaging of the cells that take glucose/[^{18}F]FDG. Figure 80 shows the difference in the metabolism of glucose and [^{18}F]FDG.

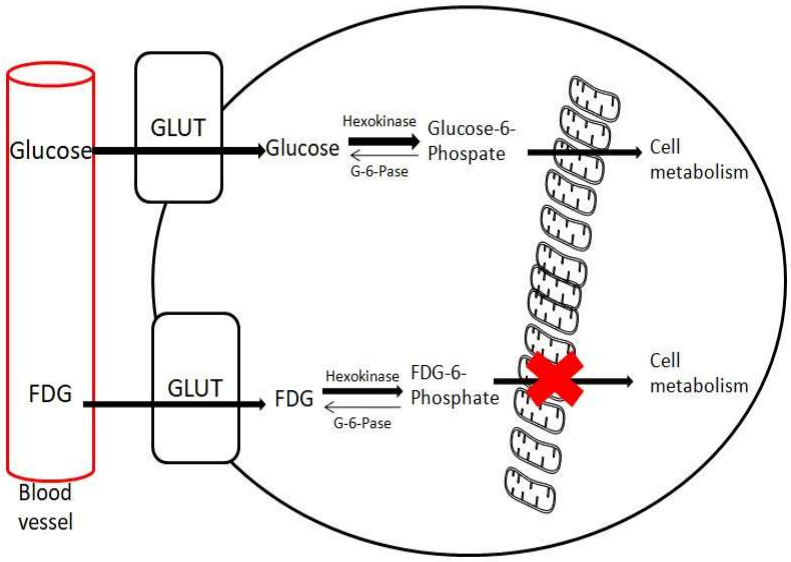


FIG. 80. Chemical structure and uptake mechanism of glucose and [^{18}F]FDG by the living cells (courtesy of N. Raviteja, Molecular Cyclotrons, India, adapted from [118]).

The primary substrate for brain is glucose and hence there is very high uptake of [^{18}F]FDG in brain cells. Though fatty acids are the primary substrate for the heart muscles, glucose is the secondary substance and taken up in large quantities by the cardiac muscles. Hence, when [^{18}F]FDG is injected to a normal person there is intense uptake in the brain and heart. Many other cells also take up [^{18}F]FDG and exercise increases its uptake in several parts of the body.

The tumour cells consume higher amounts of glucose as energy requirement of proliferating cells is far higher than normal cells. The uptake of [^{18}F]FDG increases much higher than normal cells when injected in patients having fast developing cancers. This can be measured by doing a PET imaging and measuring the standard uptake value that compare the uptake in the tumour with the uptake in normal cells. Hence, [^{18}F]FDG is a useful radiopharmaceutical for tracking glucose metabolism and also as a proliferation marker to identify fast growing cancers.

[^{18}F]FDG is used as a radiopharmaceutical for brain perfusion imaging and cardiac viability studies. But the major use of [^{18}F] as of now is for cancer imaging. [^{18}F]FDG is a very good imaging agent for cancers affecting the head and neck, lung, esophageal, breast, colorectal, lymphoma and melanoma. [^{18}F]FDG PET-CT is done for staging, therapy response monitoring, recurrence and follow up of the cancers mentioned above.

6.2. RADIOTRACERS FOR AMYLOID

Dementia is a general term associated with cognitive decline that can have a detrimental impact on daily quality of life for patients. Dementia can be caused by a variety of neurodegenerative disorders such as Alzheimer's disease, dementia with Lewy bodies, Parkinson's disease with dementia, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration and vascular dementia [119]. To effectively manage dementia patients, definitive diagnosis of the underlying neurodegenerative disorders is required because, despite frequent clinical overlap, they are underpinned by distinct clinical pathology. Historically, definitive diagnosis occurred post-mortem by identification of pathological features consistent with a given

neurodegenerative disorder. To address this knowledge gap, PET imaging of amyloid plaques has been one widely investigated approach [120].

Amyloid plaques represent one of the hallmark pathologies of Alzheimer's disease and related disorders like dementia with Lewy bodies. They are extracellular deposits composed of misfolded amyloid beta and the amyloid hypothesis of Alzheimer's disease states that accumulation of amyloid beta in the brain is the primary factor driving Alzheimer's disease pathogenesis [121]. Amyloid plaques are identified post-mortem by staining with fluorescent dyes like thioflavin-T, which were the inspiration for PET radiopharmaceuticals like [^{11}C]Pittsburgh Compound B ([^{11}C]PiB) [122]. Like thioflavin-T, PET radiopharmaceuticals targeting amyloid are thought to bind to the cross β-sheet structures of the amyloid fibrils, and thus they allow non-invasive quantification of amyloid plaque burden using PET imaging.

[^{11}C]PiB was the prototypical radiopharmaceutical for imaging amyloid plaques in neurodegenerative disorders like Alzheimer's disease and dementia with Lewy bodies, developed by Mathis and Klunk [122]. Early positive results with [^{11}C]PiB spurred development of second generation agents labelled with ^{18}F for commercialization. The longer half-life of ^{18}F (110 min) vs ^{11}C (20 min) allows production at centralized nuclear pharmacies, and distribution to satellite imaging centres without their own cyclotron or radiochemistry capabilities. There are several approved ^{18}F labelled radiopharmaceuticals targeting amyloid plaques, while others currently remain investigational in nature (Fig. 81). The first example, [^{18}F]flutemetamol (Vizamyl), is an ^{18}F labelled analog of [^{11}C]PiB, licensed from the University of Pittsburgh and commercialized by GE Healthcare [123]. Another class of amyloid agents is based upon the stilbene motif, developed by Kung and co-workers at the University of Pennsylvania [124]. Two agents have been commercialized: (i) [^{18}F]florbetapir ([^{18}F]AV45, Amyvid) was developed by Avid Radiopharmaceuticals, now a wholly-owned subsidiary of Eli Lilly [125], and (ii) [^{18}F]florbetaben ([^{18}F]AV1, Neuraceq) was developed by Avid and subsequently licensed to Bayer AG [126]. [^{18}F]flutemetamol, [^{18}F]florbetapir and [^{18}F]florbetaben are approved for clinical use around the world [127]. A challenge with all of three of these agents is that while there is pronounced specific binding to amyloid plaques in the cortical gray matter, there is also varying degrees of non-specific white matter binding that can complicate image analysis. To address this, efforts continue to develop improved imaging agents and [^{18}F]flutafuranol ([^{18}F]NAV4694, [^{18}F]AZD4694) has been the most utilized investigational amyloid agent in clinical trials to date [128]. All of the agents allow clear distinction of amyloid positive patients from amyloid negative subjects (Fig. 81).

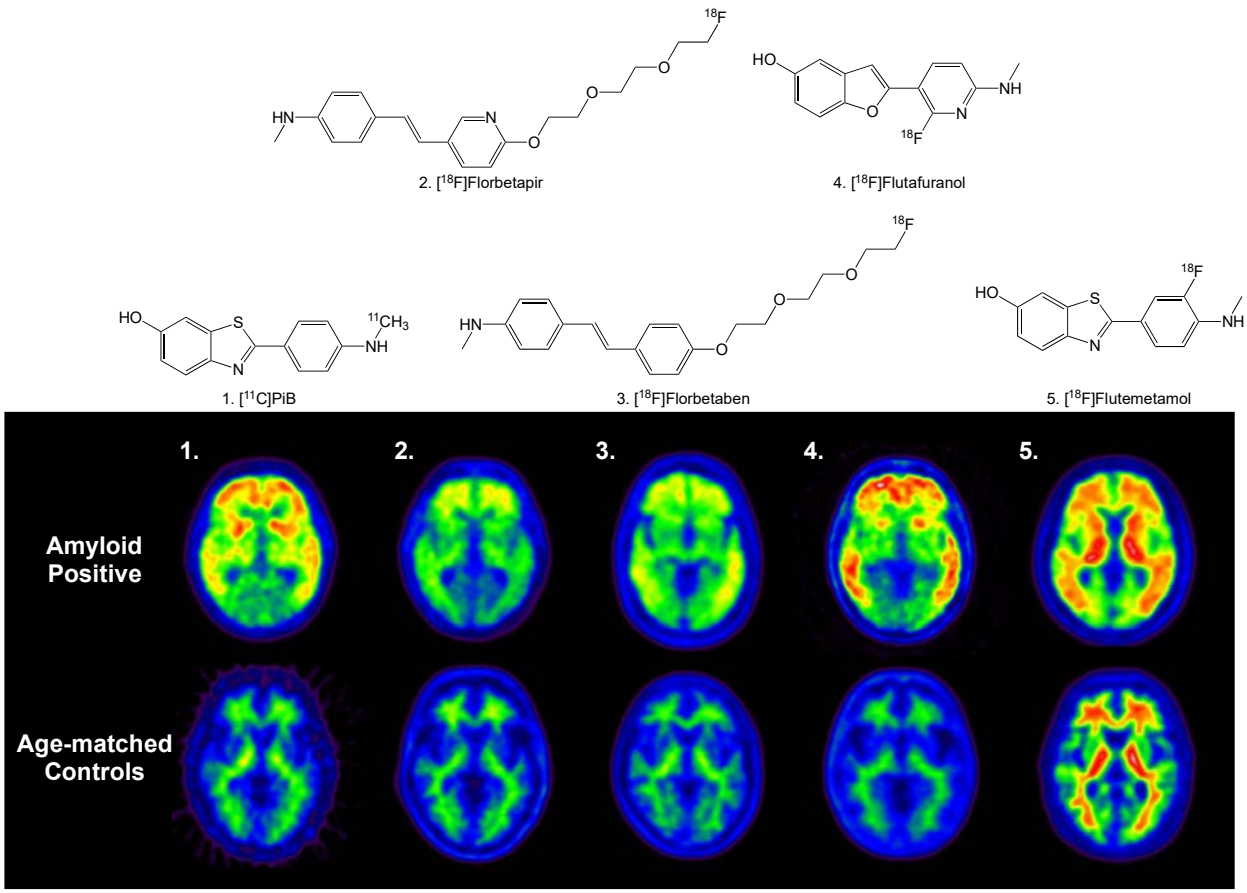


FIG. 81. Radiopharmaceuticals targeting amyloid plaques (courtesy of R. Koeppe, University of Michigan).

6.3. RADIOTRACERS FOR TAU

A number of neurodegenerative disorders are associated with the misfolding, aggregation and accumulation of protein deposits [129]. Tauopathies represent a subset of neurodegenerative disorders characterized by accumulation of abnormal types of tau protein, including Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration and frontotemporal dementia. For example, the pathophysiology of Alzheimer's disease includes both extracellular amyloid and intracellular tau neurofibrillary tangles. In the contexts of Alzheimer's disease, tau neurofibrillary tangles burden is of critical importance because it has been shown to correlate with cognitive decline [130]. Reflecting this, there has been considerable work undertaken to develop radiotracers targeting tau [131] (Fig. 82).

Tau is a phosphoprotein that stabilizes microtubules [129]. However, abnormal hyperphosphorylated tau gives rise to tau aggregates like neurofibrillary tangles. Imaging tau is complicated because there are six isoforms of tau that are sub-divided based on the number of repeats of the microtubule binding domain (3R or 4R) [132]. Tauopathies can be classified by the presence of 3R frontotemporal dementia, 4R (progressive supranuclear palsy, corticobasal degeneration) or both 3R and 4R Alzheimer's disease's tau aggregates.

Of the agents developed to date, only [^{18}F]flortaucipir ([^{18}F]T807, [^{18}F]AV1451, Tauvid) has garnered regulatory approval [133] (Fig. 82). Notably, first generation agents have limitations associated with off-target binding to monoamine oxidase ([^{18}F]flortaucipir, [^{18}F]THK5351), or brain penetrating metabolites ([^{11}C]PBB3) which need to be accounted for during image

analysis [132]. Moreover, these agents appear more selective for 3R tau. As such, several second generation radiotracers, all labelled with ^{18}F [131], are in various stages of development and translation ([^{18}F]MK-6240 (Fig. 82), [^{18}F]PI-2620, [^{18}F]RO-948, [^{18}F]GTP1, [^{18}F]PM-PBB3, [^{18}F]JNJ-311 and [^{18}F]JNJ-067). These tracers have been developed to address issues with off-target binding to monoamine oxidase, as well as to image both 3R and 4R tau.

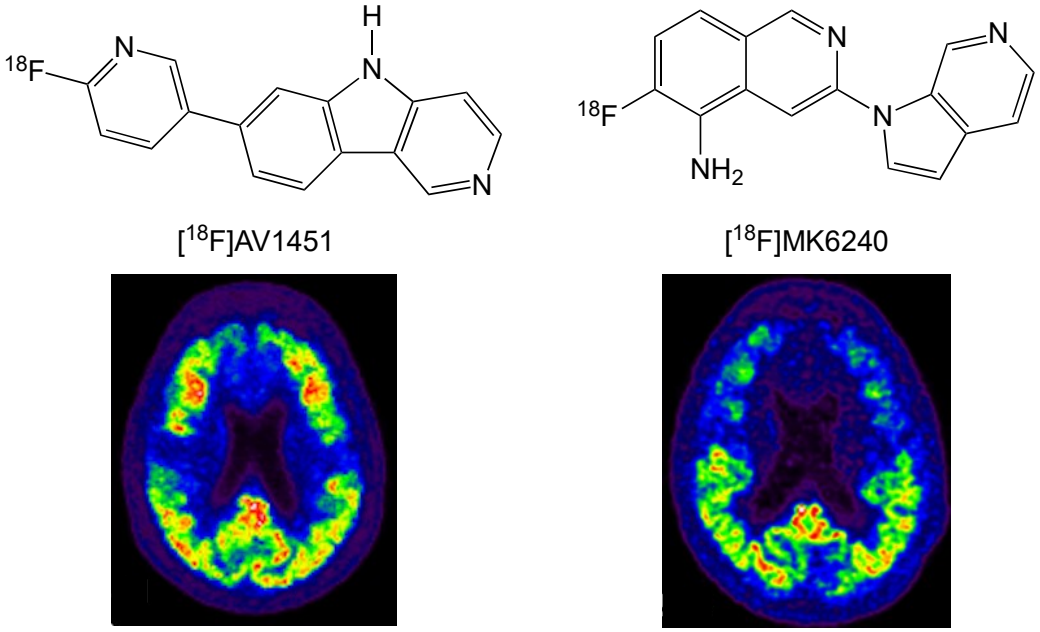


FIG. 82. Radiopharmaceuticals targeting Tau neurofibrillary tangles (courtesy of R. Koeppe, University of Michigan).

6.4. [^{18}F]FLUOROTHYMIDINE

[^{18}F]-Fluoro-3′ -deoxy-3′ -L-fluorothymidine ([^{18}F]FLT) is a proliferation radiotracer developed for imaging tumours [134]. [^{18}F]FLT is an analog of the DNA base thymidine that is taken up by cells (via both diffusion and transport via Na^+-dependent carriers). Once inside the cell, [^{18}F]FLT is phosphorylated by thymidine kinase 1 to generate [^{18}F]FLT-monophosphate ([^{18}F]FLT-MP) that is trapped in the cell (Fig. 83 (a)). Thymidine kinase 1 is upregulated in proliferating cells as it is expressed during the DNA synthesis phase of the cell cycle and, since sustained proliferation is a hallmark of cancer development and progression, there is preferential accumulation of [^{18}F]FLT-monophosphate in tumour cells [135] (Fig. 83 (b)).

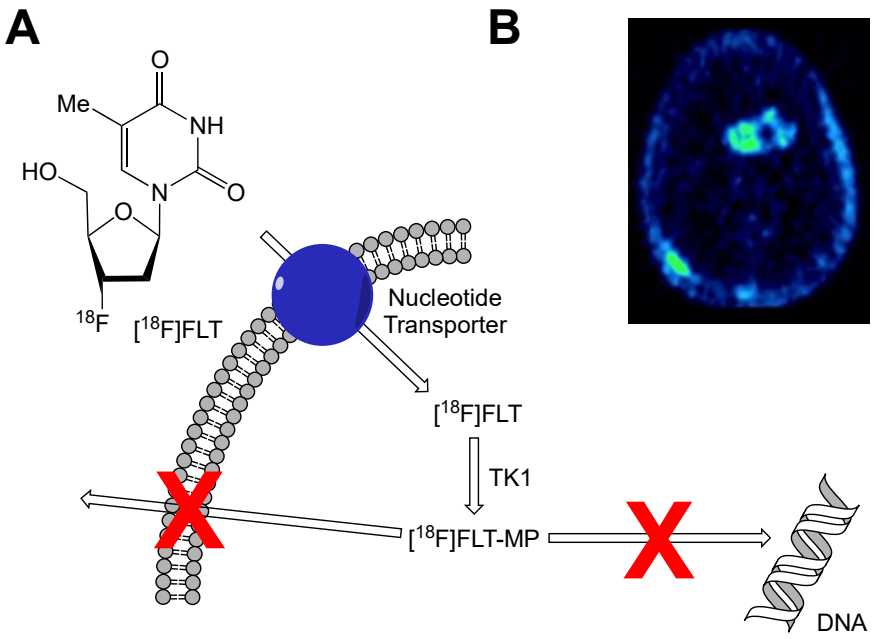


FIG. 83. (a) [^{18}F]FLT Mechanism of Action; (b): [^{18}F]FLT PET scan of a glioblastoma (reproduced from Ref. [135] with permission).

Note: TK1 = Thymidine kinase 1

The uptake of [^{18}F]FLT into tumours is rapid, and the agent has been widely used in numerous multicentre clinical studies to image different cancers including: brain tumours, breast cancer, non-small cell lung cancer, colorectal cancer, lymphoma, melanoma and sarcoma. [^{18}F]FLT is considered more of a cancer specific radiotracer than [^{18}F]FDG. Because of favourable signal to background ratio, [^{18}F]FLT has proven particularly useful for detecting brain tumours that can be difficult with [^{18}F]FDG [135]. As an imaging biomarker for cell proliferation, [^{18}F]FLT can also be employed to monitor patient response to anticancer therapy.

6.5. RADIOLABELLED AMINO ACIDS

^{18}F labelled amino acids (Fig. 84) are an important class of imaging agent that have broad applications in PET imaging [136]. They are most widely used in tumour imaging where the increased levels of amino acid transport by many tumours, stemming from increased protein synthesis due to accelerated growth, leads to accumulation of such radiotracers [137–139]. Like [^{18}F]FLT described above (section 6.4), there is low uptake due to background in the central nervous system, meaning that amino acid imaging has advantages over, for example, [^{18}F]FDG imaging of brain tumours. Other applicationsare described below in the context of individual radiotracers.

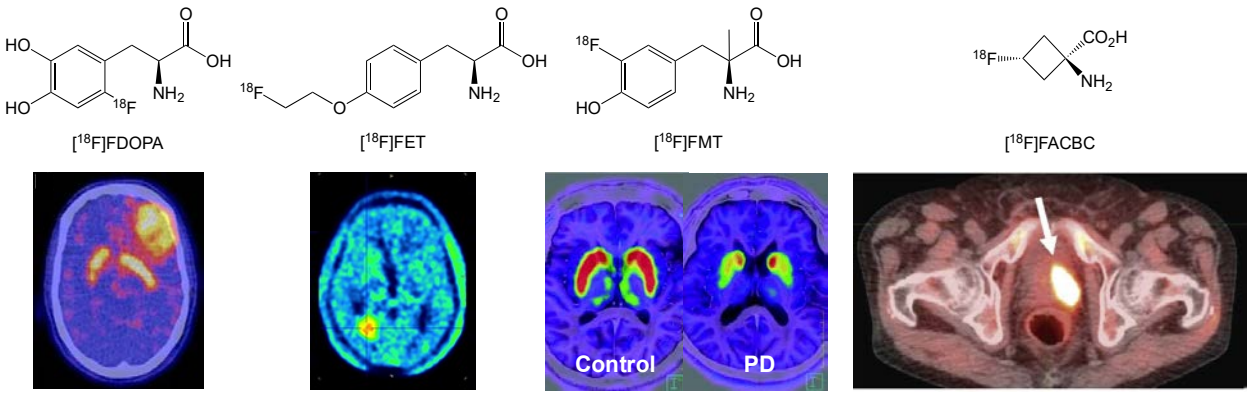


FIG. 84. Common ^{18}F labelled Amino Acid radiotracers. [^{18}F]FDOPA PET scan reproduced from [140]; [^{18}F]FET scan reprinted from [135]; [^{18}F]FMT scan reproduced from [141]; [^{18}F]FACBC scan reprinted from [142].

