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# Emerging Technologies for Decentralized Production of PET Tracers

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## 1. Introduction

### 1.1 Increasing diversity of PET

The use of Positron Emission Tomography (PET) to monitor biological processes *in vivo* (Michael E. Phelps 2000) has seen dramatic growth and acceptance in the research, pharmaceutical, and medical communities over the last few decades, with clinical PET growing from ~900,000 scans in 2004 to over 1.74 million in 2010 in the United States alone; growth in foreign markets is comparable (Muschlitz 2011). These scans are conducted in ~2,200 clinical PET centers, all providing molecular imaging diagnostics of the biology of various diseases, including cancer, Alzheimer's, and Parkinson's. Additionally, PET is a powerful tool in the drug discovery and development process, providing *in vivo* pharmacokinetics and pharmacodynamics using radiolabeled versions of new drugs. A portion of clinical PET centers support drug trials carried out by pharmaceutical and biotech companies by synthesizing these molecules. PET biomarkers can also be used to select the best treatment for individual patients. Patient stratification via PET is anticipated to increase the quality of therapeutics available to patients with a concomitant decrease in the cost of bringing these therapeutics to market. (In the current randomized approach to patient selection, ~75% of patients do not have an efficacious response to treatment.) Furthermore, PET has been widely used in preclinical research and its use in cell cultures (Vu et al. 2011) and animal models is growing dramatically due to the recent advent of preclinical PET imaging systems that are easy-to-use, compact, and affordable (Zhang et al. 2010). Coupled with the Critical Path Initiative of the FDA to partner a biomarker with each drug in clinical trials, as well as the ongoing technetium (<sup>99m</sup>Tc) shortage affecting single photo emission computed tomography (SPECT) imaging, there is an even greater demand for PET, especially so given its superior sensitivity and image quality.

The need to measure and elucidate biological processes in a non-invasive manner in functioning organisms has led researchers to develop more than 1,600 PET probes for metabolism, protein synthesis, receptors, enzymes, DNA replication, gene expression, antibodies, hormones, and therapeutics (Iwata 2004). However, the overwhelming majority of PET for the care of patients utilizes only a single molecular imaging probe, the <sup>18</sup>F-labeled

glucose analog 2-[ $^{18}\text{F}$ ]fluoro-2-deoxy-D-glucose ( $^{18}\text{F}$ ]FDG). All cells normally use glucose for a variety of cellular functions, and  $^{18}\text{F}$ ]FDG PET provides a general assessment of the alterations in glucose metabolism between healthy and diseased states. The primary use of  $^{18}\text{F}$ ]FDG is to detect, stage, and assess therapeutic responses in cancer (Weber and Figlin 2007). Limitations do exist, however, such as the difficulty in imaging tissues which normally have very high glucose metabolism, and the lack of identification of the specific biochemical pathways through which disease is occurring.  $^{18}\text{F}$ ]FDG also cannot serve as a companion diagnostic for the discovery, development, and use of new molecular therapeutics. Increasing diversity of tracers beyond  $^{18}\text{F}$ ]FDG will be needed in the clinic to provide effective diagnostics with more specificity over a greater range of disease and injury (Coenen et al. 2010)(Daniels et al. 2010).

We focus herein on the radioisotope fluorine-18 due to its many desirable chemical and physical properties that lead to excellent stability, resolution, and sensitivity, compared with many other PET radioisotopes (Lasne et al. 2002). Furthermore, the half-life (~110 min) is sufficiently long for transport from production facilities to nearby sites, and  $^{18}\text{F}$ ]fluoride in  $^{18}\text{O}$ ]H<sub>2</sub>O produced by the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  nuclear reaction is easy to handle.

## 1.2 Centralized production of tracers

With existing technology, production of PET probes requires a large capital investment in equipment and infrastructure, and high ongoing personnel and operating costs. Currently, PET probes for clinical PET service are produced in a *centralized* manner by commercial PET radiopharmacies (Fig. 1). A number of universities operate in a similar, centralized manner with a core radiochemistry facility to produce PET probes for an array of basic science and clinical research disciplines. In general, radiopharmacies contain a cyclotron to generate the positron-emitting radioisotope, dedicated probe-specific radiosynthesizers (manual and/or automated) to synthesize and purify radiotracers, and quality control (QC) testing equipment (e.g. gas chromatography (GC), analytical radio-high-performance liquid chromatography (radio-HPLC), dose calibrators, radio-thin layer chromatography (radio-TLC), etc.) to ensure safety of the patient. This workflow requires installation of cumbersome lead-shielded chemistry hoods (“hot cells”) to safely contain the radiosynthesis process, specifically allocated workspace for tasks such as QC testing, reagent qualification and storage, etc., and specially trained personnel to operate the entire process, from cyclotron operation to the aseptic dose preparation.

On December 9, 2009, the U.S. Food and Drug Administration (FDA) issued federal regulations (21 CFR Part 212) and an accompanying guidance document to establish current good manufacturing practices (cGMP) for the production, quality control, holding, and distribution of PET probes in routine clinical use. Furthermore, as of December 12, 2011, all manufacturers must register and submit a New Drug Application (NDA) or Abbreviated New Drug Application (ANDA) in order to sell and market PET probes within the U.S. Thus, in addition to the initial and recurring infrastructure and personnel costs, radiopharmacies must spend additional capital to implement and maintain compliance with federal regulations for each tracer produced.

By spreading these significant costs over many customers in the production of one probe, commercial radiopharmacies have made  $^{18}\text{F}$ ]FDG affordable and readily accessible for

clinical and research use. Due to the way tracers are made, there is almost no additional cost in increasing the size of a production run to serve more customers. A batch of [ $^{18}\text{F}$ ]FDG can be increased by simply changing the amount of the radioactive isotope at the beginning of the synthesis, a function of the bombardment time in the cyclotron with no change in the synthesis, purification, and QC steps. This implies a significant financial advantage in producing large batches of a small number of tracers rather than small batches of a large number of tracers. Due to the relatively low demand for a given new tracer, these tracers cannot be provided at a reasonable price in the current centralized model. To obtain the diversity in molecular probes to match the diversity of disciplines and biological problems being studied, research labs must make the significant investments in radiochemistry capability described above (and make their own tracers) or must obtain tracers at high cost from centralized or core production facilities.

### 1.3 Decentralized production

As technologies advance that simplify the processes involved in making tracers, an alternative, *decentralized*, approach to probe production can be envisioned (Fig. 1). In this paradigm, researchers and clinicians are enabled with the resources to produce on-demand doses of PET probe of interest *themselves*, at low cost, in an automated, user-friendly device. These technologies are aimed at synthesis, purification, and quality control of the PET tracer. Cyclotrons for production of radioisotopes such as fluorine-18 will likely still remain prohibitively expensive for widespread use, even in light of significant reductions in size and cost that have been achieved by ABT's Biomarker Generator dose-on-demand cyclotron (ABT Molecular Imaging, Inc.). Decentralized production, at least for now, will thus still rely on production of radioisotope in centralized facilities. Fortunately, both production and distribution are already well-established, and F-18 can be obtained at very reasonable cost (about \$2-4/mCi) in the United States and around the world.

In contrast to the centralized model, where the high costs of equipment, infrastructure, and personnel can be amortized over a large number of customers by producing large batches of PET tracers, decentralized production requires that *each* locally-used batch of a PET tracer to be produced economically. This paradigm shift requires a fundamental change in radiosynthesis technology such that the following goals are achieved:

- Low capital cost
- Compact size with minimal infrastructure requirements (hot-cell space, etc.)
- User-friendly operation
  - Simple setup and cleanup
  - Fully automated tracer production including synthesis, purification, quality control, and formulation
  - Operation by existing lab technicians
- Multiple runs per day (especially back-to-back production of different tracers)
- cGMP support (e.g. automated batch records, reagents available as kits)
- Integrated quality control
- Ability for end-users to customize/develop syntheses

The last point is important because it will take tremendous effort to develop synthesis protocols for the many currently known and to be discovered tracers. Flexibility in the

process enables the entire base of end-users to participate in new method development to ensure a wide diversity of tracers will become available in kit form.