Anti-1-amino-3-^{18}F-fluorocyclobutane-1-carboxylic acid ([^{18}F]FACBC, [^{18}F]fluciclovine, Axumin) is a widely used ^{18}F labelled amino acid that is approved for imaging prostate cancer [142, 143] (Fig. 84). [^{18}F]FACBC is indicated for "PET imaging in men with suspected prostate cancer recurrence based on elevated prostate specific antigen levels" [143]. [^{18}F]FACBC is a ^{18}F labelled analog of L-leucine and is a substrate for two amino acid transporters (sodium-dependent system ASC and the sodium independent system L). [^{18}F]FACBC-PET is used to localize prostate cancer recurrence, where it has similar utility to [^{18}F]fluorocholine. Nowadays, prostate cancer imaging with both agents is superseded by PSMA-PETimaging.

Another well-known ^{18}F-labelled amino acid tracer is 6-[^{18}F]fluoro-L-dopa ([^{18}F]FDOPA) [144, 145], which finds use in imaging of neuroimaging, tumours and congenital hyperinsulinism. In dopaminergic nerve terminals in the brain, [^{18}F]FDOPA is decarboxylated by amino acid decarboxylase to generate [^{18}F]fluorodopamine which is stored in presynaptic vesicles in the brain. Thus, [^{18}F]FDOPA is indicated for "use in PET to visualize dopaminergic nerve terminals in the striatum for the evaluation of adult patients with suspected Parkinsonian syndromes" [146]. [^{18}F]FDOPA also finds widespread use in tumour imaging (Fig. 84), such as neuroendocrine tumours [140, 147]. The exact mechanism of uptake of [^{18}F]FDOPA in brain tumours is not entirely understood, but over-expression of the L-type amino-acid transporter 1 (LAT1) in tumours is thought to play a role. Lastly, several studies have described use of [^{18}F]FDOPA PET in patients with congenital hyperinsulinism, and suggest the technique is extremely accurate for distinguishing diffuse from focal disease (Christiansen CD, Petersen H, Nielsen AL, Detlefsen S, Brusgaard K, Rasmussen L, Melikyan M, Ekström K, Globa E, Rasmussen AH, Hovendal C, Christesen HT. 18F-DOPA PET/CT and 68Ga-DOTANOC PET/CT scans as diagnostic tools in focal congenital hyperinsulinism: a blinded evaluation. Eur J Nucl Med Mol Imaging. 2018 Feb;45(2):250-261).

Other amino acid imaging agents include ^{18}F labelled derivatives of tyrosine and phenyalanine, which are structurally related to [^{18}F]FDOPA and are sodium-independent amino acid transport system L (LATs) substrates. For example, both [^{18}F]fluoroethyl-L-tyrosine ([^{18}F]FET) [135, 148] and L-[3-^{18}F]-α-methyltyrosine ([^{18}F]FMT) [149] have proven useful for tumour imaging, while the latter has also been used for neuroimaging in Parkinson disease(Fig. 84) [141].

Finally, there is considerable interest in imaging agents based upon glutamine after the discovery that this non-essential amino acid has a key role in tumour proliferation. Glutamine metabolism is another important metabolic pathway in tumours, and certain cancers have a

glucose consumption rate >10 times that of normal cells. To this end, Liu and co-workers [150] recently translated ^{18}F - (2*S*,4*R*)-4-fluoroglutamine into clinical studies, and obtained encouraging results in preliminary imaging studies in breast cancer patients.

6.6. UPTAKE MECHANISM OF PSMA RADIOPHARMACEUTICALS

Prostate cancer (PCa) is the highest prevalent cancer diagnosed among the human male population leading to high morbidity worldwide. The routine detection route consists of prostate specific antigen (PSA) levels determination in serum, a rectal exam, and biopsies. Unfortunately diverse PCa etiopathology might hamper the determination of the correct critical limit of PSA levels.

The visualization of prostate cancer was revolutionized by the discovery, in the early 90's, of the presence and overexpression of PSMA. It is found to be present not only in the primary disease, but also in neovascularization of metastases, lymph nodes and in the recurrent disease. Furthermore, PSMA expression varies over the different stages of the disease, with low levels in benign prostatic tumour tissue, but showed a specific increase in well-defined early stage PCa.

PSMA is a well characterized target in 'structure activity relationship' studies in medicinal chemistry, which is basically the enzyme glutamate carboxypeptidase II, an enzyme which detaches glutamate moiety from *N*-acetyl-L-aspartyl-L-glutamate [151].

The ^{111}In-labelled monoclonal PSMA antibody capromab pendetide, better known as ProstaScint, was the first U.S. Food and Drug Administration (FDA) approved PET tracer for PCa used in clinics. However, the disadvantage of ProstaScint which is binding to the intracellular binding domain (Fig. 85) is the long circulation time of the antibody, which decreases its imaging potential. Therefore, research was performed towards finding small molecular weight molecules targeting PSMA at the extracellular binding site (Fig. 85). Several tracers were developed and published with very promising results, including three main binding motifs: thiol based, phosphoric acid based, and urea based PSMA inhibitors (Fig. 86). Three PSMA targeting tracers have been clinically introduced for this purpose: [^{68}Ga]PSMA-HBED-CC, [^{18}F]PSMA-1007 and [^{18}F]DCFPyL (Fig. 86). Upon binding to PSMA, the protein PET ligand complex internalizes into the cytoplasm.

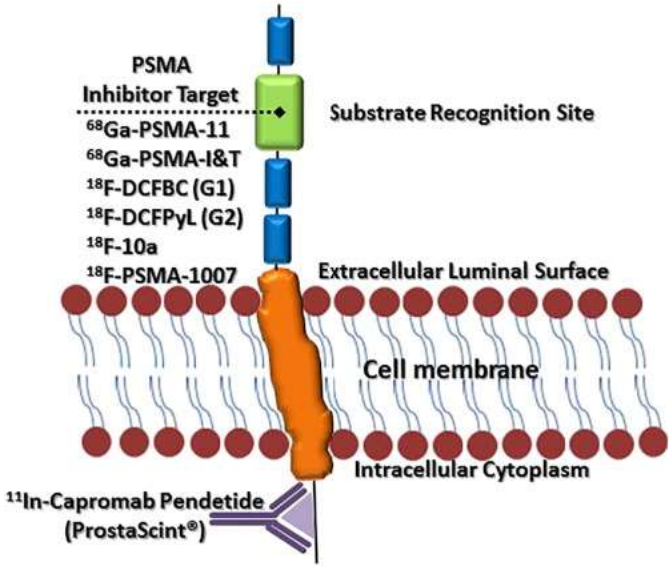


FIG. 85. Binding sites for PSMA targeted radioligands (reproduced from Ref. [152] with permission).

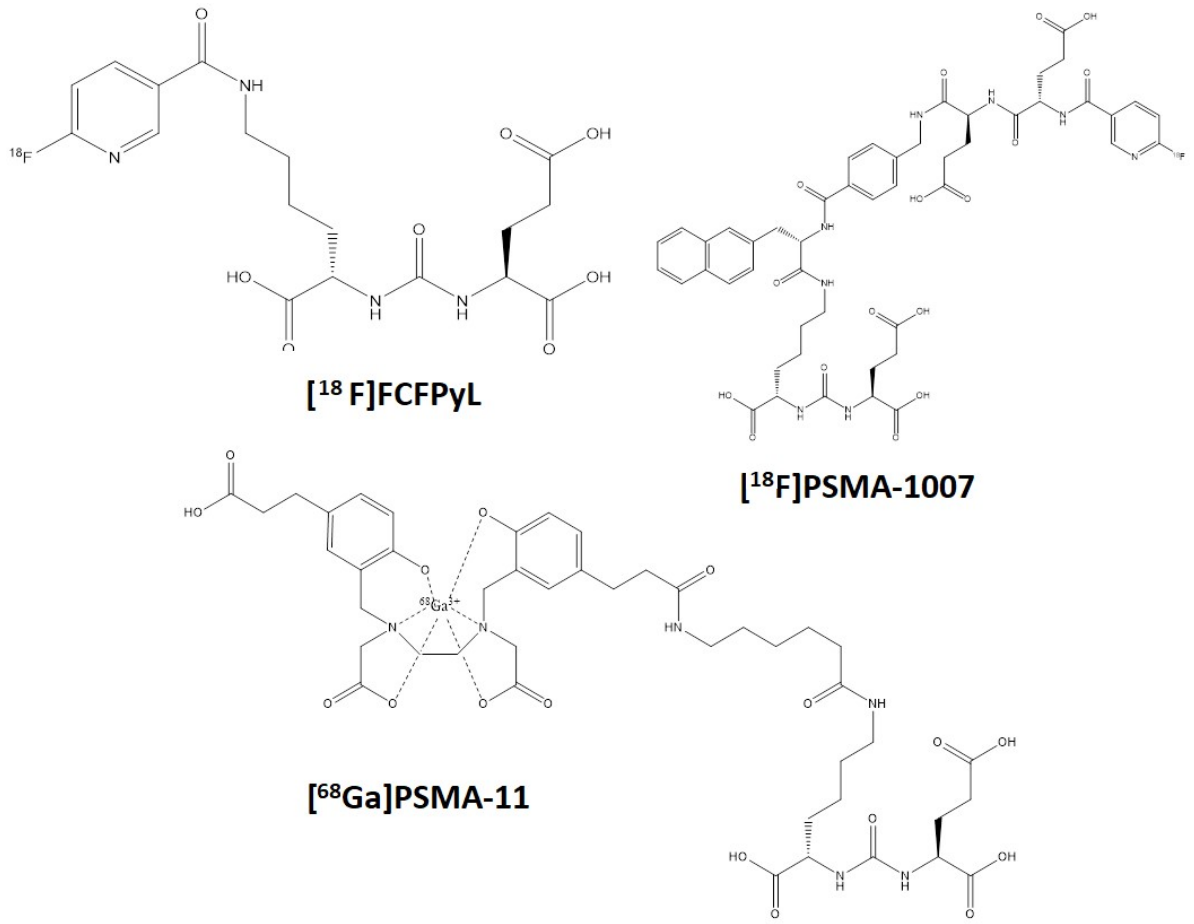


FIG. 86. Some representative chemical structures of urea based PSMA targeted radiopharmaceuticals (courtesy of M. Huiban, Invicro LLC).

[^{68}Ga]PSMA-HBED-CC was the first clinically used radiotracer targeting PSMA and it is based on the urea based glutamate-urea-lysine (Glu-urea-Lys) binding motif. Despite its high diagnostic potential, the production capacity is limited as one production yields only a few patient doses. Additionally, ^{68}Ga has a positron energy of 1.9 MeV and a higher positron range than ^{18}F (635 keV), which results in a lower resolution of the final image. This fact promoted the development of ^{18}F based PSMA tracers for PCa imaging. [^{18}F]PSMA-1007 and [^{18}F]DCFPyL became the most frequently used radiotracer in clinics all over the world.

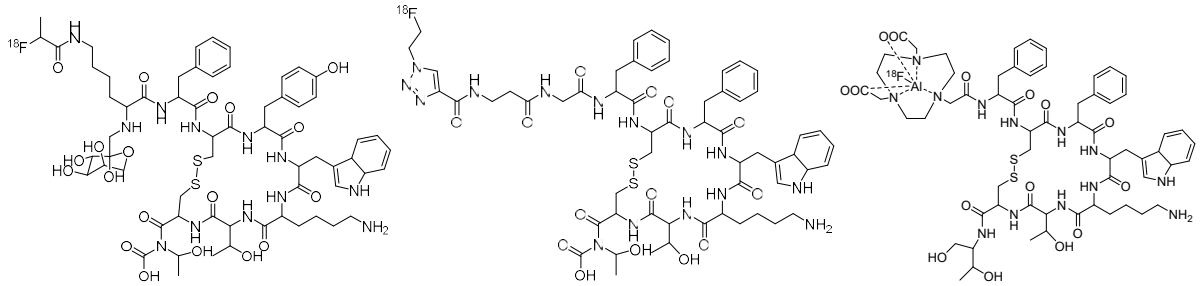
6.7. SOMATOSTATIN RECEPTOR UPTAKE MECHANISMS

Somatostatin receptors (SSTR) are G-protein coupled membrane glycoprotein receptors that are utilized in the diagnosis and treatment of neuroendocrine tumours (NET) [153, 154]. Although the list of human cancers and diseases that are known overexpressing SSTRs are extensive, some examples include gastroenteropancreatic NETs, paragangliomas, breast cancers, prostate cancers, pituitary adenomas, small cell lung cancers, medullary thyroid carcinomas, Merkel cell carcinomas and neuroblastomas [155–157]. Somatostatin (SST) is the endogenous SSTR ligand which is a neuropeptide that is produced and excreted by cells such as neuroendocrine, immune and inflammatory cells [158].It has many downstream biological effects yet it is mainly a broad inhibitor of the secretion of hormones, proliferation and cell growth [159]. SST itself is suboptimal as a pharmaceutical due to its short biological half-life (<3 min) [154, 156, 160]. Therefore, somatostatin analogs that have been generated are more

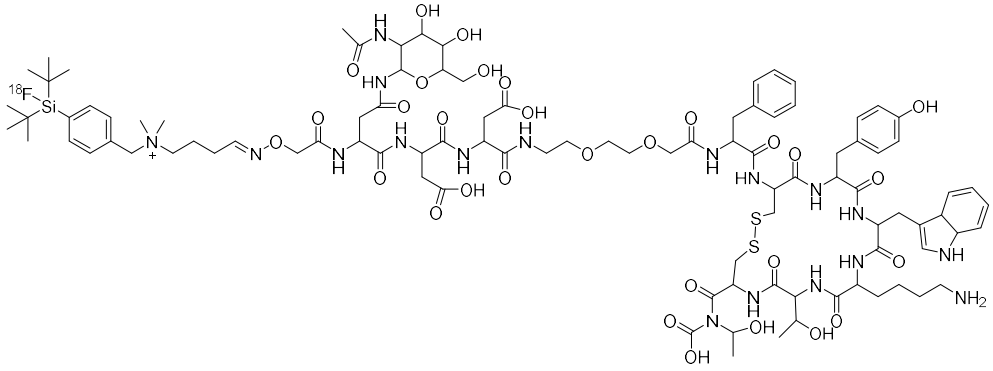
stable receptor selective agonists in vivo. The first somatostatin analogs developed were octreotide and lanreotide that were initially used in the treatment of carcinoid syndrome, a side effect due to the hypersecretion of hormones associated with NETs [161]. Octreotide derivatives are the most commonly used vectors for targeting relevant radionuclides to NET sites since octreotide has a significantly high affinity for subtype SSTR2 and a suitable biological half-life of about 72–98 min [162]. The common derivatives used are Tyr^3-octreotide (TOC), Tyr^3-octreotate (TATE) and 1-Nal^3-octreotide (NOC) [162].

The ^{111}In-labelled octreotide was the first EMA and FDA approved tracer for NETs used in clinics. ^{68}Ga-labeled somatostatin analogues have shown excellent results for imaging of NETs and better results than conventional SSTR scintigraphy [163]. Recently, a preparation of ^{68}Ga-DOTATOC formulation (SomaKIT TOC) has been introduced in the market [164].

Current investigational ^{18}F radiotracers that target SSTR are Gluc-Lys-[^{18}F]FP-TOCA, [^{18}F]FET-βAG-TOCA, Al^{18}F-NOTA-octreotide and [^{18}F]SiTATE (Fig. 87).



Gluc-Lys-[^{18}F]FP-TOCA (1) [^{18}F]FET-βAG-TOCA (2) Al[^{18}F]-NOTA-octreotide (3)



[^{18}F]SiTATE (4)

FIG. 87. Common ^{18}F labelled SSTR Radiotracers (courtesy of M. Huiban, Invicro LLC).

Targeting SSTRs with radiolabelled SSAs allows for the imaging and localizing primary NETs as well as metastases [158]. The mechanism of uptake being receptor specific binding, and receptor mediated cell internalization of the receptor peptide complex. Internalization of the radiolabelled SSA receptor complex allows for accumulation of the radiotracer in the NET cells. Visualization of the tumour localization can then be done via PET or single photon emission computed tomography (SPECT); as well, treatment is available using peptide receptor radionuclide therapy (PRRT) [156, 165]. The receptor subtype SSTR2 is a good target for nuclear imaging as well as PRRT not only because it is commonly found overexpressed at a high density on certain tumour cells, but it also has low expression on normal cells allowing for preferential image contrast and minimal off-target effects [166]. Nuclear imaging of NETs

is therefore considered as an ideal method as it can provide high specificity, low toxicity, and favourable pharmacokinetics [158]. Additionally, the higher image quality and specificity of NETs achieved through PET/SPECT compared to the widely used MRI/CT allows for earlier detection and personalized treatment plans [165, 167]. Further, PRRT is non-invasive and highly specific to cells with an overabundance of SSTRs, effectively targeting NET while minimizing damage to healthy tissues and thus adverse side effects. The ability to utilize PRRT against NET is significant as approximately 12–22% of patients are metastatic at diagnosis, limiting the possibility of surgical resection [161].

6.8. INFLAMMATION TRACERS

Inflammation is a key factor in multiple pathologies, from oncology to cardiovascular, metabolic, musculoskeletal and nervous system diseases. Imaging inflammation is therefore of great interest in order to improve disease diagnosis, guide therapeutic intervention and monitor efficiency of treatments. Though this has been a great challenge to date, as much is still to understand in terms of the underlying processes by which the immune response is activated. With regards to visualization of inflammatory diseases by nuclear imaging modalities (PET/SPECT), multiple radiopharmaceuticals have been developed to date, labelled with a wide range of isotopes some of which are still at preclinical evaluation stages. A lot of attention was also given to neuroinflammation in particular, due to its prevalence in many central nervous system disorders, including a range of neurodegenerative disorders, from which primarily ^{11}C labelled radiotracers have been identified [168]. Nonetheless, numerous ^{18}F inflammation radiotracers have also been developed over the years, and below is presented a brief overview of some examples that are currently available for clinical use (Fig. 88).

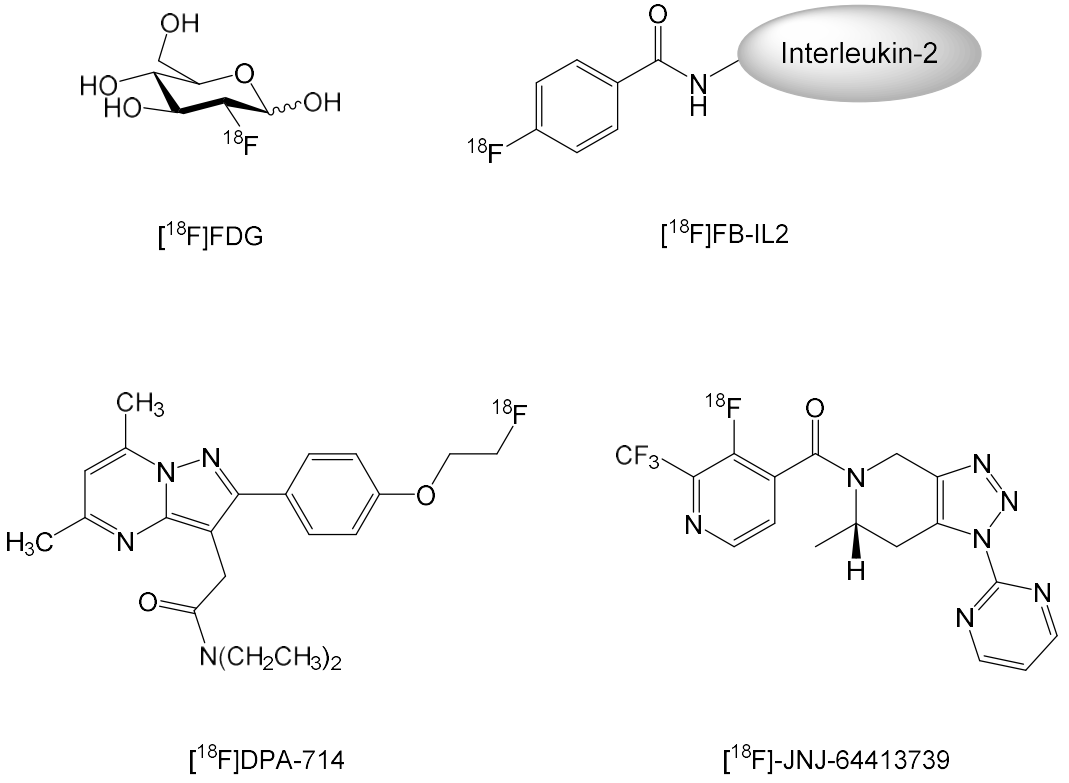


FIG. 88. Examples of ^{18}F inflammation radiotracers (courtesy of M. Huiban, Invicro LLC).