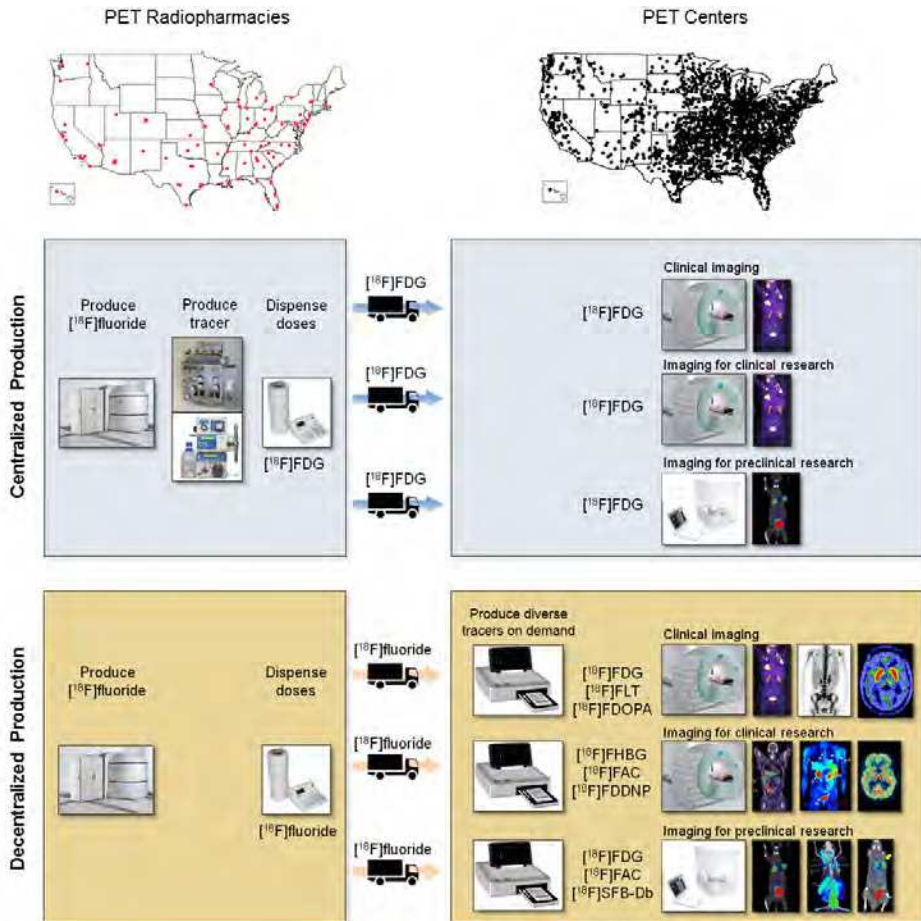


Fig. 1. Centralized and decentralized models of PET tracer production.

A number of research and development efforts in academia and industry are developing technologies that meet many of these needs. Kit-based macroscale radiosynthesizers on the market today are a starting point (Section 3.1), but ultimately microscale systems (Section 3.2) that offer fully integrated radiochemistry solutions on the benchtop will be needed. Several proof-of-concept microfluidic efforts have been described in the scientific literature and several aspects are already beginning to find their way into commercial products. In addition to cassette-based macroscale synthesizers and fully-integrated microscale systems, parallel advances in fundamental radiochemistry (Section 3.3) are resulting in increased yields under milder conditions and simplified purifications. This will increase the diversity of tracers that can be easily adapted into macroscale kit or microfluidic chip format to standardize their production.

## 2. Emerging technologies

### 2.1 Kit-based radiochemistry systems

The availability of simple-to-use kits and automated instruments has completely transformed certain areas of biology and biochemistry, putting powerful assays at the fingertips of the masses. For example, polymerase chain reaction (PCR) assays can be performed today on inexpensive equipment by staff with minimal training. In general, kits simplify assays by (i) reducing reagent preparation and setup time, (ii) reducing the possibility of human error or contamination, (iii) facilitating cleanup, and (iv) simplifying operation through automation. Similarly, kits designed for radiotracer production are beginning to put the capability to make PET tracers directly into the hands of the scientists and clinicians who need them.

Once established in kit form and made available to customers, a given synthesis becomes standardized and enables straightforward and reliable production at many different locations. A further advantage of kits is the simplification of reagent handling. Rather than individually managing multiple reagents, the customer need only to manage the kit as a single unit, greatly simplifying compliance with FDA regulations concerning production of tracers for injection into humans.

#### 2.1.1 General approach

Traditional automated radiosynthesizers are built from fixed components, including reaction vial(s), heater(s), tubing, reagent reservoirs, and electronic valves. Reagents are carefully prepared by the operator and loaded into the reagent reservoirs before the start of synthesis. The system performs automated steps to synthesize—and in some cases—purify and formulate a tracer. After production, the system may perform an automated cleaning protocol to eliminate chemical residue from all wetted components and prepare the system for the subsequent production run.

Recently, many commercial groups have made the important technical advance of developing single-use cassette-based synthesizers to dramatically simplify reagent preparation, eliminate cleaning time, and reduce system complexity by eliminating the need for cleaning. The aim of these one-probe, one-cassette synthesizers is to make PET more accessible to laboratory scientists and easy to use by general lab personnel. These systems typically require a new disposable cassette plus a set of reagents (including consumables such as purification cartridges and filters) for each production run. The cassette itself contains most, or all, of the fluid path to accomplish the synthesis. This fluid path consists of valves, a reaction vessel, reagent reservoirs and pumps, and tubing to connect F-18 source, cartridges, and the collection vial. Pinch valves or stopcock valves are generally used for flow control because they allow a clear separation between an inexpensive fluid-contacting component (i.e. the valve itself) and a more expensive valve actuator (e.g. stopcock rotator) that is part of the fixed system onto which the cassettes are installed. Similarly, pumping is often achieved using pressurized inert gas, or by controlling disposable syringes with motion actuators in the fixed system. To reduce complexity and manufacturing costs, in some systems, the fluid path is molded in a manifold structure, such as a bank of stopcock valves.

After a synthesis run, the cassette is discarded and minimal or no cleaning is required. If the scale of the run is high, there can be high level of residual radioactivity remaining in the cassette (e.g. in purification cartridges). In some systems, one must simply wait for the activity to decay to a safe level before removing the cassette and installing a new one for the next run. However, several systems are designed for multiple back-to-back runs, enabling easy and safe cassette replacement with little downtime between syntheses of the same or different tracer. For example, a wash solution may be flushed through the fluid path to a shielded waste container before cassette removal to reduce the radiation dose to the operator. Another approach, taken in the IBA Synthera, is to automatically eject the cassette (while simultaneously cutting the tubing to the collection vial), and drop the cassette into a shielded waste container to isolate the activity and protect the operator. Disposing of cassettes after synthesis also eliminates the possibility of cross-contamination from one tracer to another. It may also enhance reliability, because many components that would otherwise wear or degrade (e.g. seals) in a conventional system would be automatically replaced with fresh components for each run in a cassette-based system.

Mass-produced, probe-specific kits used on a single platform will help standardize radiosynthesis protocols and quality control methods, improving reproducibility and lowering cost. Because the cassettes are intended to be disposed, they are designed to be mass-manufacturable at a low cost relative to the reagent cost. This often implies using inexpensive plastic materials that can easily be formed by molding processes. In many cases, this introduces limitations into the range of reaction conditions (e.g. temperature, pressure, solvents, etc.) that can be implemented in the cassette. New developments in radiochemistry could mitigate this limitation by enabling reactions to be performed under milder conditions (see Section 3.3).

### 2.1.2 Commercial kit-based systems

There are currently several commercially-available systems that are designed around this concept of reagent kits (Fig. 2).

*ABT Molecular Imaging, Inc. "Biomarker Generator"*. The Biomarker Generator comprises a miniature cyclotron that produces doses of [ $^{18}\text{F}$ ]fluoride (and potentially other isotopes in the future) on demand (e.g. 20 mCi), coupled to a dose-on-demand chemistry module that uses tracer-specific disposable cassettes (ABT Molecular Imaging, Inc.). Unlike other systems, the chemistry module does not require a hot cell but rather includes integrated shielding sufficient to protect operators from the single dose level of radioactivity. Furthermore, it is coupled to an automated QC testing system. A small aliquot of the formulated tracer is diverted to the QC system and the results automatically incorporated into the batch record. The remainder of the tracer is loaded directly into special syringe body that can be used directly for injection into the subject.

*Bioscan, Inc. "F18-Plus"*. This system (Bioscan, Inc.) consists of two compact modules: an FDG-Plus module (for fluorination and deprotection processes) and a ReFORM-Plus module (for formulation) that together provide ten 3-way valves and two reactors. Preassembled sets of tubing, syringes, vials, and a stopcock valve manifold serve as disposable "cassettes." These are installed by the operator, placing components according to a fluid diagram depicted on the

front panel of the instrument. Many other systems provide the cassette as essentially a rigid assembly, which may be slightly more straightforward to install.



Fig. 2. Commercial cassette-based radiosynthesizers. (a) ABT Biomarker Generator. (b) BIOSCAN F-18 Plus synthesizer (left), disposable cassette (middle), and reagent kit (right). (c) Comecer Taddeo. (d) Eckert & Ziegler PharmTracer. (e) GE TRACERlab MX. (f) GE FASTlab. (g) IBA Synthera in operation (left) and during cassette ejection (right). (h) SCINTOMICS GRP module. (i) Sofie Biosciences ELIXYS.

*Comecer "Taddeo"*. At the time of writing, the Taddeo system (Comecer) is marketed for production of  $^{64}\text{Cu}$ -ATSM, but appears to be designed as a more universal synthesis module. Cassettes are based on a stopcock manifold with 15 zero-dead-volume valves, and two reaction vessels are supported.

*Eckert & Ziegler "Modular-Lab PharmTracer"*. The PharmTracer system (Eckert & Ziegler 2011) builds on the strengths (e.g. flexibility and extensibility) of the Eckert & Ziegler Modular-Lab system. Cassettes based on stopcock-manifolds of different sizes (i.e. different numbers of valves) can implement synthesis protocols of varying complexity. The fixed part of the system can be configured to match the desired cassette configuration. The fact that the cassettes use common, off-the-shelf components means they can be reconfigured to develop new protocols.

*GE "TRACERlab MX" and "FASTlab"*. The TRACERlab MX is currently the most widely used synthesis module with disposable cassettes. The FASTlab (GE Healthcare) is a refined version of the TRACERlab, but has highly optimized cassettes and synthesis protocols providing among the highest [ $^{18}\text{F}$ ]FDG yields in the industry. Furthermore, unlike most other cassette-based modules, reagents are pre-loaded, simplifying setup by the operator.



While this simplifies routine production, it hinders development of protocols. Post-synthesis cleaning reduces residual radiation to <0.8% of the starting activity, enabling back-to-back synthesis runs.

*IBA “Synthera”*. The IBA Synthera (IBA) performs one-pot syntheses involving up to four reagents in a compact cassette, the “Integrated Fluidic Processor (IFP)”. Tracer-specific cassettes and reagent sets are available for a number of  $^{18}\text{F}$ -labeled tracers. Synthera is one of the most compact radiosynthesizers on the market: up to three units fit in a standard mini cell, enabling modular expansion to more complex syntheses. A compact HPLC module is also available containing pump, detectors, and column that can be used for tracers that require HPLC purification. The Synthera system has a mechanism for automatically ejecting the cassette after the synthesis to enable multiple runs. The used cassette (which contains substantial residual activity on purification cartridges, etc.) drops down into a shielded waste container, permitting the hot cell to be opened with minimal radiation exposure to the operator during installation of the cassette for the subsequent run.

*SCINTOMICS “GRP (Good Radiopharmaceutical Practice)” Module*. This recently commercialized system (SCINTOMICS GmbH) for the production and dispensing of radiopharmaceuticals is based upon stopcock-manifold cassettes.

*Sofie Biosciences “ELIXYS”*. Most of the above systems are designed for “single-pot” syntheses, and increasing the number of synthesis steps to accommodate other tracers either requires user modification of the system, which may obviate the ability to use the cassette, or purchasing additional modules and thereby dramatically increasing the cost of the overall system. The design goal of the ELIXYS system (Sofie Biosciences, Inc.) was to develop a new PET radiosynthesis platform for probe discovery, development, and production that has the flexibility to accommodate a wide range of radiosynthesis conditions, including high temperatures and pressures and multi-pot reactions, to increase diversity in classes of imaging probes. After development and optimization of a tracer, the same system can be used for routine production for research or clinical use.

At the heart of the ELIXYS system is a mechanism to robotically cap the vessel with a “stopper” during sealed reaction steps (Fig. 3), thereby removing all tubing and valves from exposure to high vapor pressures generated during heating of volatile solvents to high temperatures (Herman et al. 2011)(Herman et al. Submitted). Exposure of these components leads to numerous problems that fundamentally limit the capabilities (i.e. range of pressures and temperatures possible) in other systems. Standard vials are loaded into a temperature control fixture with unique spring-loaded design to maintain good thermal contact despite natural variations in vial size. All reactors can be moved to individual positions via computer control to perform unit operations including reagent additions, evaporations, sealed reactions, transfers, and vial removal. Each reactor has an associated disposable cassette that was designed with both probe development, and optimization and routine probe production in mind; each contains up to 10 reagent vials, two cartridge purification positions, three stopcock valves, and two waste vials. By merging easy cassette configurability with an intuitive software platform, the user is able to conduct probe development and optimization and, on the same system, standardize a final protocol for routine use. Additional unique features of the system include a robot to move vials from

storage positions to one of two needle ports in each cassette for delivery into the reaction vessel. The reagent system trades off the complexity of motion by eliminating numerous valves and vastly simplifies the cassette design. A further advantage is that reagent vials remain sealed until delivered to the reaction vessel, thus preventing exposure of sensitive reagents to the atmosphere, which is critical for long multistep radiosynthesis.

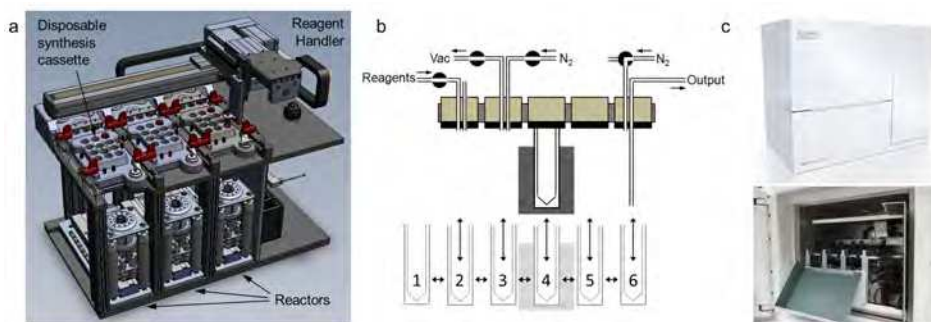


Fig. 3. Sofie Biosciences' universal synthesizer, ELIXYS. (a) 3D schematic of main components. (b) Principle of operation to circumvent issues with high vapor pressures that arise at high temperatures in volatile solvents. This reaction vessel can be moved among 6 different positions and sealed (raised) to a stopper or other interface depending on the desired operation. (c) Photograph of ELIXYS inside a mini-cell.

### 2.1.3 Millifluidic or minifluidic systems

In addition to the above commercially available systems, there are efforts underway at several companies to develop miniaturized kit-based systems based on the idea of microfluidics. The main distinction from the other, macroscale cassettes previously discussed is that these approaches strive to dramatically reduce manufacturing cost by integrating fluid path, valves, and other components directly into a large “chip”, rather than requiring assembly/connection of separate components. Although similar to microfluidics in many ways, the approaches in millifluidics/minifluidics underway generally have larger components (e.g. mm to cm) and volumes (100s of  $\mu\text{L}$ ) than the true microfluidic efforts described in the next section. Systems are in development at Trasis (Trasis)(Voccia et al. 2009) and GE Healthcare (Christian Rensch et al. 2011). In both platforms, chips are structured in a layered manner to form channels, diaphragm valves (from thin, flexible plastic sheets), inlets and outlets, etc. In the system from GE Healthcare, the diaphragm of the valve is part of the chip, while the solenoid actuator exists in the fixed system. The Trasis system employs pressurized gas delivered by an external system to actuate diaphragm valves. It appears that both systems, while more compact than traditional macroscale modules, would still have to be operated inside a hot cell.

## 2.2 Microfluidics

Unfortunately, the above macroscale systems still do not address some of the fundamental limitations to PET probe production previously discussed, namely infrastructure

requirements, high cost, and need for additional equipment for purification and QC. Another technology that looks promising in its ability to support the decentralized model of PET probe production is microfluidics. Operations for synthesis, purification, and QC can potentially be integrated into a compact, inexpensive device that implements the whole pipeline of PET tracer production beginning with a supply of F-18 from existing commercial radiopharmacies.

The use of micro-reaction technology in many areas of chemistry has grown tremendously over the past several years, due primarily to the highly precise control of reaction conditions that is possible through rapid mixing and heat transport, leading to improved reaction speeds and selectivity compared to macroscale approaches (McMullen and Jensen 2010). These advantages are particularly relevant to the production of radiotracers incorporating short-lived isotopes, leading to a reduction in the amount of required starting material (e.g. radioisotope) and thus the thickness of radiation shielding. A further advantage of microfluidics is the ability to manipulate fluids and perform reactions in extremely small volumes, which are well-matched to the minute mass quantities of radiolabeled tracers needed for PET imaging (e.g. 6 pmol for typical human scan). To exploit numerous advantages of working at small scale, a number of research groups have recently explored microfluidic technology platforms for the production of radiotracers. These efforts have been described in several excellent review articles (Arkadij M. Elizarov 2009)(Miller 2009)(Audrain 2007)(S. Y. Lu and Pike 2007)(Miller et al. 2010).