6.8.1. [^{18}F]FDG

As for tumour cells, increased glycolysis is also a feature of the immune system following activation, and therefore [^{18}F]FDG has seen wide use in imaging of inflammatory diseases. Examples of use include cardiac sarcoidosis and vasculitis. However, it should be noted that there is still a lack of evidence of the true value of [^{18}F]FDG in imaging inflammation [169].

6.8.2. [^{18}F]FB-IL2

Cytokines are cell signalling molecules that enable communication of immune cells and stimulate their movement towards sites of inflammation. Interleukin-2 (IL2) is one example of such cytokines that binds to the IL2R receptor expressed mainly on activated T cells (T lymphocytes). [^{18}F]FB-IL2 was designed by reacting recombinant human IL2 with [^{18}F]SFB, and preclinical evaluation did confirm it was specifically binding to interleukin-2 receptors expressed on activated CD25+ T cells. A GMP compliant synthesis of [^{18}F]FB-IL2 was recently proposed [78], and clinical evaluation of the tracer is currently ongoing as of early 2021.

6.8.3. [^{18}F]DPA-714

Neuroinflammation, which involves microglia and astrocytes activation, is thought to play a key role in the development and progression of neurodegenerative diseases and other brain pathologies. The main biomarker of microglial activation that has been studied to date is notably the translocator protein-18 kDa (TSPO), for which three generations of compounds have been proposed over the years, to overcome various limitations such as low brain density, lack of specificity (target expressed on other cells than the microglia) and variability in the tracer affinity for the target (due to genetic polymorphism). Clinically, the most used ^{18}F-TSPO radiotracer is probably [^{18}F]DPA-714.

6.8.4. [^{18}F]JNJ-64413739

Another marker of microglial activation that has received attention is the P2X7 receptor, a member of the purinergic family of receptors. In the brain, the P2X7 ion channel is predominantly expressed in activated microglia, but is also present in neurons and astrocytes. The promising use of the selective P2X7 antagonist [^{18}F]JNJ-64413739 in preclinical models, led to its translation to humans, where its suitability as the PET ligand was recently confirmed [170]. However further work is still needed to fully assess its potential, as limitations arising from the tracer itself (high intersubject variability) and the target (e.g. lack of cell specificity) may hamper its future use.

6.9. UPTAKE MECHANISMS OF RADIOLABELLED BIOMOLECULES (ANTIBODY FRAGMENTS, PEPTIDES)

Radiolabelled biomolecules include protein based radiopharmaceuticals ranging from small peptides to intact antibodies. These radiopharmaceuticals are mostly applied in oncology. Well known examples are somatostatin receptors, CCK, gastrin releasing peptide receptors for peptides and HER2, VEGF and PDL1 for antibodies.

Summarizing the pharmacokinetics of these protein based radiopharmaceuticals, it can be concluded that size of the protein molecule matters regarding its pharmacokinetics After the intravenous administration, factors like extravasation, diffusion, accumulation, and clearance determine tissue uptake and image contrast. For full antibodies these processes take quite long resulting in optimal scanning protocols, taking 3 to 7 days after injection. Therefore, a longer lived radionuclide needs to be used for PET imaging such as ^{124}I or ^{89}Zr. Because of the favourable characteristics of ^{18}F, fragments of antibodies (Fab) retaining the antigen binding properties have been generated.

The advantage of the use of radiolabelled Fab and $(Fab')_2$ fragment of monoclonal antibodies (mAbs) are:

(a) Shorter time interval between injection and imaging;
(b) Often better contrast in comparison with full antibodies;
(c) Lower radiation dose to patients.

However, there are still some challenges such as:

(a) Decrease of apparent binding affinity compared to full antibodies. This is typical for Fab fragments due to the loss of the avidity effect of bivalent binding;
(b) Absolute tumour uptake is often lower and kidney uptake is often increased;
(c) Both Fab and (Fab)2 are still too large to have efficient extravasation;
(d) Both Fab and (Fab)2 are still above the border of enhanced permeability and retention (45 kDa for globular proteins). Small peptides do not suffer from slow PK; they can rapidly penetrate into tissue and bind to the receptor of interest.

After binding of the protein based tracer with the receptor/antigen (=target), the combined tracer/target complex internalized into the cancer cell by endocytosis (Fig. 89) [171]. The target will undergo recycling and will be available for another interaction with a tracer. The internalized tracer can undergo 1. degradation by peptidases, 2. release the radiolabel. In most cases the radiolabel stays in the cell.

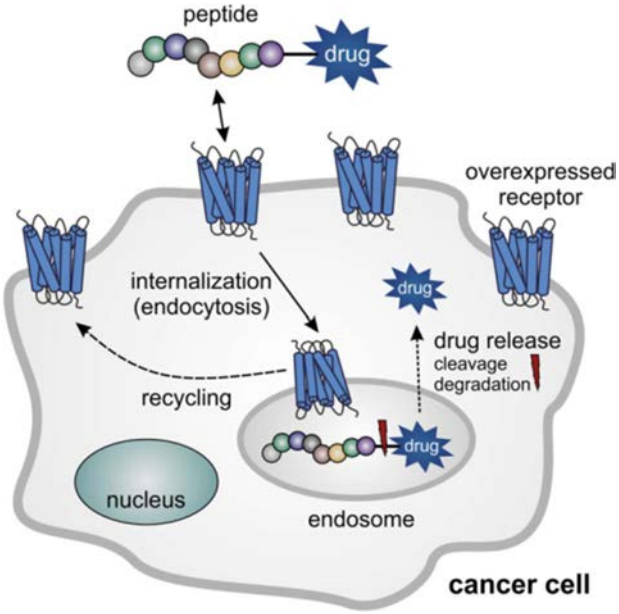


FIG. 89. Uptake mechanism of peptide/protein based radiopharmaceuticals in the cancer cell (reproduced from Ref. [171] with permission).

Another approach to address the slow PK of full antibodies is pretargeting (Fig. 90) [172], where tumour target antigen binding specificity is obtained by: (a) injecting unlabelled antibody derivatives followed; (b) by a radiolabelled low molecular weight compound that specifically binds to the antibody and is rapidly cleared from the circulation. The binding between the antibody and the radiolabelled compound should follow a bio-orthogonal fashion and can be achieved via click chemistry of ultra high affinity interactions such avidin-streptavidin.

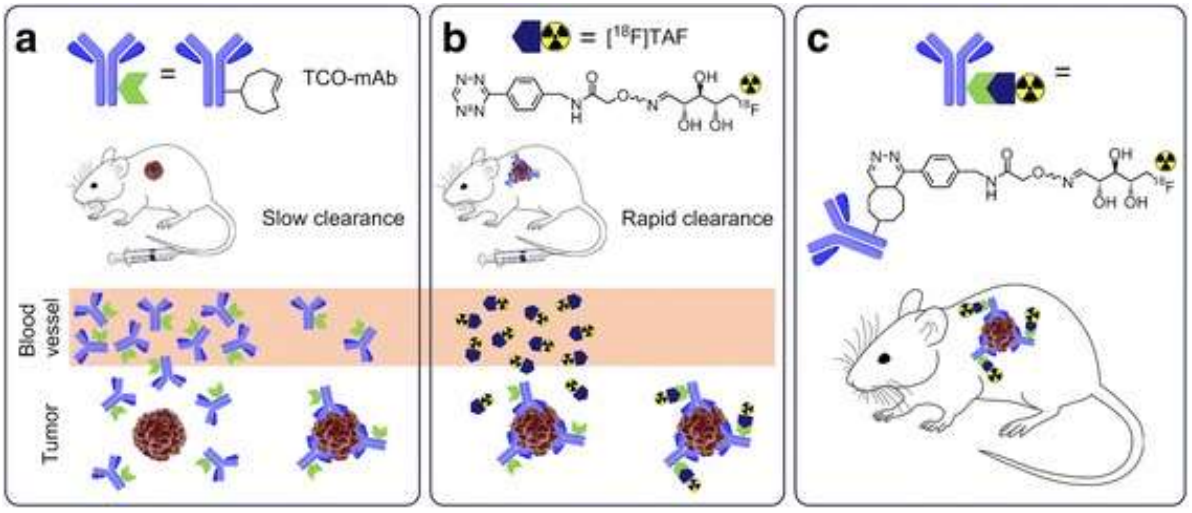


FIG. 90. Principle of pretargeting strategy (reproduced from Ref. [172] with permission).

Advantages of this approach are:

(a) Taking advantage of the biodistribution pattern of high affinity full antibodies;
(b) Short time interval between injection of radiotracer and scanning;
(c) Possibility of using ^{18}F with its favourable radiation characteristics.

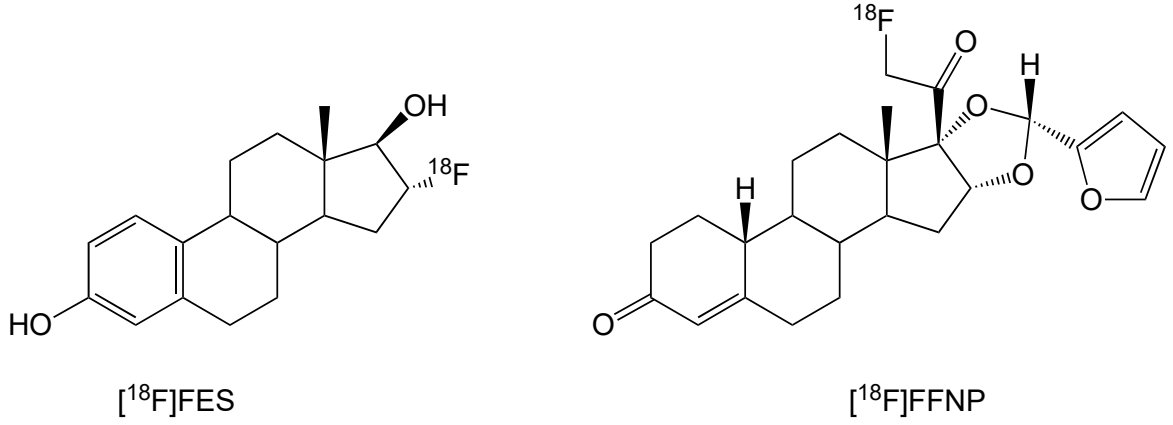
Challenges still to be addressed

(a) Non-specifically bound antibody can affect the PET image;
(b) Two iv injections required (two visits to hospital);
(c) Time intervals between injections are critical;
(d) A clearing agent using non-radioactive IEDDA counterpart can be used but involves a 3rd iv injection;
(e) 2–3 FDA or EMA approvals required for each administered agent.

6.10. HORMONE RECEPTORS

Breast cancer is the most common cancer in the women, with 2 million new cancer cases diagnosed every year. Breast cancer diagnosis are mainly based on estrogen receptor and progesterone receptor positive, ^{18}F radiolabelled steroid hormones can be used to study and image tumour expression of estrogen receptor and progesterone receptors in patients with primary and metastatic breast cancer.

16α-[^{18}F]fluoro-17β-estradiol ([^{18}F]FES) and [^{18}F]fluoro-furanyl-norprogesterone ([^{18}F]FFNP) (Fig. 91) are two promising radiopharmaceuticals for estrogen/progesterone receptors content evaluation of tumours using PET, allowing whole body receptor scanning without invasive techniques such as biopsy. The advantages of non-invasive in vivo assessment include avoiding sampling error, assessing the entire tumour volume receptor status rather than part of the tumour (addressing the heterogeneity of ER expression), assessing the status of all the lesions expressing ER, and assessing the biological activity of the receptor at diagnosis and in response to treatment. In patients with estrogen receptor expressing tumours, [^{18}F]FES-PET may prove useful for patient stratification, selection of patients eligible for hormonal therapy, assessment of estrogen receptor occupancy, response prediction and follow-up. Recently, FDA approved ^{18}FF-fluoroestradiol, manufactured by Zionexa as Cerianna, as radiopharmaceutical for ‘estrogen receptor +’ in breast cancer, as an adjunct to biopsy in patients with recurrent or metastatic breast cancer.



[^{18}F]FES [^{18}F]FFNP

FIG. 91. Structures of estrogen receptor PET-agents labelled with ^{18}F (courtesy of E. Cazzola, Sacro Cuore Hospital).

6.11. [^{18}F]FLUOROCHOLINE

Choline is used in all cells as precursor for phospholipids biosynthesis, essential as membrane component. Therefore, choline is transported into the cells, metabolized and then trapped on both normal and cancer cells. Many tumours are characterized by increased cell proliferation that include increased metabolism and increased choline demand. Choline demand originated from hyperactivity of the enzyme (choline-kinases) that is essential to create a phophatidylcholine, which is fundamental for synthesis of the cell membrane. A ^{18}F derivative of choline, that follows the same biological pathway of choline can be used for image of altered cell function.

Historically [^{11}C]Choline was used to evaluate many kinds of tumours like in the brain, lung, urinary bladder or prostate where was very effective on diagnosis. Due to the short half-life of ^{11}C (20 min), [^{11}C]Choline is very difficult to use in routine clinical care, to cover the high radiopharmaceutical demand. For this reason, several [^{18}F]fluorinated derivatives of choline were studied and [^{18}F]fluorocholine (Fig. 92) was selected as a promising fluorine analogue.

The presence of monograph in EU and USA Pharmacopoeia combined with several approved marketing authorizations in many countries make F-Choline availability easy and well spread around countries.

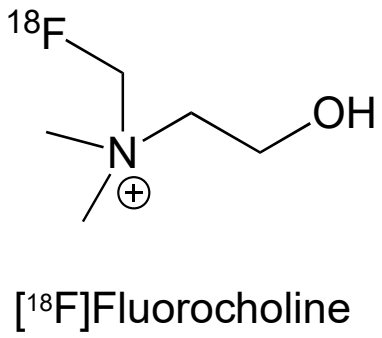


FIG. 92. Structure of [^{18}F]fluorocholine (courtesy of E. Cazzola, Sacro Cuore Hospital).

6.12. CLASSES OF PET RADIOPHARMACEUTICALS APPLIED IN HUMANS

6.12.1. PET radiopharmaceuticals for brain studies

Regarding PET radiopharmaceuticals for human brain studies several new applications to image new targets have emerged. Third generation tracers for TSPO (related to image activated microglia and neuroinflammation) [173], ^{18}F-labelled tracers for the cholinergic system, tracers to image protein misfolding (beta-amyloid, tau and alpha-synuclein for Alzheimer's disease, Parkinson's disease and other neurodegenerative diseases). Very recently also PET radiopharmaceuticals were published for Synaptic vesicle glycoprotein 2A (SV2A) receptors representing synaptic density which is a factor that changes over time during neurodegeneration [174]. Table 17 gives an overview of neuroPET radiopharmaceuticals applied in humans and appeared in literature

TABLE 19. NEURO PET-TRACERS APPLIED IN HUMANS
(courtesy of P. Elsinga, University of Groningen)

Target	Tracer	Physiological process
TSPO	DAA and PBR derivatives, [^{18}F]GE180	Antagonist

GABA	[^{18}F]Flumazenil	Antagonist
Dopaminergic system	[^{18}F]FDOPA	Vesicular storage
	[^{18}F]Fallypride	D_2 antagonist
	[^{18}F]FP-CIT, [^{18}F]FE-PE2I	Dopamine transporter
	[^{18}F]Florbetaben/florbetapir	Staining agent
NMDA	[^{18}F]GE179	Antagonist
Cholinergic system	[^{18}F]FEOBV	VAChT ligand
	[^{18}F]FP-TZTP	M_2 antagonist
	[^{18}F]ASEM, [^{18}F]A-85360,	α_7-nAChR ligands
	[^{18}F]flubatine	$\alpha_4\beta_2$-nAChR ligands
mGlu-5	[^{18}F]PSS232, [^{18}F]FPEB	Antagonist
VMAT2	[^{18}F]AV-133, [^{18}F]FP-DTBZ	Inhibitor
5-HT_{1A}	[^{18}F]MPPF	antagonist
P2X7	[^{18}F]JNJ-64413739	Antagonist
GLUT-transporters and Hexokinase	[^{18}F]FDG	Glucose consumption

TABLE 17. NEURO PET-TRACERS APPLIED IN HUMANS *('cont')*

Target	Tracer	Physiological process
Tau protein	[^{18}F]THK523, [^{18}F]AV1541	Protein misfolding in AD
Phosphodiesterase-PDE-4	[^{18}F]MNI589	Breakdown of cAMP
SV2A	[^{18}F]UCB-J	Synaptic density Ligands
Sigma	[^{18}F]fluspidine	Antagonists

6.12.2. Oncology PET tracers

Table 18 represents published PET radiopharmaceuticals that are used in oncological patients. The main development is the emerging of tracers for PSMA ([^{18}F]PSMA1007) to diagnose and treat prostate cancer [175]. Furthermore, tracers for EGFR-tk are developed for treatment follow-up. In comparison to the neurology tracers there are a few new tracers labelled with ^{11}C or ^{18}F published in the last 5 years. Physicians still use established tracers such as [^{18}F]FDG, [^{18}F]FET, [^{18}F]FDOPA and [^{18}F]FLT.

TABLE 20. ONCOLOGY PET TRACERS APPLIED IN HUMANS
(courtesy of P. Elsinga, University of Groningen, the Netherlands)

Target	Tracer	Physiological process
GLUT-transporters and Hexokinase	[^{18}F]FDG	Glucose consumption
Thymidine kinase type 1	[^{18}F]FLT	DNA synthesis
Choline synthase	[^{18}F]fluormethyl choline	Membrane synthesis
Dopamine storage	[^{18}F]fluoro-DOPA	Dopaminergic system
Amino acid transporter	[^{18}F]fluoroethyl tyrosine	Amino acid transports
Hydroxyapatite matrix	[^{18}F]sodium fluoride	Bone metastases
Androgen receptor	[^{18}F]FDHT	Prostate cancer
Estrogen receptor	[^{18}F]FES	Breast cancer

Hypoxia	[^{18}F]FMISO, [^{18}F]FAZA,	Oxygenation levels in tissue
PSMA	[^{18}F]PSMA1007	Prostate cancer
Amino acid transporter	[^{18}F]fluciclovine	Prostate cancer
Integrins, alphaV-beta3	[^{18}F]galacto-RGD	Angiogenesis

6.12.3. Cardiovascular tracers

With respect to cardiovascular PET radiopharmaceuticals most interest is in the PET imaging of heart perfusion which is affected in heart failure and infarction [176]. Traditionally [^{13}N]ammonia and [^{15}O]water are the most used tracers but because of the short half-life only hospitals with a cyclotron are able to perform such PET studies. Another option is [^{18}F]flurpiridaz, however its clinical value is not proven yet. Recently mini-cyclotrons in combination with user friendly equipment and software have been developed by industry to boost the use of [^{13}N]ammonia and [^{15}O]water. Table 19 demonstrates some unconventional myocardial tracers.

Another application is reuse of PET radiopharmaceuticals for atherosclerosis imaging. Several tracers that are already in use for oncology or neuroinflammation have found their way to probe molecular processes in atherosclerotic plaques.

TABLE 21. MYOCARDIAL PET-TRACERS APPLIED IN HUMANS
(courtesy of P. Elsinga, University of Groningen, the Netherlands)

Target	Tracer	Physiological process
Mitochondrial complex	[^{18}F]flurpiridaz	Blood flow
Several targets see table above	Na[^{18}F]F, [^{18}F]fluoromethyl choline, [^{18}F]galacto-RGD	Atherosclerotic plaques

6.12.4. Infection/inflammation tracers (excluding neuroinflammation and atherosclerosis)

PET radiopharmaceuticals to investigate inflammatory and infectious processes are relatively new [177]. Traditionally radiolabelled white blood cells are used to image these processes. Furthermore, [^{18}F]FDG is applied as a non-specific tracer to measure metabolic flair as a result of infection/inflammation. For bacterial infection imaging first PET tracers have become available based on ubiquicidine peptides (Table 20).