Microfluidic platforms generally possess a high degree of integration of fluid pathways and active components such as valves, leveraging parallel fabrication techniques such as photolithography from the microelectronics industry and sometimes using expensive materials such as plastics. Such techniques promise to minimize the cost of microfluidic devices produced in high volumes, making them well suited to the disposable one-tracer-one-chip model for decentralized PET tracer production described above.

The myriad of microfluidic platforms that have been explored for chemical reactions can be classified into three basic formats: (i) flow-through (or continuous flow), (ii) droplet or slug, and (iii) batch. In flow-through systems, streams of two or more reagents are mixed and then reacted by flowing through a capillary with a predetermined residence time unit held at a constant temperature. Synthesis of most PET tracers requires multiple reaction steps, often in different solvents. To accommodate this need, liquid-liquid extraction and other processes have been developed in continuous format to enable multi-step reactions (Sahoo et al. 2007). Droplet and slug systems are a variant of flow-through systems, in which individual droplets or slugs (with reaction volumes as low as tens of nanoliters) are separated by an immiscible carrier fluid. Batch microfluidic chip designs use microvalves to isolate small batches of reagents in chambers, providing enhanced control of small volumes including sophisticated operations such as solvent exchange and drying processes (C.-C. Lee et al. 2005)(R. Michael van Dam et al. 2008)(Arkadij M. Elizarov et al. 2010a)(Bejot et al. 2010). These chips enable multi-step organic synthesis in nanoliter to microliter volumes.

### 2.2.1 Flow-through microfluidics

In a typical flow-through synthesizer, reagents are pumped at constant flow rate (e.g. using syringe pumps) through a fluid path that first induces rapid mixing of the reagents then

maintains the mixed fluids under constant conditions (e.g. temperature) for a residence time determined by flow rate and the length/volume of the device. The fluid path may comprise a microfluidic chip or a capillary tube. Both approaches have similar channel dimensions but differ significantly in manufacturing technique. A heater or heat exchanger generally provides energy for the reaction. The large thermal mass of the chip relative to that of the small volume of liquid present within it at any given time ensures the liquid is maintained under very close to ideal isothermal conditions. Providing reaction energy by immersion in a microwave field (Issadore et al. 2009) has also been recently reported.

Flow-through synthesis of [ $^{18}\text{F}$ ]FDG has been reported by a number of groups in glass-based chips (Steel et al. 2007), polymer-based chips (Gillies et al. 2006b), and capillary tubes (Wester et al. 2008)(Ungersboeck et al. 2011). The design of Gillies *et al.* (Gillies et al. 2006b) seeks to maximize mixing and flow-rate and achieved an acceptable yield in only seconds. Commercially available flow-through radiochemistry systems include the Advion Biosciences “NanoTek” (Advion Biosciences, Inc.), and the Scintomics “ $\mu$ -ICR” (SCINTOMICS GmbH). Several other  $^{18}\text{F}$ -labeled tracers have also been synthesized in flow-through systems, including [ $^{18}\text{F}$ ]FMISO (Collier et al. 2010), [ $^{18}\text{F}$ ]fallypride (S. Lu et al. 2009), and part of the synthesis of [ $^{18}\text{F}$ ]FIAU (Anderson et al. 2010). In all cases above, solvent-exchange processes (both for drying of [ $^{18}\text{F}$ ]fluoride, and between reaction steps) were performed off-chip. Typically, this is done via azeotropic evaporation in macroscopic apparatus. The mix of microfluidic and macroscopic elements makes the systems complex and inefficient and is not easily amenable to disposable configuration. Other techniques for drying and activating the fluoride are being developed that could be more readily integrated with flow-through synthesizers. In these approaches, the eluent from the trap-and-release cartridge (C. F. Lemaire et al. 2010)(Wessmann et al. 2011) or electrochemical cell (Saiki et al. 2010)(Alexoff et al. 1989)(C. Rensch et al. 2009)(Sadeghi et al. 2010)(Sadeghi et al. 2011) is directly used in the first reaction step.

Potentially alleviating the above restriction, glass-based and polymer-based chips for continuous solvent-exchange and continuous purification are being developed by investigators involved in the Radiochemistry on Chip (ROC) project (ROC-Project). The concept of this project is to develop a modular set of chips (solvent-exchange chip, reaction chip, purification chip, etc.) that can be assembled to perform different syntheses entirely in continuous-flow format.

### 2.2.2 Batch microfluidics

In contrast to continuous flow devices, batch devices (Fig. 4) operate on a “finite” batch of reagents all at once to produce a single batch of radiotracer. This batch may be used for a single imaging study, or may be subdivided for multiple studies. Batch microfluidic devices can perform processes that cannot readily be achieved in continuous flow microsystems such as evaporative solvent exchange (including drying of [ $^{18}\text{F}$ ]fluoride, the most critical step in the synthesis of most  $^{18}\text{F}$ -labeled tracers) and efficient cartridge purifications.

In general, batch microfluidics can manipulate total volumes much smaller than in continuous flow microfluidics. Performing the radiochemistry in small volume batches in the 40nL – 60 $\mu$ L range (C.-C. Lee et al. 2005)(Arkadij M. Elizarov et al. 2010a)(Bejot et al. 2010) offers numerous additional advantages, including reduced precursor consumption,

and accelerated heating and cooling due to reduced mass of liquid. Other potential advantages (not yet established experimentally) include enhanced reaction kinetics by increased concentration of [ $^{18}\text{F}$ ]fluoride, reduced radiolysis, simpler purification and quality control, and increased specific activity (ratio of the radiolabeled to the nonradiolabeled form) due to reduced contact with material surfaces (Berridge et al. 2009) and/or reduced amounts of reagents. Batch devices are therefore the microfluidic platform of choice in building compact radiosynthesizers capable of diverse multi-step syntheses. Beyond the essential functionality for synthesis, it may also be possible to integrate into the chip basic process monitoring and quality control functionality.

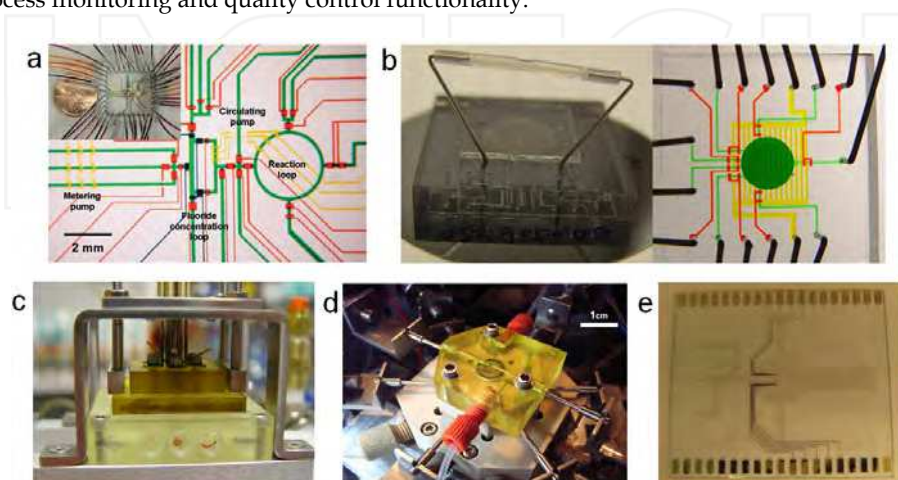


Fig. 4. Batch-format microfluidic platforms for synthesis of PET tracers. (a) Silicone rubber chip (Lee et al. 2005). (b) Scaled-up silicone rubber chip (Elizarov et al. 2010). (c) Chemically-inert pDCPD chip (van Dam et al. 2007) mounted in reagent loading interface and illustrating microvalve actuators. (d) "Open" chip (Bejot et al. 2010) mounted on reagent loading interface and illustrating mechanical actuators for integrated valves. (e) All-electronic EWOD microfluidic chip (Keng et al. 2011).

#### *Batch Microfluidics with Microvalves*

One type of batch microfluidic chips resemble, in many respects, a tiny version of a conventional radiosynthesizer, except with microchannels, microvalves, and micropumps replacing their macroscopic counterparts. The multi-step on-chip synthesis of [ $^{18}\text{F}$ ]FDG, beginning from [ $^{18}\text{F}$ ]fluoride concentration, drying, fluorination, and hydrolysis was first performed as a proof-of-principle study in a microfluidic chip made from poly(dimethylsiloxane) (PDMS) silicone rubber (C.-C. Lee et al. 2005). Since this initial report, additional efforts have demonstrated production of quantities sufficient for preclinical imaging (several mCi) by improvements in chip design and scale-up of reaction volumes from 40nL to 5 $\mu\text{L}$  (Arkadij M. Elizarov et al. 2010a). PDMS chips contain tiny microchannels (with width and depth on the order of 100 $\mu\text{m}$ ) as well as integrated microvalves and micropumps (Melin and Quake 2007) in which small volumes of reagents are manipulated to perform multistep chemical reactions. The underlying technology enables integrated manufacture of chips with tremendous sophistication. Furthermore, the

inherent permeability of siliconerubber enables a novel approach to solvent exchange processes. Reagents can be pumped into a reaction chamber, and the chamber sealed by closing valves at all inlets/outlets. As the chip is heated by an external heat source, solvent is removed by pervaporation and permeation.

Despite several publications on these elastomeric chips, device reliability is limited by the inherent incompatibilities of the silicone rubber polymer with many solvents and reagents (Mukhopadhyay 2007), and suspected interaction of the PDMS material with [ $^{18}\text{F}$ ]fluoride ion under certain conditions (W.-Y. Tseng et al. 2010), resulted in up to 95% of [ $^{18}\text{F}$ ]fluoride lost (Arkadij M. Elizarov et al. 2010a). By re-engineering the chip to increase the burst pressure of the micro diaphragm valves and replacing the PDMS with an inert poly-dicyclopentadiene (pDPCD) polymer, the radiosynthesis of [ $^{18}\text{F}$ ]FDG in sufficient quantity for human imaging was demonstrated (R.M. van Dam et al. 2007). The exploration of new materials was enabled by devising a chip architecture that did not require bonding between the device layers.

Rate of solvent evaporation is inherently limited by the gas-permeable membrane present in the above devices, and, for the 5  $\mu\text{L}$  volume in the latter reports, becomes a significant fraction of overall process time. Elizarov *et al.* (Bejot et al. 2010) reported the synthesis of N-succinimidyl-4- $^{18}\text{F}$ fluorobenzoate ([ $^{18}\text{F}$ ]SFB) in a slightly larger (60  $\mu\text{L}$ ) microreactor in which the membrane is eliminated and evaporation occurs directly from an open liquid-air interface (Arkadij M. Elizarov et al. 2011). A synthesis time of 25 min was achieved, which is substantially faster than that possible on macroscopic modules (60-100 min). The valves were re-engineered in this chip to withstand even higher pressures, but they do not easily lend themselves to a separation between disposable and fixed elements.

#### *Valveless Microfluidics*

Recently our group described a novel platform for performing batch synthesis at the microscale, based on a technology known as “electrowetting-on-dielectric” (EWOD). Constructed from inorganic materials coated with a perfluoropolymer layer, these microfluidic chips provide much greater compatibility with diverse reagents and reaction conditions for microscale chemical synthesis. Liquid manipulation is performed electronically, eliminating the need for moving parts such as pumps and valves (inherently increasing reliability), and simplifying the chip and the external control system. Furthermore, it is possible to integrate additional electronically controlled functions into the chip such as sensors to monitor liquid volumes (Gong and Kim 2008) and composition (Schertzer et al. 2010)(Sadeghi et al. Submitted) as well as heaters and temperature sensors for heating liquid droplets or evaporating solvent (Nelson et al. 2010). EWOD devices have the additional advantage of digitally-programmable fluid pathways that could readily be configured for a wide variety of microscale batch organic syntheses, optimization, or screening studies for diverse tracers.

EWOD devices belong to a class of two-dimensional (2-D) droplet-based devices that manipulate droplets using their surface tension (Abdelgawad and Wheeler 2009). A typical EWOD microchip (Fig. 5) consists of two parallel plates: (i) a substrate patterned with electrodes and coated with dielectric and non-wetting layers, and (ii) a cover plate coated with a conductor (to act as a ground electrode), dielectric and non-wetting layers. Droplets are sandwiched into a disc shape between the plates, and electrical potential is applied to

individual or multiple electrodes on the patterned substrate to achieve unit operations such as droplet generation, transport, splitting, and merging (S. K. Cho et al. 2003). The open structure of EWOD chips is particularly advantageous in achieving rapid solvent evaporations and solvent exchange. Though generally used for manipulation of aqueous samples and biochemical assays, EWOD chips can manipulate organic solvents (Chatterjee et al. 2006) and ionic liquids (Dubois et al. 2006) critical for performing chemical reactions. With this platform, radiosynthesis of  $[^{18}\text{F}]\text{FDG}$ , and 1- $[^{18}\text{F}]$ fluoro-4-nitrobenzene ( $[^{18}\text{F}]\text{FNB}$ ) has been demonstrated with high repeatability (S. Chen et al. 2010)(Pei Yuin Keng et al. 2010). So far, mCi quantities of tracer have been produced on chip (Supin Chen et al. 2011)(Pei Yuin Keng et al. Submitted), with further scale-up efforts underway.

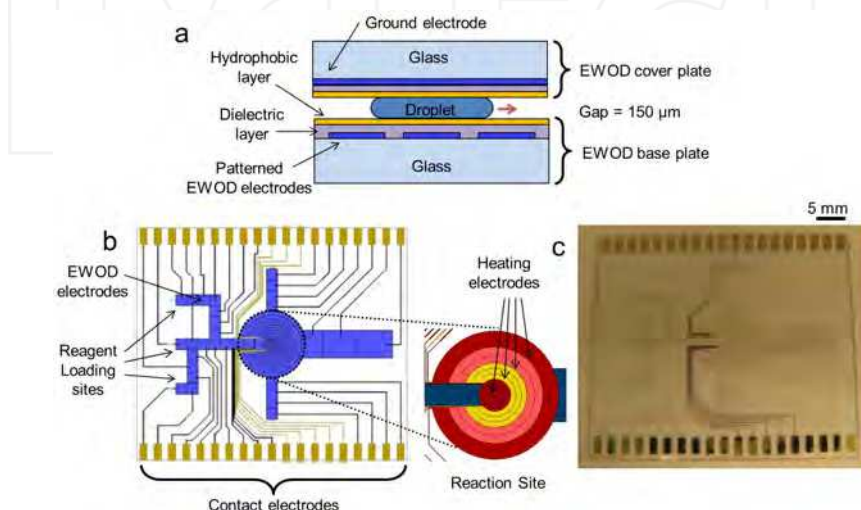


Fig. 5. Electronic microfluidic chip for synthesis of PET tracers. (a) Cross-section of electrowetting-on-dielectric (EWOD) chip illustrating a reagent droplet sandwiched between the base plate and cover plate. (b) Electrode design of a PET tracer synthesis chip. Reagents are moved from the inlet sites to the reaction site in the center of the chip. The reaction site is heated by electrodes to perform reactions or evaporations. During evaporations, vapor can readily escape from the open sides of the chip. (c) Photograph of an EWOD radiosynthesis chip. Adapted from Keng et al. 2011.

### 2.2.3 Outlook of microfluidic technologies

The main advantages of applying microfluidics to the radiosynthesis of PET tracers thus far have been reduced reaction times, higher synthesis yields, and increased throughput in reaction optimization (Pascali et al. 2010). Microfluidics can also reduce consumption of expensive precursors, increase speed of evaporations and other processes, reduce need for shielding, and potentially increase concentration of  $[^{18}\text{F}]$ fluoride to normal stoichiometric levels. While these are very important advances, microfluidics also has the potential for tremendous advances in miniaturization and integration that will reduce cost of synthesis, and potentially in the future encompass downstream processes such as purification and quality control testing.

An automated, compact, self-shielded, microscale synthesizer for the on-demand production of individual doses of PET probes in a biological or clinical laboratory setting would overcome many limitations inherent in traditional macroscale systems. First, the need for cost-prohibitive infrastructure associated with developing and bringing novel PET tracers to market will be removed. Second, because of the minute volumes, the amount and expense of cold compounds such as precursors used in radiosynthesis is decreased. Instead of ordering from a limited menu of tracers produced by a centralized radiopharmacy, a scientist or clinician would need only to purchase the benchtop microfluidic synthesizer for their existing laboratory, install their probe-specific "chip" of choice, add the associated radioisotope supplied by the commercial radiopharmacy, and push "START" on the PC control system. The integration of all liquid handling functionality and shielding within a small, self-contained device removes the need of secondary shielding (hot cells) while maintaining the safety of the end user within federal guidelines. Furthermore, a point-of-care device of this kind will address the serious issues facing SPECT by making PET a more practical clinical option and provide a convenient low-cost supply of PET probes for academic and pharmaceutical research.