TABLE 22. INFECTION/INFLAMMATION PET TRACERS APPLIED IN HUMANS
(courtesy of P. Elsinga, University of Groningen, the Netherlands)

Target	Tracer	Physiological process
Energy consumption	[^{18}F]FDG	Activated immune cells
Antimicrobial peptides	[^{18}F]Ubiquicidin peptides	Immune response
T-cells	[^{18}F]Interleukin-2	Immune response

7. REGULATORY CONSIDERATIONS

7.1. DESIGN OF GMP COMPLIANT CYCLOTRON PET RADIOPHARMACEUTICAL

PRODUCTION FACILITY

7.1.1. Introduction

The indispensability of PET-CT imaging with management of cancer as main application is driving the need for setting up new cyclotrons and PET radiopharmaceuticals production facilities all across the world. These facilities need to be set up in compliance with GMP as the products produced in these facilities are used for human administration. While there are differences in the individual GMP practices followed in different countries, it is advisable to plan the facility as per WHO/EU standards. However, the readers are advised to follow their own national guidelines.

GMP has several components such as the suitability of the premises and equipment, adequacy of the personnel and their training as well as a systematic documentation of all the processes taking place within the facility. There is also a need to have a quality management system that identifies and documents the organization structure, procedures, processes as well as the systematic actions that will be taken to ensure that the radiopharmaceuticals produced in the facility satisfy the specified quality requirements [178].

This section gives the general guidelines for the design of a GMP compliant cyclotron PET radiopharmaceuticals production facility and hence limited to the single component of GMP i.e. the 'premises'. IAEA has published a 'Technical Report Series (TRS 471): Cyclotron Produced Radionuclides: Guidelines for facility design', which gives details about setting up cyclotron-PET radiopharmaceuticals production facilities and readers are invited to refer to that publication for more details [6].

7.1.2. Scope of the facility

It is important to understand the precise scope of the facility to be set up before starting the design of a cyclotron PET radiopharmaceuticals production facility. There are different working models in existence in different parts of the world. The first and the simplest of such models is a single user facility wherein a major hospital installs a cyclotron for PET radiopharmaceuticals production to be used for in-house applications. This facility may also have some research component integrated to it with respect to the preparation and development of new tracers which are relevant for clinical use. On the other extreme are the national facilities which are run by the Atomic Energy Institutes. These facilities will plan to have very high levels of research components in addition to production and supply of radiopharmaceuticals. The research done could be not only in chemistry of new tracers but also in cyclotron, targetry etc. A third type of facility is that is operated by private operators which is solely dedicated to the commercial production of radiopharmaceuticals for distribution. These types of facilities will have very little R&D component. The planning of the above facilities will have significant differences. The design of the facility will depend on which category the new cyclotron-PET centre will fit in. However, one common point is that the new facility to be installed should comply with the elements of GMP so that the radiopharmaceuticals manufactured are approved by drug regulatory authorities for human use.

7.1.3. Products to be manufactured

It is important to clearly visualize the products that will be manufactured. If it is a single user facility inside a hospital it will be worthwhile to plan the production of ^{13}N and ^{15}O for the

preparation of some of the ultra short lived radiopharmaceuticals. If the facility is part of a research centre, it will be important to plan solid targets for production of some of the exotic PET radionuclides. The facility can also be designed exclusively for the large scale production of [^{18}F]FDG and other ^{18}F- radiopharmaceuticals only if the desire is to make a commercially viable facility. If the facility is sought for collaboration by drug industry, planning gas target for ^{11}C-production will be important. The user has to judiciously select the cyclotron, targetry and hot cells depending upon the expected output from the facility. Often equipment manufacturers will try to over sell the equipment when funding is available or compromise when funds are scarce. Hence, prudence in planning and selection of equipment is to be applied.

7.1.4. Designing the facility

Designing a cyclotron PET radiopharmaceuticals production facility is a very crucial task and it is important to avail upfront the help of an expert who already did such an exercise and running a successful facility. The exact scope of the new facility planned should be provided to the expert. There are several elements in the design of the facility related to (i) cyclotron; (ii) clean room and hot cells; (iii) QC and (iv) services. It is important to consider the workflow so that the individual rooms are planned in such a way that there is minimum movement of personnel and materials needed for the smooth operation of the facility.

A cyclotron facility will have controlled areas where the operation involves the handling of radioactivity and hence radiation safety is to be ensured. Some part of these controlled area will also have clean rooms where radiation safety and pharmaceutical safety need to be harmoniously integrated.

Offices, conference room, staff rooms, pantry, janitorial rooms and toilets, which are essential for running a facility are planned outside the controlled area so that the personnel movement inside the controlled area is only need based. The layout of the controlled area of a typical cyclotron facility is shown in Fig. 93. Only controlled areas are shown in the figure. The cyclotron installed in this facility is a self-shielded Siemens HP 11 MeV proton machine. This facility design can be taken as a guideline, however, with due modification as needed.

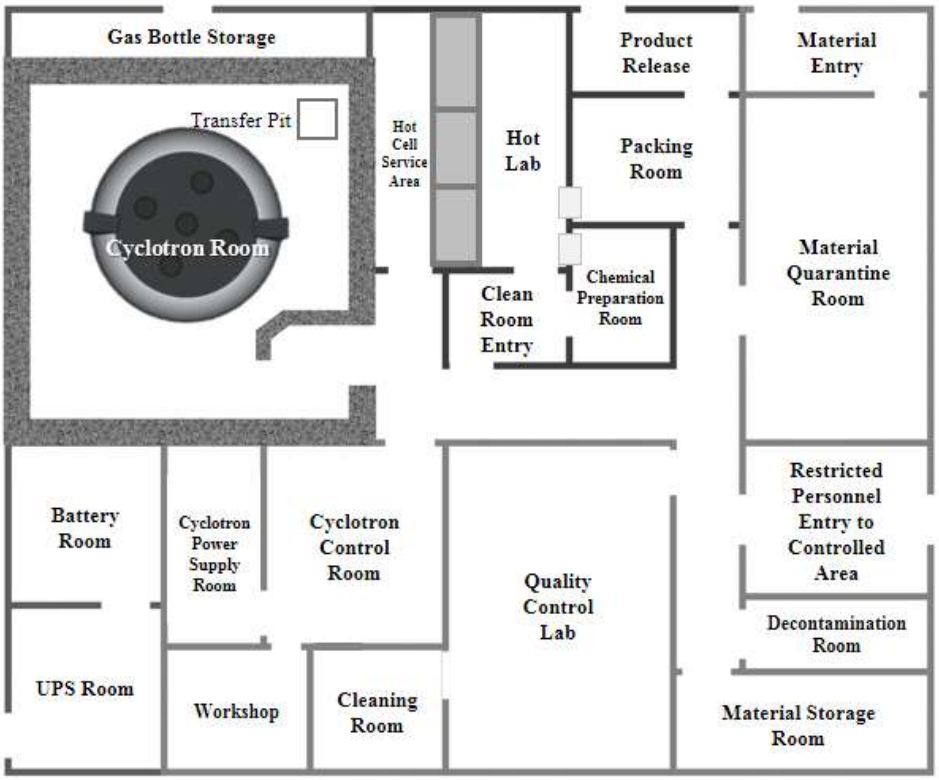


FIG. 93. The layout of a typical cyclotron PET radiopharmaceuticals production facility used for the manufacture of [^{18}F]FDG and ^{18}F radiopharmaceuticals (courtesy of Ajith, J., Molecular Cyclotrons).

A description of the different rooms needed in the controlled area is given below:

7.1.4.1. Cyclotron vault

The design of the cyclotron vault depends on the type of cyclotron, the beam energy and maximum beam current that will be used within the facility. The commercial PET cyclotrons have energy ranging from 8–20 MeV and beam current as high as 300–400 μA is available in some machines. Such machines will be making over 30 Ci (1 110 GBq) of ^{18}F in a single batch of operation and hence all radiation safety aspects need to be planned to handle such high activity in a safe manner.

It is advantageous to go for the highest beam current offered by the manufacturer as beam current enhancement subsequently could work out more expensive and the manufacturers are likely to back out from commitments. Also, permission and relicensing from regulatory agency will be essential whenever beam upgrading takes place.

Both self-shielded and unshielded options are available from most cyclotron manufacturers especially for the low beam current versions. The advantage of self-shielded machine is that it can be installed even when there is a space constraint in a facility. The bunker shielding requirement will be low when self-shielded cyclotrons are installed. Access to the cyclotron vault is possible even when the machine is in operation as the radiation dose inside the vault will be within permissible limit to allow service personnel to work for a limited time. The

concrete walls of the bunker have less chance to get activated in the case of self-shielded cyclotrons as the neutrons are absorbed by self-shields of the cyclotron.

Unshielded cyclotrons need to be installed in concrete bunkers having higher shielding often up to 200 cm or more of concrete. The inside area of the bunker can be smaller than the self-shielded cyclotrons as the space for moving self-shields also occupy space. Some of the cyclotron manufacturers provide local shielding options for targets which will be good to purchase even while taking an unshielded cyclotron. In the case of unshielded cyclotrons, there should be no other operation planned inside the cyclotron vault as the materials installed for such purpose could get activated due to the presence of secondary neutrons. Also, entry to the cyclotron vault will be allowed only after a specified time period. As an example, IBA takes a rule of thumb of no actions within 24 h.

An important decision to be taken while designing the facility is whether the cyclotron vault should have a plug door or a maze arrangement. The plug door takes less space and is moved mechanically using motorized wheels or hydraulic power system. There could be both installation and maintenance issues to this and regular servicing will be essential. A maze arrangement is relatively easy as the entry to the cyclotron vault is through multiple concrete walls. The door used in such a set up will not need heavy shielding. A maze arrangement utilizes more space as compared to the use of plug doors.

The cyclotron installation should be planned as one of the last jobs when most of the construction of the facility is completed in order to minimize the gap between the time of arrival of the machine at site and the commissioning of the facility. The cyclotron is a heavy equipment often weighing anywhere up to 20 tonnes that comes as a single piece to be installed in the specified area.

It is essential that the cyclotron vault is planned in the periphery of the facility where easy access to heavy vehicles carrying shipping containers is available. The cyclotron can be unloaded from the containers using cranes and inserted in the bunker either from the top of the vault or through the side of the bunker. In the former case the bunker including the plug on the top is fully constructed. The machine is inserted using heavy duty cranes. After inserting the machine, the plug can also be placed using the same crane. A very careful planning is needed for a successful operation. There needs to be sufficient space above the roof for the free movement of the crane. If the facility is part of a multi-floored building, it will be necessary to keep 2 to 3 floors on top free for the movement of the crane. Figure 94 shows the installation of a Siemens HP cyclotron inside a fully constructed and ready cyclotron vault (left); Unloading the cyclotron from the container using a hydra crane (top left); Heavy duty crane used for placing the cyclotron and shields inside the vault (top right); Inserting one part of the self-shield inside the vault using the crane (bottom left). Placement of the precast plug to close the cyclotron vault (bottom right).

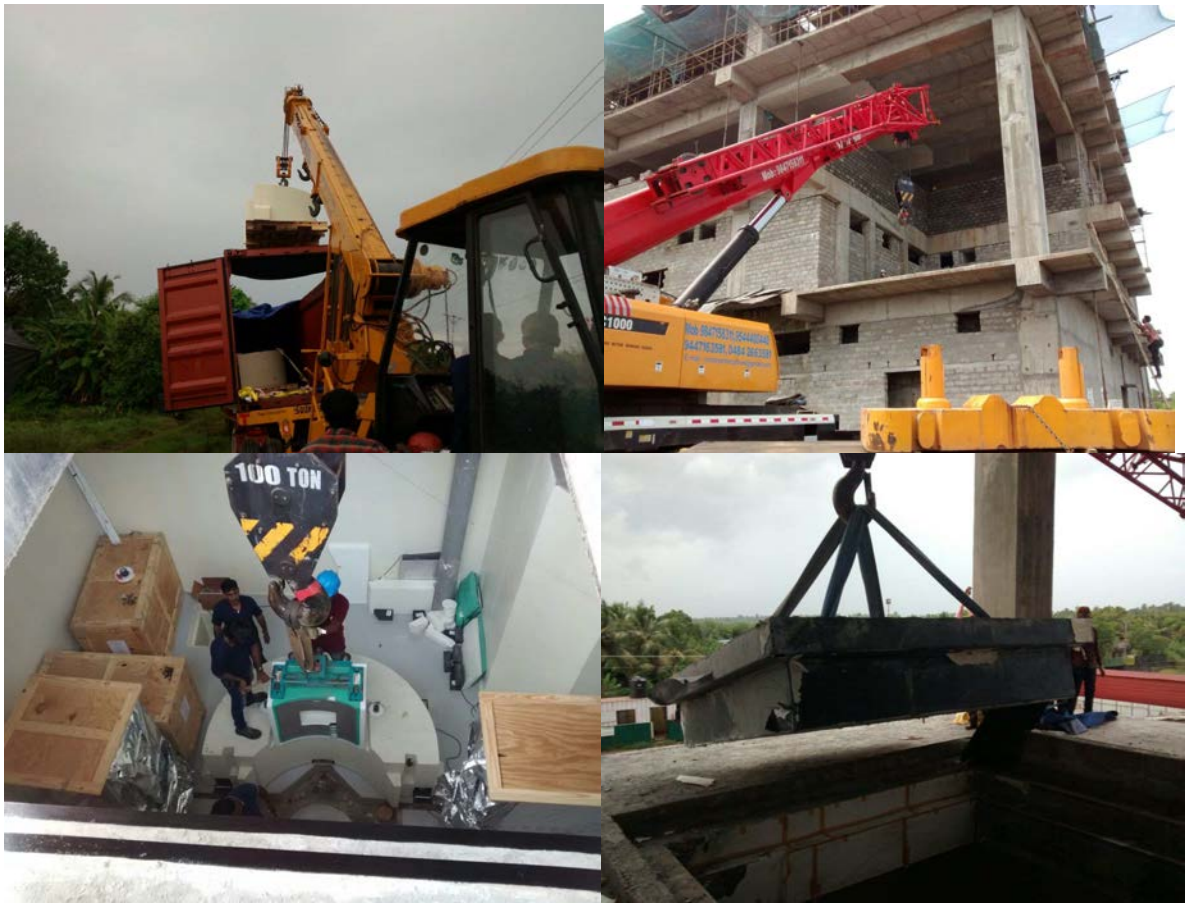


FIG. 94. Example of a cyclotron installation (courtesy of K.N.S. Nair, Molecular Cyclotrons).

The cyclotron can also be moved from the side of the bunker and the bunker construction completed after placing the machine. This could be a bit messier than the first approach as a substantial part of the concreting of the bunker has to be done after inserting the machine. It is important to decide which of the above options will be exercised at the planning stage of the facility.

Other than the cyclotron vault, other auxiliary rooms are also needed for the operation and maintenance of the cyclotron. The requirement will vary from manufacturer to manufacturer as the design and placement of auxiliary systems vary. The cyclotron manufacturers will give details about the electrical power supply, chilled water, uninterrupted power supply (UPS), compressed air, gases etc. needed for the operation of the cyclotron. All these need to be installed proximal to the cyclotron.

The support systems such as water chiller, air compressor, HVAC plant room, air handling units, air exhaust systems etc. can be placed on the first floor of the cyclotron facility.

7.1.4.2. Control room

The control room houses the workstation for operation of the cyclotron. This room will also have the workstation for monitoring the radiation safety systems installed at different places in the facility. The monitors for HVAC system could be installed in this room to make it convenient for the operator to monitor the environmental conditions before starting the cyclotron. This room should be proximal to the cyclotron bunker. Figure 95 is the control room

of a Siemens HP cyclotron, where the HVAC conditions are displayed on the monitors. Control panels of the radiation monitors inside the bunker is also installed in this room. The room also houses the workstation which gives the radiation levels of all the rooms including the stack monitors.

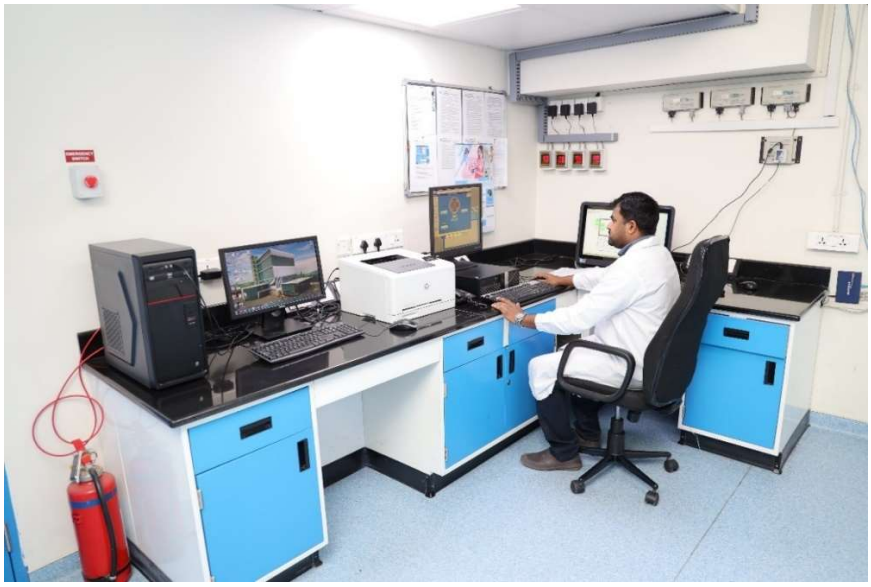


FIG. 95. Control room of the cyclotron (courtesy of M. Anees, Molecular Cyclotrons).

7.1.4.3.Workshop

The cyclotron parts such as the target, ion source, beam extraction carousal, target carousal etc. need periodic maintenance. These parts will have induced radioactivity and hence a small workshop should be planned which is equipped for handling radioactive materials. A fume hood, sink, and laboratory work benches are needed in this room. This room can also be used, or an independent small room can be built to store long lived waste such as the target foil, copper grid, used target body etc. Additionally, liquid waste arising out of the rebuild of the parts of the cyclotron can be stored for decay in the waste storage room.

7.1.4.4. Technical room

Some of the manufacturers such as Siemens have the water cabinet that circulates chilled water to the magnet, ion source and magnet attached to the cyclotron. Whereas other manufacturers have this system isolated and is kept in a room adjacent to the control room. The need of a technical room should be checked with the cyclotron supplier.

7.1.4.5. Power supply room

A relatively spacious power supply room is needed to house all the electrical panels of the cyclotron as well as the batteries for the uninterrupted power supply system.

7.1.4.6. Services for cyclotron

The cyclotron facility needs supply of chilled water (independent chiller unit), compressed air, air handling units (HVAC) units and air exhaust systems. All these occupy substantial space

and hence should be factored at the time of planning the facility. All this equipment can be housed on the first floor (roof) of the facility.

The HVAC system should meet the air quality, fresh air exchange and total air change requirements as per the class of the covered area. The climate conditions (temperature and relative humidity) and sensible heat removal should be met as per requirements of individual areas.

Substantial electrical power requirement is necessary for a cyclotron facility for radiopharmaceutical production. Power will be generally drawn from the local grid. The power supply should be backed up by power from a synchronised diesel generator installed in the facility. If power failures are expected repeatedly in the grid it is advisable to go for an uninterrupted power supply system for the entire power.

Required compressed air is generally oil and moisture free. Air quality is specified by the cyclotron manufacturer and the same should be followed. Availability of air at the specified pressure and flow rate should be ensured.

Chilled water supply should be available even if the cyclotron is in standby mode. It is advisable to provide a dedicated supply of chilled water for the cyclotron facility.

Industrial practices for fire safety should be planned for this facility also. Security of the facility should be ensured by proper access control for authorised personnel and CCTV system.

7.1.4.7. Clean room and hot cells

The radiopharmaceuticals production should be done in clean rooms of appropriate class. Ideally one small and a large Class C clean rooms are needed for PET radiopharmaceuticals production. The small clean room will be used for material preparations to be used inside the hot cell. A laminar flow bench will be provided in this room to provide a Class A handling facility for material preparation including reagents, kits and glass wares needed for production (Fig. 96). This room can also be used for housing the incubators for microbiological QC. This room should be approachable from the change rooms and should have a pass box opening to the clean room housing the hot cells.

FIG. 96. Clean room Class C for reagent preparation with the laminar flow bench (courtesy of J. Ajith, Molecular Cyclotrons).

A Class C clean room is needed for setting up the hot cells. The hot cells are installed in an adjacent (parallel) room such that they open to the Class C clean room. The clean room size can be optimized to have the minimum volume which helps in efficient air handling and maintenance. All walls in this room should be smooth and easily cleanable.

The synthesis hot cells are of Class C and the dispensing hot cell is of Class A type as injectable radiopharmaceuticals are prepared here. A Class C transfer box made of PMMA (poly methyl methacrylate) is attached to the dispensing hot cells for entry of vials, rubber closures etc. for dispensing the finished products. Figure 97 shows the hot cells installed in a clean room C class. Each chamber in the hot cell accommodates one synthesis module. The radiopharmaceutical is dispensed, radioactivity measured and dropped to the lead/tungsten container. Maximum care is taken in the design of the hot cells such that the exposure to the operators is minimum.

FIG. 97. Four mini hot cells installed in a Class C clean room (courtesy of J. Ajith, Molecular Cyclotrons).

The radioactivity in the dispensed vials are sealed with rubber closure and aluminium caps and assayed using a dose calibrator which is installed in the bottom of the hot cell. After measurement of radioactivity the vials are dropped into lead containers kept in a drawer on the bottom of the hot cell. The lead containers are automatically closed by dropping the lid over it while pulling the drawer out (Fig. 98). After insertion of the vial, the closure of the lead pot falls automatically over the container. The containers are screw tightened and moved to the packing room.

FIG. 98. Packaging of dispensed radiopharmaceuticals inside lead/tungsten containers (courtesy of K. Dilshad, Molecular Cyclotrons).

It is highly advisable to have a service corridor behind the hot cells. Electrical cabinets of the hot cells and all service lines should be in the service gallery accessible easily for maintenance. Figure 99 shows the backside of the hot cell and the service corridor. The terminals of the gases needed for operation of the hot cells and the synthesisers are installed in this corridor and the gases taken using tubing to the different hot cells. The ^{18}F transfer lines pass underneath the service corridor to the different hot cells in shielded trenches.

FIG. 99. The hot cells and the service corridor of a PET radiopharmaceutical production facility (courtesy of M. Anees, Molecular Cyclotrons).

7.1.4.8. Packaging room

The radioactive package and despatch room is designed adjacent to the production clean room (Fig. 100). The finished radiopharmaceuticals packed in lead/tungsten containers are transferred to the packaging room through a pass box and further packed in transport containers. The release of the packages is done after radiation monitoring. The packaging room is connected to an ante room where packages are handed over to the drivers for despatch.

FIG. 100. Packaging and dispatch room (courtesy of K. Dilshad, Molecular Cyclotrons).

7.1.4.9. Quality control laboratory

The QC laboratory should be sufficiently large to accommodate all the QC equipment such as GC, TLC, MCA, Endosafe equipment etc. A Class D condition will be desirable in this laboratory. Figure 101 shows a typical QC laboratory. The microbiological controls can be done in QC laboratory or preferably in the material preparation class C clean.

FIG. 101. QC laboratory of a PET radiopharmaceutical production facility (courtesy of J. Ajith, Molecular Cyclotrons).

7.1.4.10. Material transfer and quarantine room

As per GMP all materials received for production need to be temporally quarantined till it is released for production. This room could have three separate areas:

(a) For storing biochemicals and kits in cold storage;
(b) Storage of solvents and acids;
(c) Packaging materials.

Entry to this room could be through a material entry corridor. The materials and chemicals released for production are stored in another room from where it is released for use.

7.1.4.11. Laboratory glassware preparation room

This room will have a sink, oven and ultrasound cleaning machine to be used for cleaning and sterilization of glass ware, which are used for production and QC of the radiopharmaceuticals.

7.1.4.12. Decontamination room

A decontamination room having a sink for hand, face and eyewash and a shower for body wash is needed within the facility and preferably adjacent to the entrance of the facility. This area will be used for personnel decontamination which might be needed due to accidental contamination.

7.1.4.13. Entrance room to the controlled area

The nuclear regulatory body will stipulate that the entire cyclotron PET radiopharmaceuticals production facility is access controlled. It is better to plan a medium sized entrance room. The room is partitioned using a barrier (Fig. 102). Shoe covers are put or laboratory shoes worn before crossing over the barrier. A hand and foot monitor as well as a contamination monitor are kept in this room. This room also can have the cupboard for hanging over coats which are worn before the entry to the controlled area. All staff exiting from the controlled area should do a personnel monitoring before leaving the facility.

FIG. 102. Entrance to a cyclotron PET radiopharmaceuticals production facility (courtesy of K.N.S. Nair Molecular Cyclotrons).

7.1.4.14. Services area

The HVAC system, chiller, compressor, air exhaust system, etc. are housed on the roof of the facility. A fairly large area will be needed to house these units and hence to be planned from the beginning. The air from the hot cell and cyclotron vault are filtered through HEPA and carbon filters and then exhausted through a stack. The stack height should be at least three metres above the roof of the facility if there are no taller buildings around. Otherwise the stack should be three metres above the tallest building nearby. User should follow appropriate national code/ regulatory guidelines for this purpose.

7.2. RADIATION SAFETY CONSIDERATIONS WHILE DESIGNING A NEW FACILITY

The safety of the public and occupational workers is the primary criteria while designing a new facility. The design should be done such that the occupational workers and public should not exceed the dose limits. The dose limits for occupational workers and public are given in Table 23[4].

TABLE 23. ANNUAL DOSE LIMITS FOR PERSONAL EXPOSURE
(courtesy of E. Cazzola, Sacro Cuore Hospital)

	Occupational Worker	Public
Whole body	20 mSv	1 mSv
Lens of Eye	20 mSv	15 mSv
Extremities and skin	500 mSv	50 mSv

Despite the above dose limits, the overriding principle in designing a new facility is the ALARA principle which stipulates that the radiation dose to individuals and public should be as low as reasonably achievable. However, in any case it should be within the above annual limits stipulated. It should also be noted that the government or the regulatory body may have established other dose limits for occupational exposure and public exposure in the country.

The operation of a cyclotron PET radiopharmaceutical production facility involves dealing with radiation and radioactive materials and hence the facility has to be designed anticipating the potential hazards and effective measures taken to minimize the radiation risk.

The cyclotron vault and hot cells are designed such that the public areas have <0.5 μSv/h radiation dose. The occupation areas are designed such that the exposure is <10 μSv/h, the idea being a person working in that area for eight hours per day for 250 days will not exceed 20 mSv per annum.

Radiological safety demands differential pressures in various rooms in order to ensure that air flows from cleaner areas to more potentially contaminable areas to reduce spread of

[4] This is a simplified table. More detailed dose limit requirements can be found in:
- Radiation Protection and Safety of Radiation Sources: International Basic Safety Standards, IAEA Safety Standards Series No. GSR Part 3, IAEA, Vienna (2014).
- Radiation Protection and Safety in Medical Uses of Ionizing Radiation, IAEA Safety Standards Series No. SSG-46, IAEA, Vienna (2018).

contamination in the facility in case of any incidents of spillage, etc. The cyclotron room will be at the maximum negative pressure and the production clean rooms positive compared to adjacent areas.

7.2.1. Radiation shielding of the cyclotron

(a) A cyclotron while in operation generates high radiation fields. Most of the cyclotrons now in operation for PET isotope production are negative ion machines. The ion source generates H^- ions which are accelerated inside the cyclotron tank. Being negative ions, it does not induce radioactivity. The beam is extracted by pulling out two electrons from the negative hydrogen ion and allowed to hit the target. The flux of protons at this stage is 6.25×10^{12} μA of beam current.

(b) The beam while hitting the targets make secondary neutrons and alpha particles due to (p.n) and (p.α) reactions. Alpha particles being short range are of no consequence while operating a cyclotron facility. However, the neutrons produced are a significant radiation risk. The secondary neutrons need to be absorbed in the shield in the case of self-shielded cyclotron or in the vault in the case of an unshielded cyclotron. The cyclotron vault of the unshielded cyclotron will be designed with initial layers of boronated concrete. Natural boron has two isotopes ^{10}B (19.9%) ^{11}B (80.1%). Boron-10 has a very high neutron absorption cross section and undergoes $^{10}B(n,\alpha)^7Li$ reaction thereby reducing the neutron flux and the dose outside the cyclotron vault.

(c) When a 40 μA beam of 18 MeV hits a water target a neutron flux of 3.55×10^{11} is reported to be produced. The neutrons produced are isotropic with a maximum energy of 15 MeV and peak energy of 2 MeV while using an 18 MeV cyclotron [179]. The neutron energy could vary depending on the proton beam energy. The neutrons lose energy due to interaction with surroundings and then induce (n,α) reactions in the target and the surroundings. The secondary gammas emitted can increase the radiation dose inside the vault. A 18 MeV cyclotron while in operation at 2×150 μA beam current can produce up to 180 Sv/h at 1 meter distance from the target [179]. This radiation dose exists while the machine is in operation and will reduce to lower levels when the beam is off. Multiple levels of access control are provided in a cyclotron by connecting the door to interlocks so that the vault door does not open when the cyclotron beam is 'on' or otherwise the beam is killed when the door is opened accidently.

When ^{18}F is produced, the radiation dose due to the photons emitted by the decaying ^{18}F keeps increasing depending on the irradiation time. In self-shielded cyclotrons the target will be inside the shield whereas in the case of unshielded cyclotrons the target is exposed. There will be substantial radiation dose due to the ^{18}F available in the water target. One Ci (37 GBq) of ^{18}F in the target will give 50 Sv/h at surface and 5 mSv/h at one meter distance. It will be advantageous to have local shielding around the cyclotron target in case of unshielded cyclotrons, at least to take care of the neutron dose. Interlocks will also be provided such that the door to the vault does not open when the radiation dose within the cyclotron vault is above a certain set level.

Cyclotron operation also produce radioactive gases due to the activation of the air inside the vault due to neutrons. Hence, the air from the cyclotron vault is exhausted and discharged through the stack after filtration through HEPA and carbon filter.

7.2.2. Radiation safety considerations during synthesis

The synthesis of ^{18}F radiopharmaceuticals are done inside the hot cells using automated modules. The shielding thickness of the hot cells vary from 50–100 mm lead depending on the activity to be handled. Radiation interlocks should be in place in the hot cells such that the doors do not open till the radiation dose within the hot cell is less than the set levels.

During synthesis radioactive gases are produced inside the hot cells. As a safe practice, the exhaust of the hot cell during synthesis is connected to delay-decay tanks which collects the radioactive gases (Fig. 103). In this figure, four tanks with 350 L capacity each which are filled serially using a compressor which is part of the system. Each tank is pressurized up to 15 bar before automatically switching over to the next tank. The discharge of the gas is done through HEPA and carbon filter after 16–20 h by the time near complete decay occurs. The delay-decay tank system can be installed inside the cyclotron vault in a self-shielded machine whereas it needs to be kept in a separate adequately shielded room in the case of unshielded cyclotrons.

FIG. 103. Delay-decay tanks used in a cyclotron facility (courtesy of K.N.S. Nair Molecular Cyclotrons).

7.3. PHARMACEUTICALS SAFETY CONSIDERATIONS

A facility for manufacturing radiopharmaceuticals should be both particulate and microorganism free. All injectable radiopharmaceuticals should be prepared under Clean Class Room A conditions. The clean rooms should have a graded and progressive entry and exit with appropriate changing rooms. The entry is from Class D to C to A. GMP design of a radiopharmaceuticals production facility needs to take care of pharmaceutical safety without compromising radiation safety.

The following are essential aspects to consider at the time of design to ensure pharmaceutical safety:

— Microorganism and particulates should not enter the work area;
— Production and dispensing areas are operated under positive pressure;
— Appropriate levels of clean rooms are needed depending on the operations envisaged;
— Preparation of chemicals and kits should be done in laminar flow benches;

— Flow of materials and personnel into the clean room should be separate;
— Air shower (AS) before personnel entering class B to remove the particulate contamination
— Entry of personnel to class A should be done after gowning and Air shower
— Entry of personnel to class C should be through change rooms (CR).
— Pass boxes (PB) should be provided between the rooms of different grades for material movement
— Bulk solution can be prepared in class C
— Sterility assured by dispensing in class A after sterile filtration (SF) or Autoclaving

Figure 104 shows the logistics applied for personnel and material entry exit to a clean room facility [180].

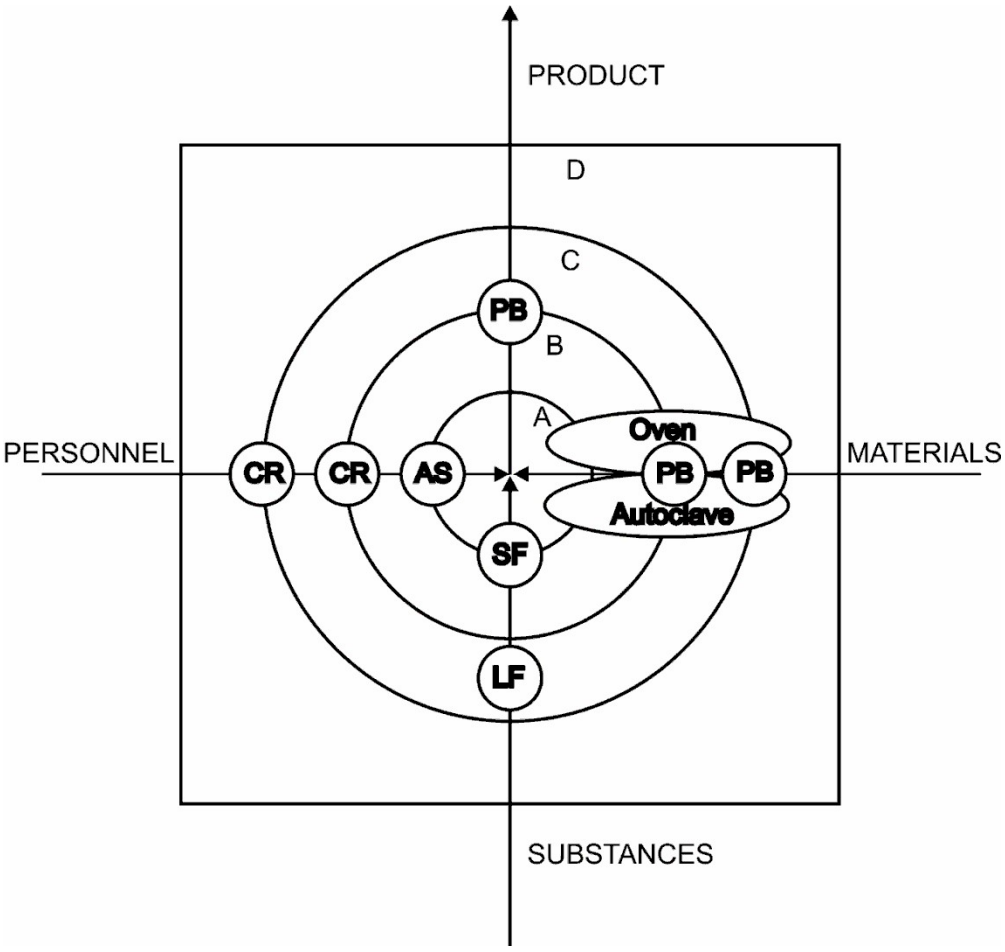


FIG. 104. Personnel and Material entry and exit to the clean rooms ((reproduced from Ref. [180] with permission).

Note: A, B, C, D indicates the different types of Clean class rooms. CR: Change room; AS: Air shower, LF: Laminar flow; SF: Sterile filtration; PB: Pass box.

The air handling system in a clean room area has to be designed such that stipulation with respect to both particulates and microorganisms are achieved by ensuring adequate filtration and air changes within the facility. Permissible number of particulates of different sizes in different clean class areas is given in Table 22 and the permissible levels of microorganisms is given in Table 23.

TABLE 24. PERMISSIBLE LEVELS OF PARTICLES IN DIFFERENT TYPE OF CLEAN CLASS ROOMS [180]

ISO Class	Grade (Class)	Maximal permitted number of particles / m^3 air			
		At rest		In operation	
		Particles ≤ 0.5 μm	Particles ≤ 5 μm	Particles ≤ 0.5 μm	Particles ≤ 5 μm
5	A (100)	3 500	<1	3 500	<1
6	B (1 000)	3 500	<1	350 000	<1
7	C (10 000)	350 000	2 000	3 500 000	20 000
8	D (100 000)	3 500 000	20 000	not defined	not defined

TABLE 25. PERMISSIBLE LEVELS OF MICROORGANISMS IN DIFFERENT CLASS TYPE OF CLEAN ROOMS [180]

Grade (Class)	Recommended limits for microbial contamination			
	Air sample cfu/m^3	Settle plates (diam. 90 mm), cfu/4 h	Contact plates (diam 55 mm), cfu/plate	Glove print 5 fingers cfu/glove
A (100)	< 1	< 1	< 1	< 1
B (1 000)	10	5	5	5
C (10 000)	100	50	25	-
D (100 000)	200	100	50	-

7.4. REGULATORY ASPECTS

Regulatory aspects on radiopharmaceuticals preparation are strict and combine the pharmaceuticals regulations to radiation protection. To make this picture more complex some aspects like transport regulations are internationally managed, while pharmaceutical regulations are managed at national level. This complex set of regulations requires high competence and investments in a business that has low return of investment compared to the normal pharmaceutical business. In this framework the radiopharmaceutical productions and delivery are located, and many different approaches are developed to fulfil to local and international legislation aspects.

Many different entities play key roles in this scenario like Industry, University and Hospital that need to balance and combine their mission and insure radiopharmaceutical availability.

7.5. THE WORLDWIDE STATUS OF ^{18}F RADIOPHARMACEUTICALS PRODUCTION

Radiopharmaceuticals preparations are based on three scenarios:

(a) Radiopharmaceuticals with a marketing authorization;
(b) Radiopharmaceuticals to be used on clinical trials;
(c) Radiopharmaceuticals prepared as extemporaneous preparation.

Radiopharmaceuticals with marketing authorization are the only products that will classify worldwide, because the regulations to be followed for pharmaceutical products, are the same used for pharmaceutical preparation with some peculiarities related to the decay and radiation exposure.

It is more difficult to harmonize the regulatory aspects in production of radiopharmaceuticals for clinical trials, e.g. in the European Union all the members need to align with a new regulation for clinical trials 'EU clinical trial regulation (531/2014)' that include also radiopharmaceuticals.

In this publication different approaches are indicated for:

(a) Radiopharmaceuticals for therapy;
(b) Radiopharmaceuticals for diagnosis.

The first are classified like pharmaceuticals that need to be produced under GMP condition while the second category can be produced in GMP or under local regulation.

Extemporaneous preparation is the most important way of production of radiopharmaceuticals in hospitals. This approach allows to make radiopharmaceuticals that are included in Pharmacopoeia for internal use. Several monographs for ^{18}F radiopharmaceuticals are available in the European Pharmacopoeia. All of these are listed on Table 24 and represent well established radiopharmaceuticals, some new tracers became emerged the last five years in nuclear medicine and are promising to be the next generation of radiopharmaceuticals (Table 25).

TABLE 26. WELL ESTABLISHED ^{18}F RADIOPHARMACEUTICALS
(courtesy of P. Elsinga, University of Groningen)

Tracer	USP	EP
[^{18}F]FDG	√	√
[^{18}F]FCH		√
[^{18}F]FDOPA	√	√
[^{18}F]NaF	√	√
[^{18}F]FET		√
[^{18}F]FLT		√
[^{18}F]FMISO		√
[^{18}F]F-PSMA1007		√

TABLE 27. NEW ^{18}F RADIOPHARMACEUTICALS
(courtesy of P. Elsinga, University of Groningen)

Tracer	Regulatory statement
[18F]fluciclovine	registered
[18F]FES	registered
[18F]Flutemetamole	registered
[18F]Florbetapir	registered
[18F]Florbetaben	registered
[18F]-CTT1057	Under registration
[18F]Flurpiridaz	Under registration
[18F]FDCFPyL	Under registration
[18F]FPSMA-1007	Under registration

7.6. SHIPMENT/DISTRIBUTION OF ^{18}F TRACERS

Because of the increasing popularity and availability of ^{18}F radiopharmaceuticals, there is an increasing demand for these PET tracers as also hospitals without a cyclotron and radiochemistry facilities acquire PET cameras. These hospitals will purchase these PET tracers either through a commercial delivery or receive it as a friendly shipment from another hospital with production facilities. These deliveries are subject to regulations that are different from country to country and can be dependent on the fact that shipment have a commercial purpose and be regarded as an industrial production (see section 7.5 and 7.6). Legislation with respect to definitions of industrial production and marketing authorization is currently evolving rapidly. Legislation may adapt to protect the investments made by the industry, but on the other hand, health care costs to perform PET scanning should remain as low as possible.