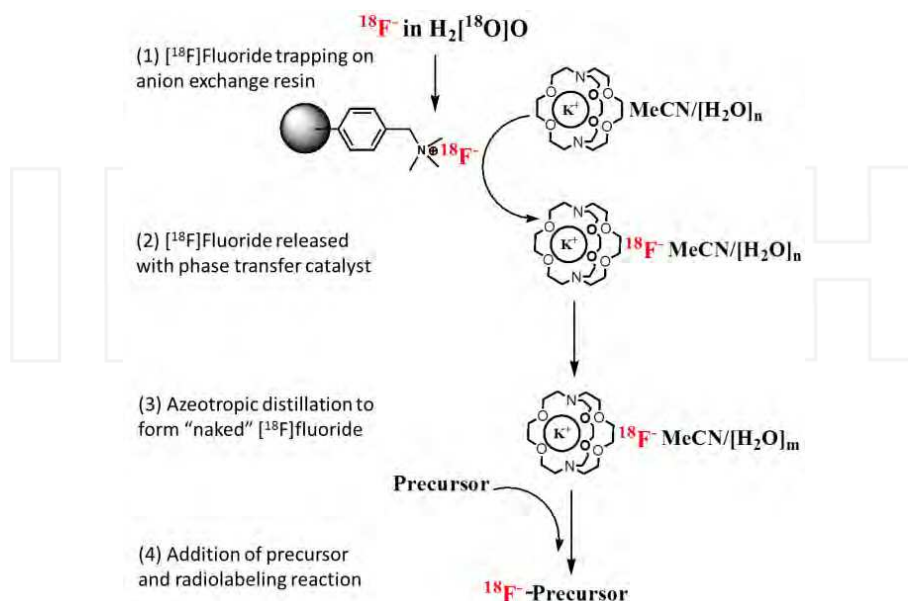
### 2.3 Advances in radiochemistry

Recent advancements in microreactor and microfluidic radiosynthesizer technologies have triggered much investigation in modifying conventional radiochemistry approaches to yield PET tracers with higher yield and selectivity, shorter reaction times and milder reaction conditions. Taking advantage of these technology developments and automated high-throughput methods, radiochemists can now perform dozens of parallel optimization experiments in a single day, which was not possible a decade ago. This has catalyzed the discovery of new probes and radiosynthetic approaches. These investigations have also led to the emergence of HPLC-free chemistry, which is critical in realizing the decentralized production of PET tracers because it eliminates the need for expensive HPLC equipment and associated specialized personnel. In addition to fine-tuning reaction parameters, the use of ionic liquid and bulky alcohol as a solvent has also shown increased labeling efficiency, selectivity, and cleaner reaction product, which could facilitate automation, reducing the overall radiosynthesis time and simplifying purification using Sep-pak cartridges. Radiosynthetic strategies utilizing milder reagents and reaction conditions (reduced temperatures and pressure, reduced the use of solvents, etc.) are often more amenable to be performed in reaction systems made from polymeric materials such as disposable kits or microfluidics. These advances represent another avenue for enabling decentralization.

The state-of-the-art [ $^{18}\text{F}$ ]fluoride ion ( $[^{18}\text{F}]\text{F}^-$ ) radiolabeling approaches (Scheme 1) involve nucleophilic substitution of a precursor bearing a good leaving group (e.g., triflate and mesylate) in an aprotic organic solvent (usually acetonitrile, dimethyl sulfoxide or dimethylformamide). (Cai et al. 2008) The fluoride anion ( $^{18}\text{F}$ ) is generated and delivered in  $[^{18}\text{O}]\text{H}_2\text{O}$  from the cyclotron, in which the fluoride anion is strongly hydrated by water molecules, hindering its nucleophilicity. To achieve highly reactive fluoride nucleophiles for substitution reactions, the fluoride anion is first transferred from the aqueous phase to the organic phase by complexing with a phase transfer catalyst such as Kryptofix ( $\text{K}_{222}$ ) plus potassium carbonate as a weak base, or tetrabutylammonium hydroxide or tetrabutylammonium bicarbonate. Subsequently, trace amounts of water are removed via



repeated cycles of azeotropic distillation with acetonitrile. The activated  $[^{18}\text{F}]\text{KF}/\text{K}_{22}$  complex is readily resolubilized in aprotic organic solvent for nucleophilic substitution reactions.



Scheme 1. General radiosynthetic scheme using no-carrier-added  $[^{18}\text{F}]\text{fluoride}$  ion generated from the cyclotron. The first step involves fluoride trapping on an anion exchange resin and release with a phase transfer catalyst. Water is removed via repeated cycles of azeotropic distillation to form the reactive ("naked") fluoride. Then a solution of precursor dissolved in aprotic solvent is added for the radiolabeling reaction.

Traditionally, macroscale radiosyntheses were designed to be performed in inert atmosphere, under anhydrous conditions, or other controlled environments, with evaporations and reactions taking place within a sealed reactor. The majority of fluorine-18 radiolabeling reactions are optimized at high temperature (usually above the boiling point of acetonitrile, which results in high vapor pressure built up in the reaction vessel), excess mass of precursor, and the use of harsh reagents. Along with these stringent reaction conditions, the majority of radiosynthetic strategies require HPLC purification to remove the excess precursors and undesired side products. Hence, radiosyntheses of PET probes have necessitated skilled radiochemists, and specialized, complex, and expensive synthesis modules--factors that have limited the availability of radiotracers. These conventional strategies are not easily implemented onto microfluidic chips (as described in Section 2.2.3) due to the difficulty in performing evaporations at the microscale and other factors.

Due to the increasing demand for PET probes and recent progress of microfluidic synthesis platforms (Miller 2009)(Arkadij M. Elizarov 2009), there is much interest on developing novel, simplified, robust and efficient radiosynthetic strategies to enable facile implementation of radiosyntheses both in the macro- and microscale synthesis modules. In

this section, we focus on recent developments in (i) HPLC-free radiosynthesis method, (ii) alcohol and ionic liquid catalyzed radiosynthesis, and (iii) enzymatic fluorination using nucleophilic fluoride ion. The scope of this section is limited to selected (radio)chemistry strategies that have the potential to be implemented with current state-of-the-art automated microreactor and microfluidic platforms.

### 2.3.1 HPLC free radiochemistry

Current F-18 PET tracer synthesis protocols, with [ $^{18}\text{F}$ ]FDG as the main exception, require HPLC purification due to the formation of radiolabeled or toxic side products that cannot be easily removed via solid phase extraction (SPE) cartridges. Thus, current methods necessitate purification via HPLC to meet the cGMP/FDA regulations before the tracer can be administered into humans (S. Yu 2006). Due to the disadvantages of using HPLC, it is desirable to modify current radiosynthetic strategies to use simpler purification methods, such as SPE. Such modification could potentially reduce the total synthesis time and radiation exposure by eliminating the need for concentrating the purified product before formulation of the PET tracer into injectable doses.