Another discussion is the choice or the requirement to acquire ^{18}F radiopharmaceuticals from industry whenever these are available or if an equivalent PET tracer can be purchased instead of in-house production, to save time, resources and maintain cost-efficiency.

With an increasing demand for ^{18}F radiopharmaceuticals, mainly FDG, batch related costs are similar for production for one patient or for multiple patients (materials, QC and all kind of tests are a constant factor). It may therefore be more resource efficient to centralize production of several ^{18}F radiopharmaceuticals and ship it to nearby PET imaging centres. Besides batch related costs one should bear in mind the investments for running a GMP production facility, and costs of a cyclotron. Also, a building or space is required. Additional costs going alongside with commercial shipment need to be considered. Also, in several countries it is required to set a registration dossier for radiopharmaceuticals which is costly and time consuming as well. Furthermore, health authorities may consider if legislation/regulations still allow small scale preparation for human use.

The drawback of course is the dependency on availability of the tracers supplied, and in addition having an own cyclotron offers additional options for other radionuclides and thus a wider panel of PET radiopharmaceuticals.

Being an academic PET facility, one may question whether to produce [^{18}F]FDG in-house or to purchase it while focusing on research activities. An often used argument is that commercial production requires a different mindset than an academic environment, and involves

specialized personnel with respect to logistics and economics. On the contrary, in many PET centres [^{18}F]FDG is the central motor of PET tracer production and largely contributes in maintaining ^{18}F related expertise.

The current situation is that commercial networks in place (North America (Siemens, Cardinal Health, Illinois Health and Service), Europe (Siemens, Alliance Medical, GE)), shipping ^{18}F-tracers: FDG, PSMA, NaF, florbetapir, flutemetamol, fluciclovine. These tracers need to be approved by FDA, EMA or other regulatory agencies.

7.7. CONCLUSION

Design of a cyclotron PET radiopharmaceuticals production facility should take care of both national and international regulations with respect to both radiation protection and GMP. It will be ideal to involve both radiation and drug regulators from the very beginning of the project. There are conflicting requirements while integrating radiation safety and GMP. However, by applying essential design elements this can be achieved. Availing the help of experts who already went through the drill of designing and constructing cyclotron PET radiopharmaceuticals production facility will be appropriate to save both time and money loss.

The number of commercial networks and number of hospitals using PET cameras and accompanying assortment of radiopharmaceuticals are continuously expanding and demonstrating the high potential of and demand for ^{18}F radiopharmaceuticals in nuclear medicine.

8. SPECIFIC SYNTHESIS PROTOCOLS

This section provides examples of protocols for the preparation of some of the most used ^{18}F radiopharmaceuticals. These protocols are based on established procedures used for daily manufacture of clinical grade batches of these radiopharmaceuticals, and provides information on synthesis, purification and reformulation, and analytical methods.

8.1. PROTOCOL FOR SYNTHESIS OF FDG

2-[^{18}F]fluoro-2-deoxy-D-glucose, [^{18}F]FDG or simply FDG is the most commonly used positron emitting radiopharmaceutical. The hydroxyl group in the 2 carbon position of glucose is replaced with ^{18}F to get FDG (Fig. 105).

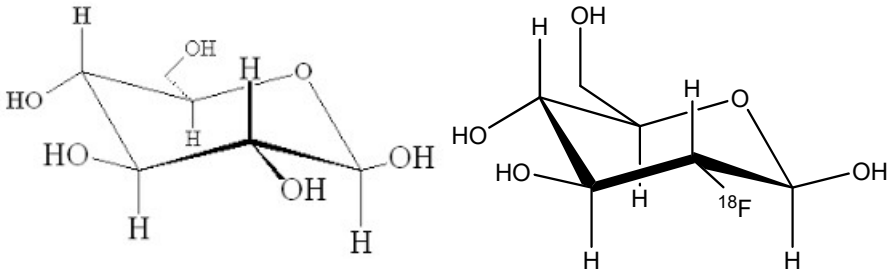


FIG. 105. Glucose (left) and [^{18}F]FDG (right) (courtesy of M.R.A Pillai, Molecular Group of Companies).

The IAEA publication '[^{18}F]Fluorodeoxyglucose (FDG): Guidance for Facility and Production' gives complete information about the manufacture of FDG under GMP conditions and readers may refer to the same for details [181]. The synthetic scheme of FDG is shown in Fig. 106.

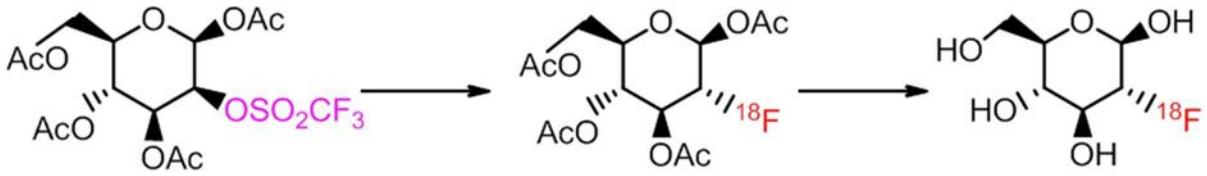


FIG. 106. Synthetic scheme for FDG(courtesy of M.R.A Pillai, Molecular Group of Companies).

Mannose triflate (1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose) is the precursor used for the production of FDG [182]. Mannose triflate is a sugar molecule containing the leaving group, trifluoro methane sulfonyl (also called triflate) at the two carbon in the molecule and the other four hydroxyl groups are blocked by acetylation.

During synthesis, the triflate group is replaced by the nucleophile, [^{18}F]fluoride. This is an S_N2 or bimolecular substitution reaction leading to the inversion the stereochemical centre of precursor mannose into glucose. The triflate group is converted to trifluorosulfonic acid (CF_3SO_2OH) and removed during the purification step. In subsequent step, the protective acetyl groups are then removed by acid or base hydrolysis to get FDG.

The irradiated ^{18}O-enriched water from the cyclotron is passed through an ion exchange column QMA which collects the fluoride ions and the enriched water is recovered. The column is eluted with Krypofix/potassium carbonate in acetonitrile: water (95:5) solution and the eluent collected in the reaction vessel and heated to remove water and acetonitrile. The temperature at this stage may go up to 85–95^{0}C. A repeated addition of acetonitrile takes place and solvent is completely evaporated. The precursor, mannose triflate dissolved in acetonitrile is added to the reaction mixture and allowed to react for 3 to 5 min at 85–95 ^{0}C. Subsequently, hydrolysis is done by addition of HCl or NaOH.

A series of purification steps are followed by passing through different columns which include:

(a) Cation exchange (AG-50 resin) column to remove all cationic impurities and Kryptofix;
(b) Ion retardation (AG-11 resin) column to neutralize the acid (applicable for acid hydrolysis);
(c) Alumina column to remove anions including unreacted fluoride;
(d) C-18 hydrophobic column to remove the unhydrolyzed FDG.

Purified FDG is recovered by passing water for injection. At this stage a small amount of ethanol (~3%) is also introduced to reduce radiolysis and improve the stability of the product. The FDG produced has very high purity and retains its RCP (>97%) during its shelf life. A shelf life of 6 hours is often given the manufacturers.

8.2. 16-α-[^{18}F]FLUOROESTRADIOL ([^{18}F]FES)

Cyclotron produced [^{18}F]fluoride is azeotropically dried with acetonitrile and allowed to react with 3-O-methoxymethyl-16,17-O-sulfuryl-16-epiestrio (MMSE) in acetonitrile at 100ºC for 10 min.

The protecting groups are removed by acidic hydrolysis with followed by evaporation of the solvent at 100ºC. The product can be purified by SPE and in this case the crude reaction mixture was diluted in water and purified by three different pre-conditioned cartridges (Oasis WAX, Sep-Pak C18 and Oasis HLB). After trapping and washing, the product was passed through collected aqueous ethanol on solution after passage on Alumina-N cartridge.

On HPLC purification the product is dissolved in 60% aqueous methanol and passed through an Alumina-N cartridge to remove the unreacted [^{18}F]fluoride and any particulates. The product is further purified by reversed phase HPLC and formulated in 10% ethanol in 0.9% NaCl. The formulated product is sterilized by filtration over a 22 μm filter into a multidose vial.

The recommended dose of [^{18}F]FES is 3 MBq/kg (allowable range: 100–225 MBq) at the time of injection, and the mass of injected drug substance is <5 μg (<17 nmol). A representation of its synthesis is shown in Fig. 107.

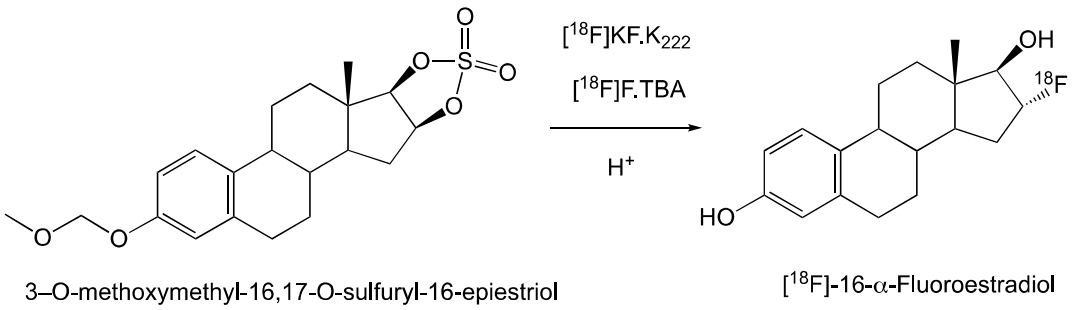


FIG. 107. Synthesis of 16-α-[^{18}F]Fluoroestradiol ([^{18}F]FES) (courtesy of E. Cazzola, Sacro Cuore Hospital).

8.3. [^{18}F]FLUOROCHOLINE ([^{18}F]FCH)

Cyclotron produced [^{18}F]fluoride is azeotropically dried with acetonitrile. For [^{18}F]fluorocholine production, two different pathways can be followed (Fig. 108).

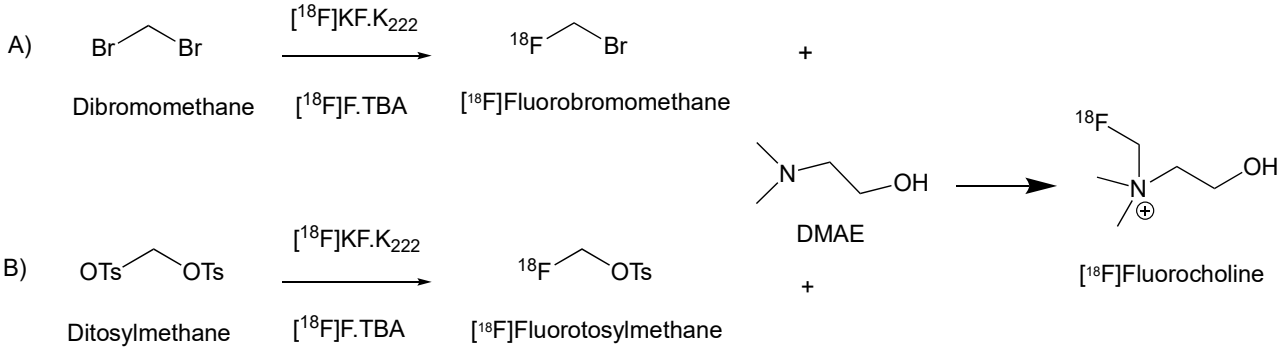


FIG. 108. Reaction scheme for the synthesis of [^{18}F]Fluorocholine ([^{18}F]FCH) (courtesy of E. Cazzola, Sacro Cuore Hospital).

The gas phase synthesis after azeotropic drying continues with a dibromomethane/acetronitrile solution in the reactor to generate the [^{18}F]FCH_2Br gas at 85–95°C for 10 min. The [^{18}F]fluorobromomethane is purified from dibromomethane by distillation over Sep-Pak Plus silica cartridges to allow to react with DMAE previously loaded on tC18 or loop. The [^{18}F]FCH trapped on tC18 was removed with ethanol and trapped on CM to allow washing with water and elution with 0.9% saline.

Different approaches can be used for liquid phase synthesis where to [^{18}F]fluoride, methylene bis(toluene-4-sulfonate) is added to obtain a solution containing [^{18}F]fluoromethyl 4-methylbenzesulfonate intermediate which is heated for 3 min at 115°C. A solution of 2-DMAE and DMF (1:1) is added to the reactor and let to react at 105°C for 12 min.

The crude reaction mixture is diluted in water, then purified through Sep-Pak tC18 and Oasis HLB cartridges, [^{18}F]FCH is trapped on two CM cartridges. The final product is collected after washing with diluted ammonia hydroxide, ethanol and water with 0.9% saline. Sterile filtration using 22 μm filter can be used in both cases.

The recommended dose of [^{18}F]FCH is 3 MBq/kg (allowable range: 100–225 MBq) at the time of injection, during QC assessment DMEA (1.0 mg/V) can be assessed using gas chromatography, as reported on EU FMC monograph or under HPLC as reported on USP monograph. Dibromomethane (0.1 mg/V), can be assessed by gas chromatography, no validated methods are available in FCH monograph regarding the tosylate synthesis route.

8.4. SYNTHESIS OF [^{18}F]PSMA-1007

The radiosynthesis of [^{18}F]-PSMA-1007 can be performed using GMP compliant synthesis modules including Synthera Synthesis Module from IBA. Cyclotron-produced [^{18}F]F^- is trapped on an QMA exchange column (Fig. 109).

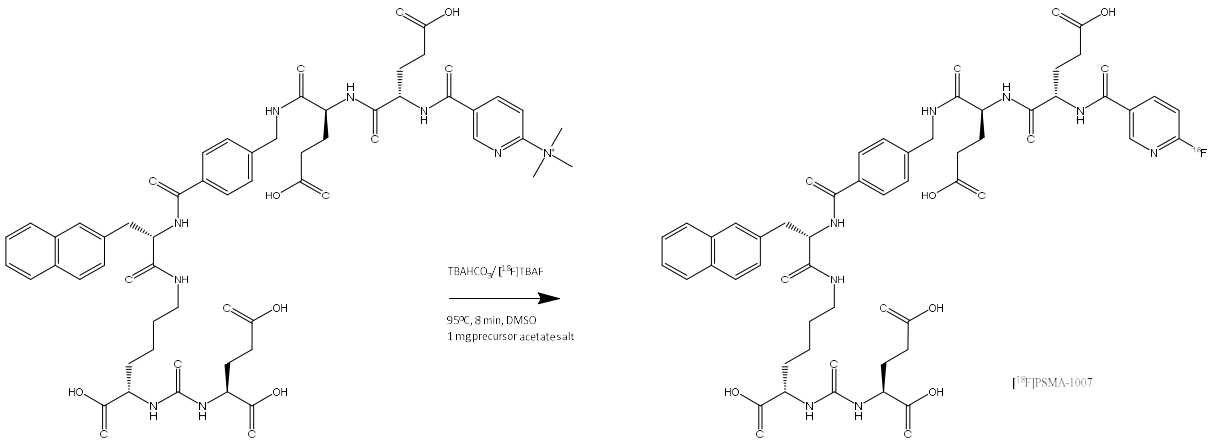


FIG. 109. Reaction scheme of [^{18}F]PSMA-1007 (courtesy of P. Elsinga, University of Groningen).

The [^{18}F]F- is eluted using the $TBAHCO_3^-$solution and subsequently [^{18}F]TBAF is transferred to the reaction vial. After azeotropic drying with acetonitrile, precursor solution (5-((S)-4-carboxy-1-((S)-4-carboxy-1-(4-((S)-1-((S)-5-carboxy-5-(3-((S)-1,3-dicarboxypropyl)ureido)pentylamino)-3-(naphthalen-2-yl)-1-oxopropan-2-

ylcarbamoyl)benzylamino)-1-oxobutan-2-ylamino)-1-oxobutan-2-ylcarbamoyl)-N,N,N-trimethylpyridin-2-aminium 2,2,2-trifluoroacetate) in DMSO is added. The reaction mixture is heated for 5 min at 95 °C (Figure 8.5). After the reaction is completed, 10 ml 5% EtOH solution is added and the mixture is transferred to the C18 cartridge. The product remains then on the C18 column and is purified via SPE fraction elution steps. First washing step is done with 20 ml 5% EtOH. Next, the purified product is eluted with 6 ml 25% EtOH and is purified again using a PS-H+ column. Sterilization is done via a filtration using a Cathivex-GV filter. The end product is collected in a sterile vial containing 12 ml PBS and 300 mg ascorbic acid. The final solution is 17 ml and contains 7 % EtOH.

The recommended dose of [^{18}F]PSMA-1007 is ranged between 150–300 MBq (3 MBq/kg) with a molar activity of >25.000 GBq/mmol. Therefore, the mass of injected drug substance is negligible. The recommended dose of 3 MBq/kg is based on the fact that this dose is used for all ^{18}F based radiopharmaceuticals at the UMCG.

8.5. PRODUCTION PROTOCOL OF THE ANDROGEN RECEPTOR ANTAGONIST [^{18}F]FDHT

Cyclotron produced [^{18}F]fluoride is allowed to react with 16α-[[(trifluoromethyl)sulfonyl]oxy]-3,3-(ethylenedioxy)-androstan-17-one (4 mg) in acetonitrile (0.5 ml) at 55ºC during 5 min (Fig. 110). After evaporation of the solvent, the ketone in the intermediate is reduced with $NaBH_4$ (5mg) in ethanol (1 ml), followed by hydrolysis of the acetal protecting group with HCl at 85ºC during 15 min. The product passed through an Alumina N cartridge and an Acrodisk filter to remove the unreacted [^{18}F]fluoride and any particulates. After neutralization with sodium acetate, the product is purified by reversed phase HPLC and formulated in 10% ethanol in 0.9% NaCl. The formulated product is sterilized by filtration over a 22 μm filter into a multidose vial.

The recommended dose of [^{18}F]FDHT is 3 MBq/kg at the time of injection, and the mass of injected drug substance is <5 μg (<16 nmol).

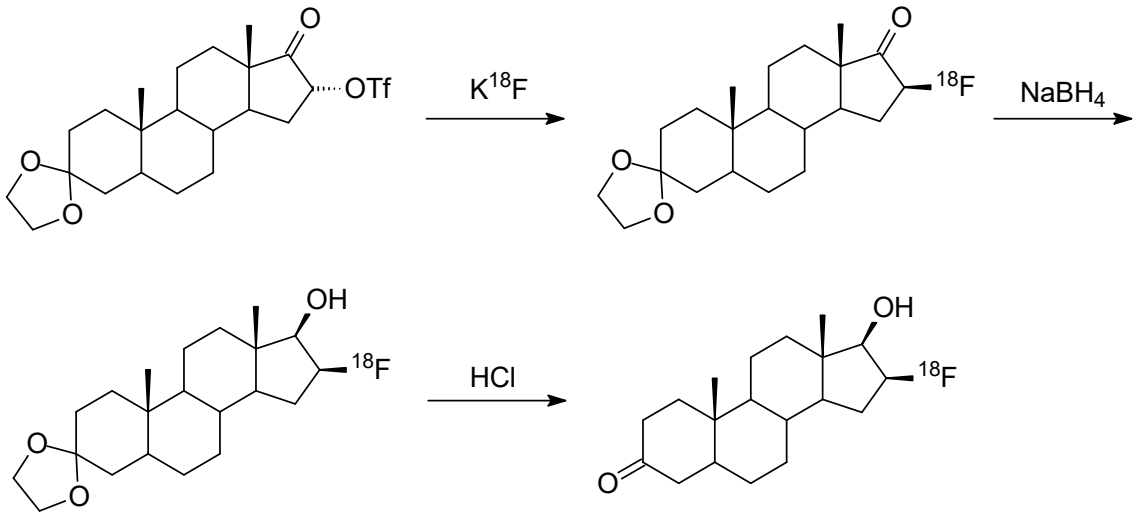


FIG. 110. Schematic of the radiosynthesis of [^{18}F]fluorodihydrotestosterone (courtesy of P. Elsinga, University of Groningen).