Recent examples have demonstrated the radiosynthesis of 3'-deoxy-3' [ $^{18}\text{F}$ ]fluorothymidine [ $^{18}\text{F}$ ]FLT and 1- $\alpha$ -D-(5'-deoxy-5' fluoro-(1S,2R,3S,4S)arabinofuranosyl)-2-nitroimidazole ([ $^{18}\text{F}$ ]FAZA) followed by cartridge purification to obtain > 99% pure radiotracers (Nandy and Rajan 2009)(Nandy and Rajan 2010). In the simplified radiosynthesis of [ $^{18}\text{F}$ ]FLT, Rajan *et al.* utilized a precursor with minimum-blocking groups, 5'-O-(4,4'-dimethoxytriphenylmethyl)-2,3'-anhydro-thymidine (DMTThy), followed by a single alumina cartridge purification to yield [ $^{18}\text{F}$ ]FLT with >95% radiochemical purity (Nandy and Rajan 2009). The key to eliminating complicated HPLC purification was to reduce the amount of side products such as the leaving group and protecting group that were liberated during the substitution and deprotection reactions. (Nandy *et al.* 2010) Using the anhydrothymidine precursor (Machulla *et al.*), the Najan group successfully synthesized and purified [ $^{18}\text{F}$ ]FLT using the custom-made alumina cartridge, which contained 7700 mg of dry alumina (compared with ~600 mg in a typical Sep-Pak alumina cartridge).. Although the anhydrothymidine precursor requires higher synthesis temperature, longer reaction time, and has slightly lower radiochemical yield in comparison to the nosylate-FLT precursor, the ability to purify the crude product using a single alumina cartridge is particularly attractive. Later, the Tang group further optimized the radiosynthesis conditions using the nosylate-FLT precursor to obtain pure [ $^{18}\text{F}$ ]FLT with up to 40% radiochemical yield in 35 minutes using a series of Sep-Pak cartridges; thus eliminating the needs for HPLC purification.(G. Tang *et al.* 2010)

One of the well-known base catalyzed competing reactions is the bimolecular elimination (E2) reaction, where the leaving group is positioned anti-periplanar to a hydrogen attached to the adjacent carbon. Due to the large excess of base used in a typical radiochemistry reaction in comparison to the [ $^{18}\text{F}$ ]fluoride anion, the majority of the precursor undergoes elimination reaction.(Chirakal *et al.* 1995) However, the presence of a large amount of side product complicates HPLC purification. Specifically in this version of [ $^{18}\text{F}$ ]FLT synthesis, the elimination by-product, 2',3'-didehydro-3'-deoxythymidine, an anti-HIV drug (Stauvidin), is permanently incorporated into DNA and results in cytotoxicity.(Grierson and Shields 2000)

By controlling the concentration of base and the precursor-to-base mole ratio, an optimal labeling efficiency could be achieved with minimal side products. In a quantitative investigation by Shuehiro *et al.*, the group showed that 300 ng of impurities is produced under typical [ $^{18}\text{F}$ ]FLT synthesis conditions where the concentration of  $\text{K}_{222}$  and  $\text{K}_2\text{CO}_3$  were 74 mmole and 40 mmole, respectively. As the base concentration of the phase transfer catalyst and base were reduced to 10 mM and 7 mM, respectively, the impurities decreased to 1 ng while achieving similar labeling efficiency. (Suehiro *et al.* 2007) This quantitative report not only showed that higher labeling efficiency could be achieved by reducing the base concentration, but also showed the potential to facilitate automation by enabling purification using SPE cartridges due to the reduced amount of impurities. Applying such methods to other tracers could similarly result in HPLC-free protocols for their production.

### 2.3.2 Solid phase supported radiosynthesis

The development of solid phase solid supports since 1970 has revolutionized peptide synthesis and combinatorial chemistry of libraries of small molecule analogues. (Ellman 1996) In solid phase organic synthesis, one of the reagents or substrates is bound onto a polymer solid support, while the second reactant (in solution) is flowed through the functionalized solid support. The advantages of solid phase organic synthesis relative to solution phase synthesis include ease of purification, selective product cleavage, recycling of precious catalyst and enabling polymer bound toxic reagents to be handled safely. (Früchtel and Jung 1996) In radiochemistry, large excess of precursor is typically needed to achieve high yield (>60%). To overcome the challenges in isolating the final product from the large excess of precursor, several groups have investigated a new radiosynthetic platform based on nucleophilic cleavage of solid-supported precursor using [ $^{18}\text{F}$ ]fluoride ion (LUTHRA *et al.* 2003)(Frank Brady *et al.*)(L. J. Brown *et al.* 2007) with 70-91% labeling efficiency. In this strategy, a PET probe precursor with a sulfonate linker is attached on a solid phase support, which can be cleaved upon nucleophilic substitution by [ $^{18}\text{F}$ ]F $^-$ . In principle, only the substituted intermediate will be cleaved into the solution phase for the subsequent deprotection reaction, thus minimizing the amount of unreacted starting material in the reaction mixture. However, this solid phase approach has not been widely utilized or commercialized for other PET probes. The limited progress in this area could be due to the multistep synthesis to prepare the solid phase linkers. Additionally, this approach utilizes a perfluorinated linker to activate the nucleophilic substitution reaction, which could arguably decrease the specific activity of PET tracers (Chyng-Yann Shiue *et al.* 1985).

The Gourvener group has worked out an alternative strategy to the solid phase approach based on the fluororous detagging of the precursor upon nucleophilic fluorination to simplify purification (Bejot *et al.* 2009). Taking advantage of the strong partition efficiency of the perfluorinated compound from aqueous and organic phases, fluororous phase extraction has been widely utilized in organic synthesis to facilitate purification and recovery of catalysts. Unlike the solid phase approach, in which the reaction mixture is heterogeneous (*i.e.*, precursor on the solid phase while the nucleophile is in the solution phase), the fluororous detagging strategy is a solution-phase approach with the advantages of rapid reaction kinetics and simplified purification via selective phase separation from liquid. In this strategy, several alkylperfluorosulfonate tags with prosthetic groups such as reactive epoxide, azide, alkyne, and triflate were synthesized and radiolabeled with the

$[^{18}\text{F}]\text{KF}/\text{K}2.2.2$  complex in acetonitrile. The radiolabeled product was collected via fluororous phase solid extraction (FPSE), in which the unreacted excess precursor tag with the fluororous phase remained on the FPSE cartridge, while the radiolabeled PET probe was eluted with high efficiency (Fig. 6). Additionally, the group has also demonstrated the synthesis of  $[^{18}\text{F}]\text{FMISO}$ ,  $[^{18}\text{F}]\text{fluoroethylcholine}$ , and *cis*-4- $[^{18}\text{F}]\text{fluoro-L-proline}$  in 53%, 84%, and 42% radiochemical yields, respectively. These results showed that the fluororous detagging strategy could potentially be integrated onto microfluidic device as a universal radiosynthesis platform for the preparation of a wide range of PET probes (Fig. 6).

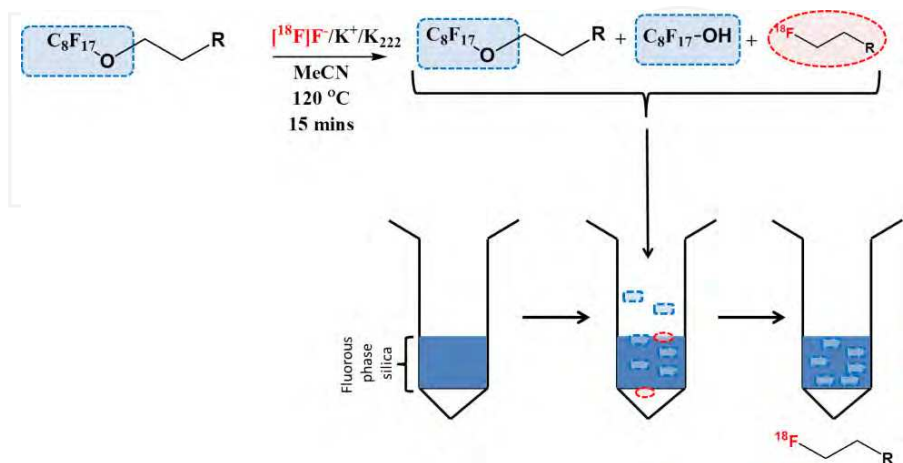


Fig. 6. Fluororous phase synthesis of  $[^{18}\text{F}]\text{F}$  prosthetic groups in acetonitrile followed by fluororous solid phase extraction (FSPE). The reaction mixture is passed through a fluororous silica gel to collect only the fluorinated product.

### 2.3.3 Alcohol and ionic liquid catalyzed radiosynthesis

The conventional radiolabeling strategies using either quaternary ammonium or Kryptand/ $\text{K}^+$  as phase transfer catalysts to solubilize the fluoride anion into aprotic organic solvent require vigorous drying steps. This drying, solvent exchange and fluoride activation process is one of the most challenging and time consuming steps in radiosynthesis. In a typical F-18 radiolabeling reaction in an aprotic solvent, multiple cycles of azeotropic distillation are needed to completely remove the hydration shell around the anion to increase the nucleophilicity. To date, only a few types of batch microfluidic chips are capable of performing all the required radiolabeling processes including the drying step on chip, while flow-through microfluidic chips have utilized macroscale drying methods. Since 2002, Chi and his group have successfully demonstrated the (radio)fluorination of alkyl mesylate and  $[^{18}\text{F}]\text{FDG}$  using  $\text{CsF}$  in the presence of ionic liquid as catalyst (Oh et al. 2011)(D. W. Kim et al. 2002). Ionic liquids are fused salts at room temperature; this class of material has been investigated as an alternative to conventional organic solvents due to their unique characteristics: (1) good solvent for a wide range of reagents, which is ideal in solubilizing different component of reagents into a single phase, (2) polar solvent but non-coordinating, which is very optimal for accelerating nucleophilic substitution reactions, and

(3) non-volatile, which is advantageous to prevent solvent evaporation and pressure build-up during fluorine-18 labeling reactions, the majority of which are performed at high temperature. In radiochemistry, this ionic liquid approach presents a new avenue, which has allowed n.c.a fluoride-18 substitution reactions to be performed in the presence of significant amounts of water (50  $\mu$ L). As reported by Kim *et al.*, high radiolabeling yield could be attained in shorter time (as rigorous drying steps could be eliminated) at higher temperature with reduced side products. This synthetic strategy could potentially be applicable in current flow-through microfluidic technologies (as discussed in section 3.2.1) integrated with an anion exchange cartridge, in which the trapped fluoride-18 could be released with a mixture of phase transfer catalyst in aprotic solvent containing microliter volume of water to achieve reactive n.c.a. fluoride-18 ion, without additional drying steps. Current limitations of the ionic liquid strategy include high temperature synthesis (120 °C), which may not be applicable for all kinds of precursors, and the need of specialized ionic liquid for a specific reaction.