8.6. SYNTHESIS OF [^{18}F]ALF-NOTA-OCTREOTIDE ([^{18}F]OC) AND [^{18}F]SITATE FOR NEUROENDOCRINE TUMOR IMAGING

Octapeptide octreotide (Sandostatin®, OC) and Tyr^3-octreotate (TATE) are two important SST-antagonists (SSTA) which selectively interact with both SSTR2 and SSTR5 and are excellent ligands for NET therapeutic treatment [183]. Both labelling methods can be performed in a kit like manner and have been applied to SSTA radiotracer syntheses.

The synthetic scheme of preparation of [^{18}F]OC and [^{18}F]SiTATE is shown in Fig. 111 and 112.

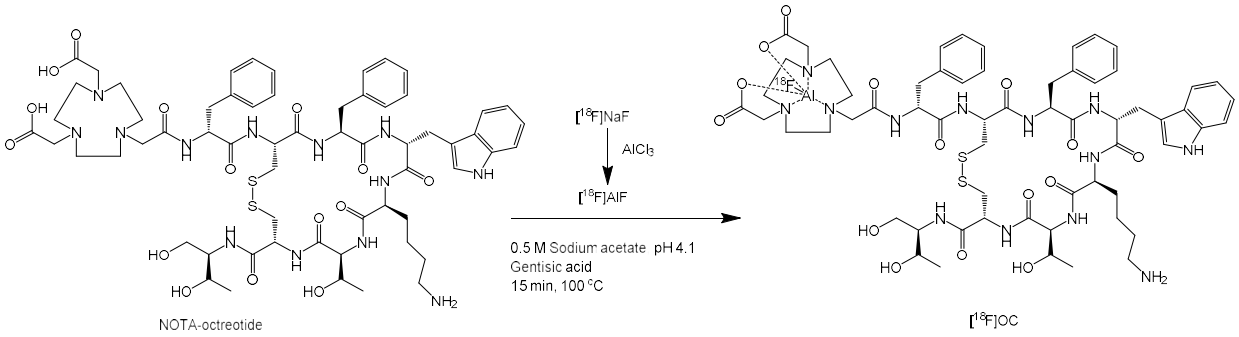


FIG. 111. Synthesis of [^{18}F]OC.

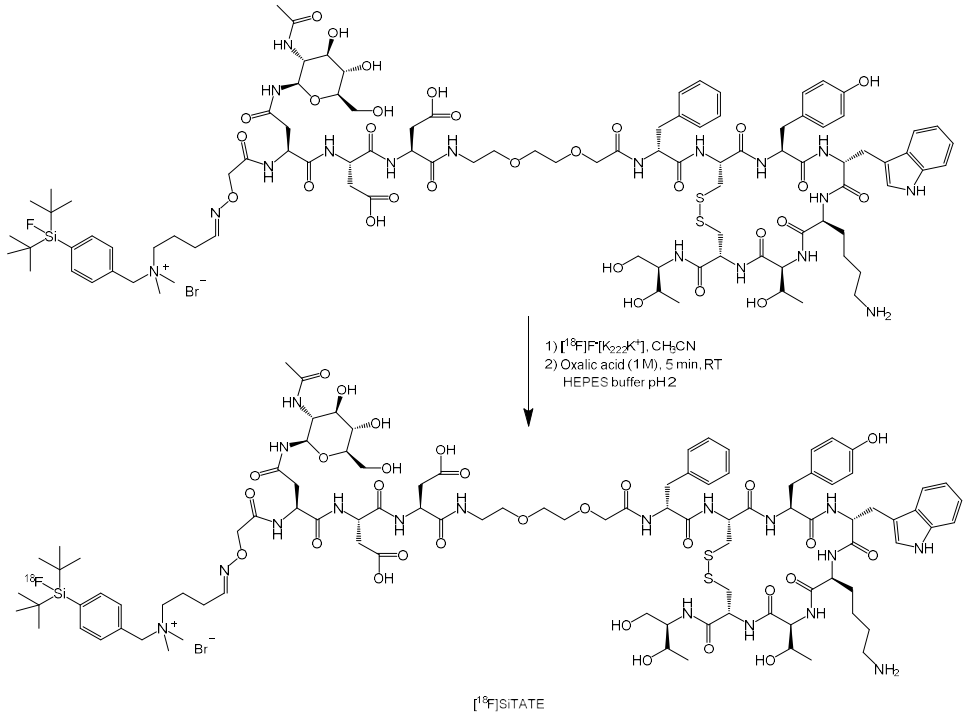


FIG. 112. Synthesis of [^{18}F]SiTATE.

8.6.1. Synthesis of [^{18}F]OC

[^{18}F]OC can be prepared by following the optimized automated synthesis by Frederik Cleeren through an AllInOne® synthesis module system (Trasis, Ans, Belgium) [154], or a manual synthesis, [184–187] which is the focus of this protocol .

Preparation of [^{18}F]AlF:

(a) An aqueous [^{18}F]fluoride solution (4–6 GBq) is passed through a Chromafix PS-HCO_3 cartridge;
(b) The cartridge is washed with 3 mL of metal-free water, rinsing the [^{18}F]fluoride trapped on the QMA of target related impurities (e.g. metal contaminations);
(c) The washing step is followed by the elution of [^{18}F]fluoride from the QMA with 100 µL 0.9% NaCl saline solution;
(d) A variable volume of 2 mM $AlCl_3$ in 0.1 M sodium acetate buffer, pH4, is added to the [^{18}F]NaF solution to achieve 8.5 µL $AlCl_3$ per GBq [^{18}F]fluoride;
(e) [^{18}F]AlF is formed by stirring for 2 min at room temperature under nitrogen atmosphere.

^{18}F labelling of [^{18}F]OC: The precursor NOTA-octreotide trifluoroacetate is commercially available from Advanced Biochemical Compounds (ABX). A prepared solution containing 10–50 µL peptide precursor (10 mg/mL NOTA-octreotide) in 0.5 M sodium acetate (pH4.1) and 6 mg/mL gentisic acid is added to the freshly prepared [^{18}F]AlF solution. The reaction mixture is kept at 100°C for 15 min.

Purification and formulation of [^{18}F]OC:

(a) The reaction mixture is cooled to rt, purified using reversed phase HPLC with a C18 column (4.6 × 100 mm) with a flowrate of 2 mL/min using the following gradient: mobile phase A: 0.1% *v*/*v* trifluoroacetic acid (TFA) in water; mobile phase B: 0.1% *v*/*v* TFA in acetonitrile; 0-5 min, 3% B; 5-35 min, 20%-25% B;
(b) HPLC analysis shows that two radioactive [^{18}F]OC peaks with retention times (R_t) of 17.4 and 19.8 min are obtained, corresponding to two stereoisomers of the [^{18}F]AlF complex;
(c) These fractions are collected and diluted 2-fold with water, then adsorbed onto an Oasis HLB cartridge (Waters, Milford, Massachusetts, USA);
(d) Acetonitrile and TFA are washed from the cartridge with 3 mL of water;
(e) The [^{18}F]OC is eluted from the cartridge with 200 µL 50% ethanol, twice;
(f) The eluate is passed through a 0.22 µm sterile filter (Millex-GV, 0.22 µm, PVDF, 13 mm, Merck KGaA, Darmstadt, Germany) and collected to afford the final drug product which is taken for QC.

8.6.2. Synthesis of [^{18}F]SiTATE

The production of [^{18}F]SiTATE can be prepared by following a manual synthesis [188–191], or an optimized automated synthesis on a Scintomics GRPTM 2V type® module [192]. The module can be obtained from Scintomics ATT (Germany) and it is geared towards [^{18}F]SiTATE production on a routine basis. The manual method is the focus of this protocol and can be easily performed in a kit-like manner [188, 191].

_ENREF_16Drying of [^{18}F]fluoride: _ENREF_19Aqueous [^{18}F]fluoride (1–5 GBq) in cyclotron water is passed through a SPE Sep-Pak QMA Carbonate cartridge (46 mg; Waters, Milford, Massachusetts, USA). The trapped [^{18}F]fluoride on the cartridge is dried by passing 20 mL of air and then 5 mL of dry acetonitrile through the cartridge. The [^{18}F]fluoride is eluted using a freshly prepared solution composed of a lyophilized mixture of K_{222} (41 mg, 110 µmol, Merck, Darmstadt, Germany) and KOH (5.6 mg, 100 µmol) dissolved in 500 µL dry acetonitrile.

Labelling of [^{18}F]SiTATE:

(a) The synthesis of the labelling precursor SiTATE described herein uses the commercial available precursor (ABX, Germany) which can also be synthesized by solid-phase peptide synthesis (SPPS) following protocols previously described [192–194];
(b) To 25 nmol of precursor and 25 µL of a 1 M solution of oxalic acid in dry acetonitrile is added the [^{18}F]fluoride solution;
(c) The isotopic exchange reaction is allowed to proceed at ambient temperature for 5 min.

Purification of [^{18}F]SiTATE:

(a) The isotopic exchange reaction is quenched by adding 9 mL of 0.1 M HEPES quench buffer (pH2) to the reaction mixture;
(b) It is then slowly passed through a Sep-Pak C18 cartridge.
(c) After the product is trapped, the loaded cartridge is washed with phosphate buffer (0.05 M, pH 2, 10 mL) to remove the reactants and solvent.
(d) The labelled product peptide is eluted from the cartridge using 200–500 µL of ethanol.
(e) To this solution, isotonic saline is added to dilute the mixture to give a final ethanol concentration of 10% or less which can be used for injection after sterile filtration.

8.7. SYNTHESIS OF [^{18}F]FDOPA (ELECTROPHILIC AND NUCLEOPHILIC)

8.7.1. Electrophilic substitution

[^{18}F]fluorine is prepared with a cyclotron in a two stage process: first the target is loaded with [^{18}O]oxygen gas and irradiated with high energy protons to promote the nuclear reaction: ^{18}O (p,n) ^{18}F; secondly, and after [^{18}O]oxygen gas has been recovered (cryotrap), the target is loaded with a mixture of argon gas containing up to 5% fluorine gas and irradiated to promote isotopic exchange to finally obtain [^{18}F]fluorine.

[^{18}F]fluorine is then delivered to a reaction vessel loaded with 6-trimethylstannyl L-Dopa precursor (60 mg) dissolved in silver stabilised deuterated chloroform (5 mL) standing at -10°C. The reaction mixture is stirred at 5°C for 10 min. 4M HCl (1 mL) is added and the vessel is heated to 65°C to evaporate the deuterated chloroform (Fig. 113). After 10 min the temperature is reduced to enable vacuum addition of the remaining 4M HCl (3 mL). The mixture is heated to 100°C for 15 min to deprotect the product before raising the pH with 2M NaOH and loading onto the HPLC injection loop for purification. The product is purified by semi-prep HPLC, using an Agilent PLRP-S column (100 Å, 10 µm, 25 × 300 mm) eluted with a 70 mM Sodium di-hydrogen phosphate buffer at a flow rate of 10 mL/min. The fraction corresponding to [^{18}F]FDOPA is collected in a vial containing L-Ascorbic Acid (10 mg) and 0.1 M Di-Sodium Hydrogen Phosphate (0.5 mL). The resulting formulated solution of [^{18}F]FDOPA is filtered through a 0.22 µm sterile filter into its final sterile container.

Typically doses in the range of 100–200 MBq are injected, and the mass of injected drug substance is <15 mg (in accordance to EP monograph 01/2008:1918).

The monograph also requires the product to be checked for enantiomeric purity. The method provided in the monograph is based on use of thin layer chromatography, but this analysis can also be performed by HPLC.

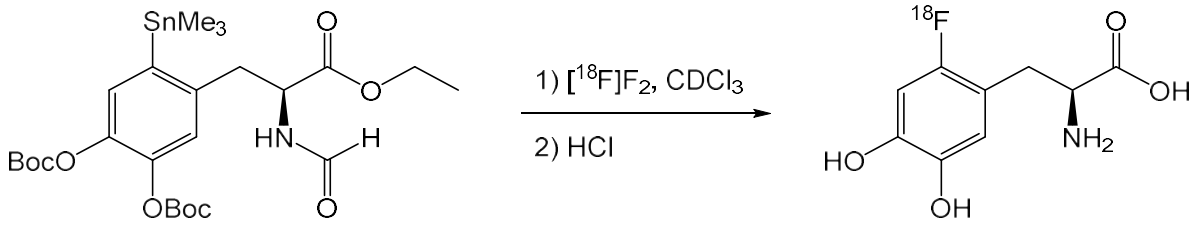


FIG. 113. Synthesis of [^{18}F]FDOPA following the electrophilic ^{18}F-fluorination route (courtesy of M. Huiban, Invicro LLC).

8.7.2. Nucleophilic Substitution

Several nucleophilic synthesis routes are under development. Here one promising method is presented in Fig. 114. Cyclotron produced [^{18}F]fluoride is trapped on a QMA cartridge, and released into the reactor using a 0.075M TBA bicarbonate solution (0.75 mL). The solution is evaporated to dryness, and [^{18}F]fluoride is further azeotropically dried twice with acetonitrile. A solution of the precursor (30 mg) in DMSO (1 mL) is added, and the reaction mixture is heated to 130°C for 8 minutes. The crude mixture is transferred to a Chromafix C18ec cartridge to remove DMSO and unreacted [^{18}F]fluoride, and the radiolabelled intermediate is eluted back in the reaction vessel using acetonitrile. Acetonitrile is evaporated, and the reaction vessel temperature reduced to 55°C. A solution of mCPBA (15 mg) in acetonitrile is added, and the reactor is heated to 65°C for 10 min to promote the Baeyer-Villiger oxidation. The crude mixture is cooled down to 50°C, and an ethanolic solution of HCl is added (consisting of 1 mL ethanol and 1.8 mL of 30% HCl solution). The mixture is heated to 50°C for 20 min to fully deprotect the product. The crude mixture is diluted with the formulation buffer (consisting of a mixture of phosphate buffer, ethanol, ascorbic acid and EDTA) and transferred onto a C18 cartridge and a HR-P cartridge connected in series. The cartridges are washed with water and phosphate buffer. [^{18}F]FDOPA is eluted off the cartridges with the formulation buffer. The resulting formulated solution of [^{18}F]FDOPA is passed through a WAX cartridge and an alumina light cartridge connected in series and then filtered through a 0.22 μm sterile filter into its final sterile container.

FIG. 114. Synthesis of [^{18}F]FDOPA following the nucleophilic ^{18}F-fluorination route (courtesy of M. Huiban, Invicro LLC).

8.8. SYNTHESIS OF [^{18}F]FLT (3'-DEOXY-3'-[^{18}F]FLUOROTHYMIDINE)

Several synthetic methods for the production have been published. All of them have their pros and cons. The two prominent routes use as precursor are a nosylate protected with Boc and dimethoxytrityl groups (DMTr-Nosyl-lyxothymidine) [195] (Fig. 115) or an anhydrothymidine precursor [50]. In this section the first method is described.

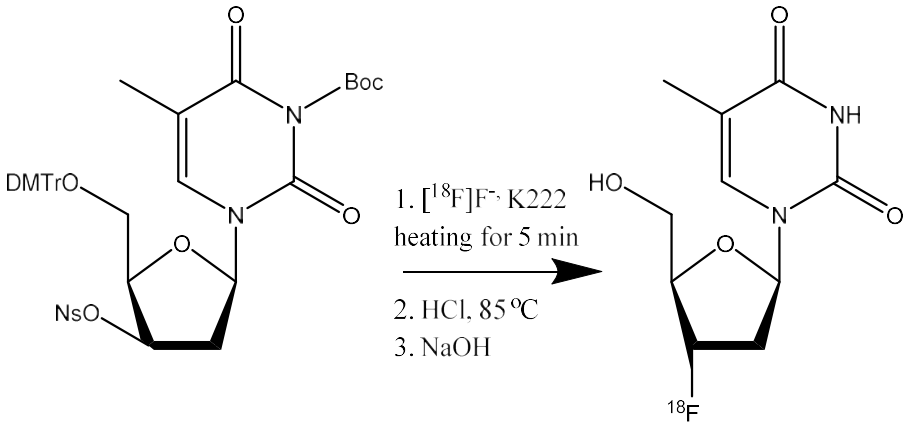


FIG. 115. Synthesis of [^{18}F]FLT via the DMTr-nosyl-lyxothymidine approach.

Cyclotron produced ^{18}F is trapped in a pre-conditioned QMA cartridge, separating the enriched water. [^{18}F]fluoride is eluted by a K_2CO_3 / Kryptofix mixture and transferred to the reactor. [^{18}F]Fluoride is dried by azeotropic distillation by additions of dry acetonitrile. After obtaining dry [^{18}F]F$^-$, DMTr-Nosyl-lyxothymidine dissolved in 2 mL of anhydrous acetonitrile is added followed by heating at 100–130°C for 5 minutes. After [^{18}F]fluorination, the crude mixture is allowed to cool down to 85°C. The hydrolysis step is performed with 1.5 mL of 2M HCl for 5 minutes at 85°C. The mixture is cooled and loaded onto PS-H + and HLB pre-conditioned cartridges. Then, HLB is washed with water for injections. The elution is done with 3 ml NaOH 0.1 M through HLB and alumina cartridges, obtaining [^{18}F]FLT.

8.9. PROTOCOL FOR SYNTHESIS OF [^{18}F]SODIUM FLUORIDE

[^{18}F]Sodium fluoride ([^{18}F]NaF) was introduced as a tracer for bone imaging in the 1960s and was approved by the FDA in 1972. It is straightforward to synthesize from [^{18}F]fluoride [196]. Production can be accomplished manually, on a fixed tube synthesizer or using a commercially available cassette solution.

[^{18}F]Fluoride is produced in a cyclotron:

(a) The [^{18}F]fluoride (as a solution from the cyclotron in [^{18}O]H_2O) is delivered through an in-line Plus-CM cation exchange cartridge in order to remove any positively charged recoil nuclei generated alongside ^{18}F in the cyclotron target and diluted with sterile water for injection (10 mL);
(b) This solution is passed through a QMA-light Sep-Pak to trap the [^{18}F]fluoride;
(c) The QMA cartridge is washed with additional sterile water for injection (10 mL) to remove residual [^{18}O]H_2O, and then the cartridge is dried (e.g. with an argon stream);
(d) [^{18}F]Fluoride (as [^{18}F]NaF) is then eluted from the Sep-Pak cartridge with 0.9% sodium chloride (10 mL);
(e) The saline solution is passed through a 0.22 μm sterile filter into a sterile dose vial (vented with an appropriate sterile vent filter;
(f) The yield of [^{18}F]sodium fluoride is recorded, and the product undergoes QC using standard methods, including radio-HPLC on an instrument equipped with electrochemical and radioactivity detectors.

The recommended dose of [^{18}F]NaF is 300–450 MBq (8–12 mCi) as an intravenous injection. The European Pharmacopeia sets a limit of maximum 4.52 mg of fluoride per maximum recommended dose in millilitres.

8.10. PROTOCOL FOR SYNTHESIS OF THE TAU TRACER [^{18}F]AV1451

[^{18}F]AV1451 ([^{18}F]flortaucipir, [^{18}F]T807, Tauvid) was introduced as a tracer for tau imaging and was approved by the FDA in 2020. It is straightforward to synthesize from a Boc-protected precursor using [^{18}F]fluoride, as show in Figure 8.12 [197]. Production can be accomplished on a fixed tube synthesizer or using a custom cassette on a module equipped with an HPLC unit.

The synthesis of [^{18}F]AV1451:

(a) It proceeds through a nucleophilic aromatic substitution (S_NAr) reaction using a nitro (or triakylammonium) precursor (Figure 8.12);
(b) [^{18}F]Fluoride is azaetropically dried and reacted with the AV1451 precursor at 130°C for 10 min. If the Boc-protected nitro precursor is used, these fluorination conditions also deprotect the Boc group [197];
(c) After synthesis, the reaction mixture is cooled, diluted with HPLC mobile phase and purified by semi-reparative HPLC;
(d) The HPLC fraction corresponding to [^{18}F]AV1451 is collected and concomitantly diluted with 50 mL of sterile water;
(e) The resulting solution is passed through an Oasis HLB cartridge, which is then washed with 10 mL of sterile water. [^{18}F]AV1451 can be eluted with 0.5 mL of EtOH for injection and diluted with 9.5 mL of sterile saline to bring the final formulation volume to 10 mL.
(f) The formulated [^{18}F]AV1451 is passed through a 0.22 μm sterile filter into a sterile dose vial (vented with an appropriate sterile vent filter).
(g) The yield of [^{18}F]AV1451 is recorded and the product undergoes QC using standard methods, including radio-HPLC to confirm molar activity and RCP.

The recommended dose of [^{18}F]AV1451 is 370 MBq (10 mCi), administered as an intravenous bolus injection in a total volume of 10 mL or less. The maximum mass dose is 20 μg per subject.

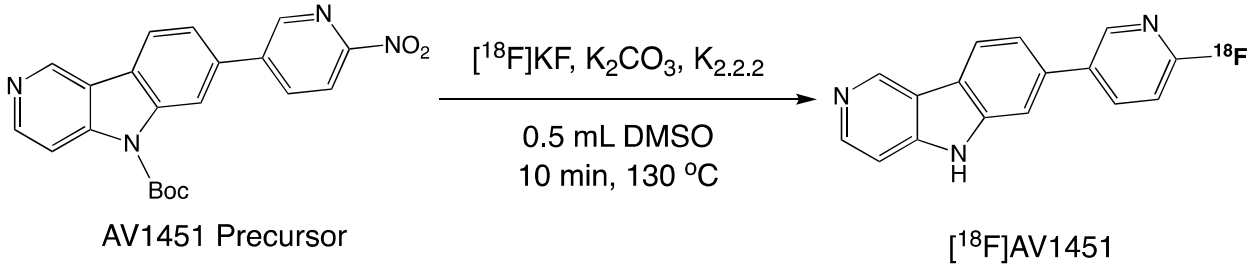


FIG. 116. Synthesis of [^{18}F]AV1451 (courtesy of P. Scott, University of Michigan).

8.11. PROTOCOL FOR SYNTHESIS OF THE BETA-AMYLOID TRACER [^{18}F]FLUTEMETAMOL

[^{18}F]Flutemetamol (Vizamyl) was introduced as a tracer for amyloid imaging and was approved by the FDA in 2013. It is straightforward to synthesize from a protected precursor

using [^{18}F]fluoride, as show in in Fig. 117 [198]. Production can be accomplished on a fixed tube synthesizer or using a custom cassette.

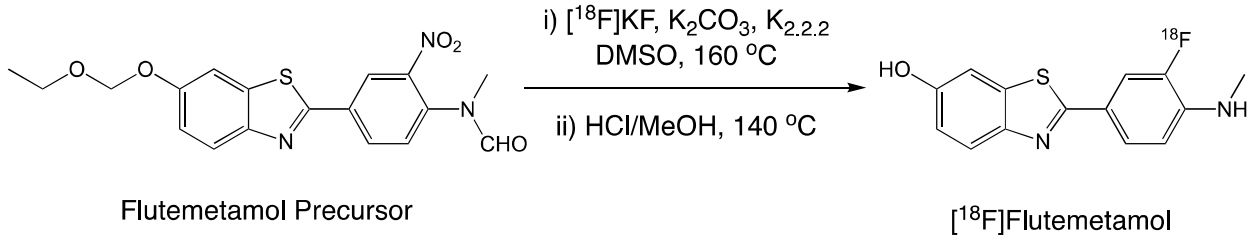


FIG. 117. Synthesis of [^{18}F]Flutemetamol (courtesy of P. Scott, University of Michigan).

The synthesis of [^{18}F]flutemetamol:

(a) It proceeds through a nucleophilic aromatic substitution (S_NAr) reaction using a nitro precursor (Fig. 117);
(b) [^{18}F]Fluoride is azaetropically dried and reacted with the flutemetamol precursor at 160°C;
(c) Following radiolabelling, subsequent acid hydrolysis at 140°C removes the phenol and amine protecting groups;
(d) After synthesis, the reaction mixture is cooled, and [^{18}F]flutemetamol can either be purified by semi-preparative HPLC [198] or solid phase extraction [199];
(e) The purified and formulated [^{18}F]flutemetamol is passed through a 0.22 μm sterile filter into a sterile dose vial (vented with an appropriate sterile vent filter);
(f) The yield of [^{18}F]flutemetanol is recorded and the product undergoes QC using standard methods, including radio-HPLC to confirm molar activity and RCP.

The recommended dose for [^{18}F]flutemetamol is 185 MBq (5 mCi) in a maximum dose volume of 10 mL, administered as a single intravenous bolus within 40 seconds. The maximum mass dose is 20 μg.

9. FUTURE TRENDS

After the thorough description of the current status of ^{18}F production, ^{18}F radiochemistry, clinically used ^{18}F radiopharmaceuticals and regulatory aspects, it is a challenge to look into the future and to identify new trends and make some predictions in a time where many uncertainties are tied with new challenges. The uncertainties are related to the position and role of nuclear medicine in healthcare, and the role of industry in commercial distribution of radiopharmaceuticals versus in-house production by hospitals.

9.1. TRENDS IN NUCLEAR MEDICINE AFFECTING ^{18}F RADIOPHARMACEUTICAL PRODUCTION

Nuclear medicine becomes increasingly important in the diagnosis and follow-up during the treatment of patients with respect to numbers and indications. Besides this there is an expanding demand for radiopharmaceuticals to diagnose new molecular targets as they are often closely connected to novel treatments. In this respect, nuclear medicine becomes an important tool in patient stratification to determine which patient receives the right dose at the right time. Within

nuclear medicine PET-CT imaging is the fastest growing medical imaging technology in this millennium and this trend is likely to continue. The first total body of PET cameras are being installed and will add more challenges and opportunities. Patient dosimetry can be reduced by injecting up to 40-fold less radioactivity. On the other hand, prolonged scans are possible, and molar activity could be less acquiring similar PET data. It can be questioned if alternative imaging or diagnostic technology will evolve in the future that will compete/replace PET based molecular imaging. In 2019 a SWOC analysis of nuclear medicine has been performed and is summarized in Fig. 118 [200]: nuclear medicine is strong in molecular imaging and more sensitive than MRI, there are good opportunities for new imaging biomarkers/tracers and theranostics. Radionuclide therapy will become one of the pillars in cancer therapy. In contrast to the ever-expanding opportunities of nuclear medicine, formal training of staff is a weakness and amongst disciplines like radiopharmaceutical scientists (radiochemistry and radiopharmacy) there is worldwide shortage of trained personnel.

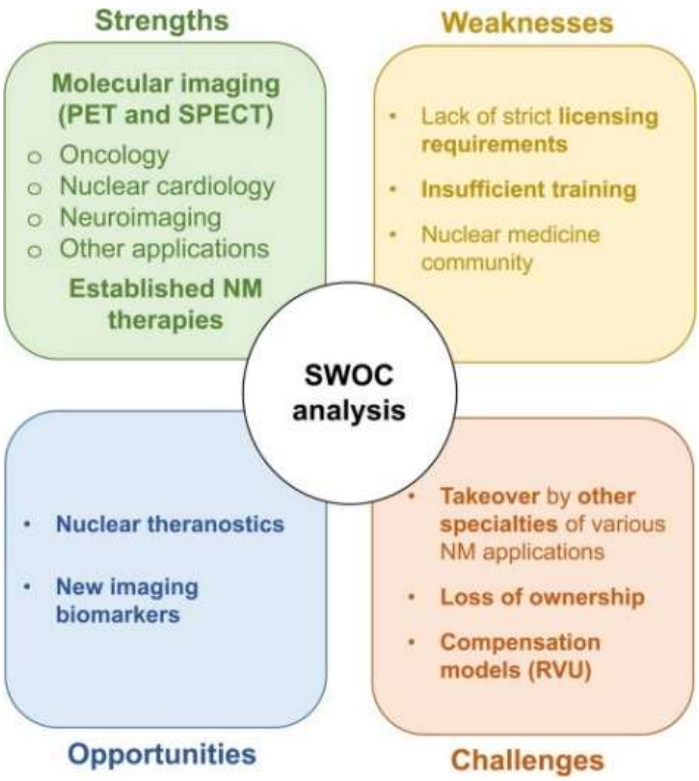


FIG. 118. SWOC analysis of nuclear medicine (reproduced from [200] with permission courtesy of [Society of Nuclear Medicine and Molecular Imaging © 2019]).

9.2. AVAILABILITY OF ^{18}F

Related to production and availability of ^{18}F the following trends can be observed:

Medical cyclotrons produce higher amounts of radionuclide, up to few hundreds GBq and in some cases even TBq. The requirements for beam currents are also increasing with latest machines having proton beam current 300 μA or even more. Almost all these cyclotrons use water targets to yield fluoride ($[^{18}F]F^-$) which is used for nucleophilic syntheses. It is expected that the application of ^{18}F will continue to increase in the future. On one hand, there is the development of tabletop models of a cyclotron catering FDG production for single hospitals. These limited cyclotrons have low proton energy and current that are nowadays combined with

cardiac perfusion tracers [^{13}N]NH_3 and [^{15}O]water. Whether having such a cyclotron is attractive depends on the business model of the hospital (running costs, personnel) in comparison to buying patient doses externally. On the other hand, the current trend is to increase the beam current to make as much ^{18}F as possible (industrial production). In many cases these cyclotrons may also be used to produce besides ^{11}C, ^{13}N and ^{15}O also new positron emitters such as ^{89}Zr, ^{64}Cu, ^{68}Ga and ^{43}Sc and ^{44g}Sc, opening new possibilities.

In specific situations generators offer radionuclides to sites without cyclotron. Radiopharmaceutical production is then restricted to small scale in-house productions in hospitals. Hospitals have the choice from centralized delivery of radiopharmaceuticals and/or production using generators. Using a generator may in specific cases be cheaper as less expensive infrastructure is required, such as a cyclotron with vault, and requiring fewer hot cells.

New positron emitters such as ^{43}Sc,^{44}Sc, and^{152}Tb are emerging. Their availability is currently limited. Scandium radionuclides can be an interesting alternative for ^{99m}Tc having a half-life in the same magnitude. These radionuclides can form attractive theragnostic pairs with Sc and Tb radionuclides with therapeutic properties. To make these radionuclides successful, their availability needs improvement.

Availability of ^{68}Ga may become easier by the introduction of liquid targets. In case liquid targets for radiometals (e.g. ^{68}Ga) will experience a breakthrough, it might become more attractive to use ^{68}Ga in specific situations as much higher amounts of ^{68}Ga can be produced compared to a ^{68}Ga generator. For some time, there was the perception that ^{68}Ga tracers might take over ^{18}F, especially in the case of peptide labelling because these tracers would become available easily for hospitals without a cyclotron and without a well equipped laboratory and well trained staff. On the other hand, GMP compliant ^{68}Ga generators have become much more expensive and put high cost burden on the exploitation knowing that such a generator needs to be replaced every nine months or so. However, other challenges are presented with this paradigm including competing demands for cyclotron time. ^{18}F remains to have favourable characteristics summarized in section 3 that will keep ^{18}F a very attractive radionuclide for many reasons. For commercial distribution of ^{68}Ga radiopharmaceuticals the half-life of 68 min will remain a hurdle.

9.3. ^{18}F RADIOCHEMISTRY

As described in section 5 a plethora of [^{18}F]fluorination methods have been developed and in combination with a wide choice of automated synthesis systems many of these methods are more easily available for PET centres. What will be the new developments in chemistry? Kit type labelling as being done for ^{99m}Tc was considered to be a way to move ^{18}F radiopharmaceuticals forward. With emerging technologies as described for Silicon and Boron ^{18}F-labelling, these methodologies are getting closer to a full kit like labelling procedure. Since the breakthrough developments in the mid 2000's, the heteroatom based ^{18}F methodologies as well as the Al based radiochemistry have grown substantially. Both, leaving group and isotopic exchange approaches continue to be developed for the boron and silicon methods, whereas the aluminium strategy has benefited from the application of new chelating moieties. The use of these methods to label peptide probes is becoming more common, with many performing well in clinical trials. The simplicity and practicality of these methods represent a major advantage over the classical carbon-fluorine bond formation reactions, and they may soon be used to create compounds to replace some standard clinical tracers used for PET imaging.

In addition, an increasing number of automated late stage [^{18}F]fluorinations provide another way to disseminate ^{18}F radiochemistry. This type of radiochemistry is still being optimized. Currently, most of the late stage strategies for the radiofluorination of heteroarenes are still in the exploratory phase. However, the promising new possibilities of these strategies made radiochemists to pursue these metal catalysed reactions. Metal catalysed late stage [^{18}F]fluorinations made a great impact on PET radiochemistry providing ^{18}F labelled compounds that could not be synthesized before with high molar activities. The main challenge for the late stage [^{18}F]fluorinations is the translation from small scale manual syntheses to large scale automated productions. Besides previously mentioned chemical reasons, also radiolytic processes play an important role.

Regarding [^{18}F]trifluoromethylation reactions, these have not been translated to clinical applications yet, though two methods allowing direct labelling of unmodified peptides are very interesting, and hold great promise for ^{18}F labelling of biomolecules.

An electrophilic ^{18}F labelling method, yielding radiotracers with high molar activity is still not available. Electrophilic substitution reactions are overall very limited in use, and are associated with technical challenges in particular as related to the high reactivity of reagents such as [^{18}F]fluorine, and are therefore not the first choice for [^{18}F]fluorinations nowadays despite the fact that these synthetic routes are often used in organofluorine chemistry. As electrophilic fluorinations are the main synthesis strategies in organic and medicinal chemistry, there is a great need for ^{18}F analogous radiochemistry approaches to allow simplified access to ^{18}F radiotracers which are otherwise difficult to obtain.

PGs will continue to play an important role in radiochemistry and PET tracer manufacturing and its full potential to access clinically relevant and accepted radiopharmaceuticals should be further pursued.

Microfluidic systems are currently experimental. Its potential needs to be further explored and the advantage over cassette based, fixed tube or hybrid systems needs to be demonstrated. It can be expected that microfluidic systems continue to develop and will play an important role in the radiopharmaceutical manufacturing facilities of the future.

Dissemination of ^{18}F labelling technology was hampered by lack of trained personnel for a long time as the mainstay of [^{18}F]fluorination being nucleophilic substitution was considered as troublesome requiring anhydrous conditions. Overall, aliphatic (S_N2) and aromatic (S_NAr) nucleophilic substitution with [^{18}F]fluoride remains a method of choice. At present, this method is well established for the majority of routine ^{18}F radiotracer productions and is employed in most commercially available synthesis platforms. Some of the new technologies are still fairly recent and it is therefore difficult to evaluate their full impact yet, but they are likely to contribute to the preparation of novel radiopharmaceuticals in the future.

9.4. EMERGING MOLECULAR TARGETS AND ^{18}F RADIOPHARMACEUTICALS

Development of radiopharmaceuticals is still a time consuming process, but more and more ^{18}F radiopharmaceuticals find their way to human application and even approval for clinical care. Requirements for success of a radiopharmaceutical are:

(a) Availability of radionuclide, robust and easy radiosynthesis;
(b) Clinical impact on decision making;

(c) Option for shipment (commercial interest for and boosting by industry),
(d) Cost saving for health care, and
(e) A profitable business model.

^{18}F radiopharmaceuticals meet these requirements and have the potential to dominate the PET imaging market. A nice example is the situation with PSMA targeted tracers where [^{18}F]PSMA-1007 partly took over the role of [^{68}Ga]Ga-HBED-CC-PSMA because the opportunity to scan more patients and to ship the radiopharmaceutical to other hospitals as well.

Several upcoming and established ^{18}F radiopharmaceuticals are described in the different sections. FDG is and will stay still the main player for the next decade. An interesting tracer that might replace FDG partly is FAPI (fibroblast-activation-protein inhibitor) as it does not require resting time for the patient after injection. Currently, ^{68}Ga labelled analogues are being used in clinical studies, but also the first ^{18}F analog of FAPI-74 is under development as the NOTA precursor can be [^{18}F]fluorinated using the Al[^{18}F]F strategy.

9.5. LEGISLATION

There is an emerging trend to apply risk based approaches in radiopharmaceutical production rather than strict rules by not taking into account specific situations. This is caused by several reasons: first, ‘one rule serves all’ is detrimental for advancing the field of radiopharmaceutical development and leads to a decreasing the number of clinical trials, and second, by increased awareness of authorities on the specificities of radiopharmaceuticals. This requires appropriate training for staff but also the regulatory authorities. This is also true for the radiopharmaceutical production and analytical methods employed to synthesize radiopharmaceuticals. Trends towards specific exemptions for radiopharmaceuticals when prepared and used under very specific circumstances do not hold for industrial production.

9.6. ACADEMIA AND INDUSTRY

A plethora of ^{18}F labelled radiopharmaceuticals have been developed and translated into clinical use around the world, as highlighted in sections 6 and 8. Nevertheless, clinical PET imaging is still dominated by [^{18}F]FDG, with millions of scans occurring worldwide each year. As such, PET infrastructure has been built to manufacture, distribute and scan [^{18}F]FDG and it remains the mainstay of commercial nuclear pharmacies, as well as any academic PET Centres that produce it for in-house use. However, the recent approvals for additional radiopharmaceuticals with application in neurology (amyloid and tau agents, [^{18}F]FDOPA) and oncology ([^{18}F]fluciclovine, [^{18}F]FDOPA, [^{18}F]Na, [^{18}F]fluoroestradiol), as well as the expected approval of agents for cardiac ([^{18}F]flupridaz) and prostate cancer [^{18}F]PSMA-1007, [^{18}F]DCFPyL), are necessitating changes to the traditional [^{18}F]FDG manufacturing paradigm. Both commercial manufacturers and academic facilities are evolving to produce multiple ^{18}F radiopharmaceuticals on a daily basis, often in addition to new ^{68}Ga labelled radiotracers. For academic labs, the scheduling challenges are often further exacerbated by busy research schedules and the need to label increasingly complex radiopharmaceuticals for new imaging targets or drug molecules for pharmaceutical industry collaborations. This presents an exciting future for radiochemists, but amplifies the urgent need for reliable ^{18}F production, new and efficient labelling reactions, and the next generation equipment for synthesis and QC testing that in many cases are introduced throughout this work.

9.7. TRAINING/EDUCATION OF STAFF

To cope with the described trends, training of radiochemistry and radiopharmacy staff is very important, but at this moment there is lack of proper (undergraduate and postgraduate) education, and shortage of staff. International professional societies such as the Society of Radiopharmaceutical Sciences (SRS), have initiated taskforces that in principle came up with the several recommendations [201].

9.8. CONCLUSIONS

Flourine-18 radiopharmaceuticals continue to play an important role in the detection, follow-up and management of human patients with almost perfect application in nuclear medicine. Nucleophilic fluorination remains the most applied labelling technology best fitting the existing synthesizers and trained staff allowing automation, well provided by the industries and compliant with radiation level regulations. New tracers for oncology, cardiovascular and specially in central nervous system diseases are developed and more under development, demonstrating the continuation of nuclear medicine/PET reliance on ^{18}F. Application of new fluorination methods based on fluoride anion have resulted in very interesting results especially together with click-chemistry and microfluidics techniques, suggesting the future introduction into commercial synthesisers. Rapidly development on the installation and application of medical cyclotrons worldwide, raise the awareness on the wise choice of machine, installation design and construction of these facilities. This publication provides a detailed overview of the above mentioned items in the field to support Member States worldwide in the production and application of ^{18}F radiopharmaceuticals, as the most important and widely used PET tracers is of great benefit for health care and society.

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ABBREVIATIONS

ASU	automated synthesis module
CRP	coordinated research project
CT	computed tomography
DMAE	dimethylamino ethanol
FDA	U.S. Food and Drug Administration
GLUT	glucose transporter
GMP	good manufacturing practice
HPLC	high performance liquid chromatography
HVAC	air handling units
IL2	interleukin-2
K_{222}	kryptofix 222
LG	leaving group
MRI	magnetic resonance imaging
NET	neuroendocrine tumours
PET	positron emission tomography
PG	prosthetic group
PRRT	peptide receptor radionuclide therapy
PSMA	prostate specific membrane antigen
QC	quality control
QMA	quaternary ammonium chloride polymer
RCP	radiochemical purity
RCY	radiochemical yield
SPE	solid phase extraction
SPECT	single photon emission computed tomography
SST	somatostatin
SSTR	somatostatin receptors
TBA	tetrabutyl ammonium
TCO	trans-Cyclooctenes

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