As mentioned earlier, the nucleophilicity of the fluoride anion decreases in the presence of protic solvents, such as water and alcohol, due to the strong hydrogen bonding interaction. However, recent investigation into bulky, tertiary non-protic alcohols (e.g.: t-butyl alcohol) has shown acceleration of the fluorination reaction rate using a nucleophilic fluoride source (e.g.: CsF, TBAF) while suppressing the formation of elimination by-products, thus have the potential to eliminate the needs of HPLC for the final purification. Furthermore, the resulting nucleophilic TBAF(t-butyl alcohol) complex is easy to handle, which facilitates automation. This strategy has been explored in the radiosynthesis of [ $^{18}$ F]FDG, [ $^{18}$ F]FLT, [ $^{18}$ F]F-FP-CIT, and [ $^{18}$ F]FMISO with a 2-3-fold increase in radiochemical yield in comparison to conventional methods reported in the literature.(D. W. Kim et al. 2010)(B. S. Moon et al. 2006) In these studies, the fluorination efficiency increases with the increase of steric hindrance of the bulky alcohol in the order of hexyl alcohol > t-amyl alcohol> t-butyl alcohol. Based on x-ray crystallography, the tertiary alcohol is postulated to enhance substitution efficiency and selectivity by the limited solvation of the fluoride anion, yielding a “flexible fluoride anion.”(D. W. Kim et al. 2008) Additionally, the reactivity of the leaving group is enhanced by hydrogen bonding of the tertiary alcohol with the oxygen on the mesylate or triflate group. Under this protic environment, side reactions such as elimination, hydroxylation and intramolecular alkylation reactions, could be suppressed. Both the ionic liquid and the bulky alcohol strategies are particularly attractive for implementation of radiochemistry on microfluidic chips due to the high yield, short reaction time (~5 min), high chemoselectivity, and ease of handling the t-butyl alcohol / fluoride (TBAF) complex in comparison to the anhydrous TBAF in aprotic solvent.

### 2.3.4 Enzymatic radiofluorination

In nature, enzyme catalysis is highly stereoselective and efficient, lowering the activation barrier and thus enabling milder reaction conditions, shorter reaction times, and simplified purification methods. Recent development and discovery of the fluorinase enzyme (O’Hagan 2006)(Dong et al. 2004) has demonstrated the radiofluorination of (S)-adenosyl-L-methionine (SAM) and [ $^{18}$ F]fluoride anion with 95% radiochemical yield and 1 million-fold rate enhancement (Fig. 7a).(H. Deng et al. 2006) Based on the mechanistic studies of the fluorinase enzyme, substrate stabilization appears to be the most critical parameter in this

C-F enzymatic catalysis. Additionally, both crystallographic and theoretical studies suggest that the enzyme dehydrates the solvated fluoride ion through cooperative hydrogen bonding on the serine moieties in the active site (Fig 7b). These hydrogen bonding interactions around the naked fluoride ions decrease the calculated activation energy from 92 kJ/mol to 53 kJ/mole, which can explain the dramatic rate acceleration.(O'Hagan 2006) However, the fluorinase enzyme is extremely specific to the SAM substrate and thus is not applicable to any other substrates. To increase structural diversity of fluorinated biomolecules based on the fluorinase catalytic reaction, a base-swap biotransformation has been demonstrated in a single pot to yield 5'-fluorinated uridine derivatives.(Winkler et al. 2008) Although the fluorinase enzyme has shown potential in accelerating radiolabeling reactions and has the potential to be immobilized into microfluidics (Krenkova and Svec 2009), practical application of enzymatic catalysis is currently limited by their short shelf life, and their intolerance to harsh conditions such as non-aqueous environment and extreme temperatures, which currently hinders application to other fluorination reactions.

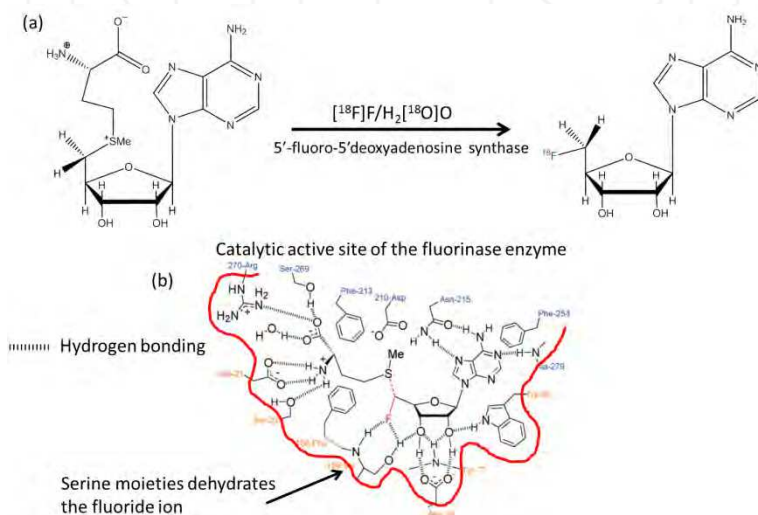


Fig. 7. (a) Enzymatic catalysis of (S)-adenosyl-L-methionine (SAM) and  $[^{18}\text{F}]\text{F}^-$  to 5'-deoxyadenosine (5'-FDA) in using the fluorinase enzyme. (b) Illustration of cooperative hydrogen bonding interactions at the active site of the fluorinase enzyme,  $[^{18}\text{F}]\text{F}^-$  fluoride ion, and the SAM substrate as deduced by x-ray crystallography. Reproduced from O'Hagan 2006.

### 3. Conclusions

With the number of PET tracers with established research and clinical value increasing, the centralized model of PET tracer production will no longer be able to meet these demands. A paradigm shift to incorporate decentralized production is essential in order to enable imaging scientists to use desired tracers on demand in research or clinical settings. To enable decentralized production, new technologies and automation are needed that simplify tracer production, reduce the cost, size and complexity of equipment required, and eliminate the

need for highly skilled radiochemists in routine tracer production. Kit-based radiosynthesizers are a significant step in this direction, with many excellent systems already on the market. Microfluidic technologies under development will further miniaturize and reduce costs of kits, as well as provide fundamental advantages in speed, yield, and perhaps specific activity. In parallel with engineering developments, advances in radiochemistry are making possible syntheses under simpler and milder conditions, thus facilitating their automation. Even with the advances to date, PET tracer production remains expensive and complex and out of reach of most labs. Further developments in miniaturized and automated purification as well as quality-control testing will be instrumental in bringing PET tracer production to the benchtop.

#### 4. Acknowledgments

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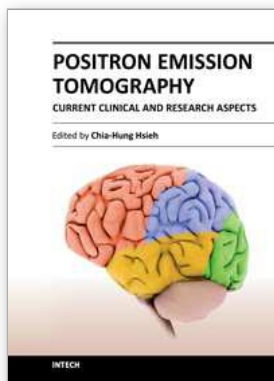
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## **Positron Emission Tomography - Current Clinical and Research Aspects**

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This book's stated purpose is to provide a discussion of the technical basis and clinical applications of positron emission tomography (PET), as well as their recent progress in nuclear medicine. It also summarizes current literature about research and clinical science in PET. The book is divided into two broad sections: basic science and clinical science. The basic science section examines PET imaging processing, kinetic modeling, free software, and radiopharmaceuticals. The clinical science section demonstrates various clinical applications and diagnoses. The text is intended not only for scientists, but also for all clinicians seeking recent information regarding PET.

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