## MICROFLUIDIC RADIOSYNTHESIS: ELECTROCHEMICAL PHASE TRANSFER FOR DRYING [18F]FLUORIDE

**C. RENSCH*[1], C. BOELD[1], B. BACHMANN[1], S. RIESE[2], G. REISCHL[3], W. EHRLICHMANN[3], N. HEUMESSER[3], M. BALLER[1] and V. SAMPER[1]**

1. GE Global Research, Garching b. Munich, Germany; 2. GE Healthcare, Germany; 3. University Hospital Tuebingen, Radiopharmacy, Tuebingen, Germany

**Objectives:** Ion exchange trapping of [$^{18}$F]fluoride followed by azeotropic drying is a common method of performing separation of [$^{18}$F]fluoride from $H_2{}^{18}O$ into an aprotic solvent. Rapid azeotropic drying is challenging to integrate in a closed microfluidic synthesis system because of the size and property requirements placed on the gas permeable membrane. Electrochemical phase transfer is an attractive alternative technique because it avoids the drying step. The objective of the work described is to perform electrochemical phase transfer of [$^{18}$F]fluoride from $H_2{}^{18}O$ into DMSO, followed by labeling of [$^{18}$F] fluoromisonidazole[1] (FMISO) to validate the process and to compare the results of measured and predicted ion motion inside the microfluidic electrochemical reactor.

**Methods:** A 20$\mu$l microfluidic reactor with a glassy carbon anode was constructed. The anode-cathode electrode distance was ~125 $\mu$m. The set-up was heated by electric heater cartridges. The electrical potential and currents were controlled by a potentiostat. 500$\mu$l of $^{18}$F$^-$ containing water with activities between 50 MBq and 600 MBq were pumped through the reactor by a syringe pump, at a controlled flow rate, while various trapping potentials were applied. Subsequently, the reactor was dried using Acetonitrile and DMSO. Finally, a release potential was applied to the reactor and the $^{18}$F$^-$ was released into a kryptofix 222/$K_2CO_3$/DMSO mixture. The release of $^{18}$F$^-$ with time, its efficiency labeling [$^{18}$F]FMISO and water concentrations of solutions were measured and associated with the parameters under user control. A Karl-Fischer titrator was used to quantify the water content. The trapping pattern on the anode was imaged using an electronic autoradiograph and compared with a physical model that predicted the motion of ions in an electric field.

**Results:** Voltages > 7 V consistently resulted in trapping efficiencies of > 90 % (n = 12). Release efficiencies > 50% could be achieved using different combinations of temperature (r.t. to 120 °C), voltage (0 to 10 V), and concentrations of reagents and precursor. The highest measured labeling yield was 87 % (n = 5). The results show that the distance traveled by the $^{18}$F ions before they were captured on the anode was consistently longer than predicted. Electrical current versus time results and autoradiography of the anode provided indirect evidence that this discrepancy could be ascribed to the generation of gas by electrolysis of water. Water content results showed a good inverse correlation with labelling yield, as did chemical concentrations.

**Conclusions:** The reported electrochemical phase transfer experiments demonstrated the feasibility of applying this method in a microfluidic synthesizer. Using [$^{18}$F]FMISO as model tracer, demonstrated that the technique is applicable to labelling reactions that are more water sensitive than [$^{18}$F]FDG. The analytical model incorporating a compensation factor for electrolysis, was also shown to have useful capabilities in predicting necessary reactor lengths.

**Research Support:** This project/work has been funded in part by a grant from the program Lead Projects in Medical Technology of the Bavarian Ministry of Economic Affairs, Infrastructure, Transport and Technology (Germany).

# DEVELOPMENT OF ONE-FLOW SYNTHESIS METHOD FOR N-SUCCINIMIDYL 4-[F-18] FLUOROBENZOATE ([F-18] SFB) USING MICROREACTOR FOR 3-STEP-REACTION

**H. KIMURA*[1], K. TOMATSU[1], H. KAWASHIMA[2], H. NAKANISHI[3], E. OZEKI[3], H. SAIKI[3], R. IWATA[4], Y. KUGE[5], M. ONO[1] and H. SAJI[1]**

1. Kyoto University, Graduate School of Pharmaceutical Sciences, Kyoto, Japan; 2. Kyoto University, Graduate School of Medicine, Kyoto, Japan; 3. Technology Research Laboratory, Shimadzu Corp, Kyoto, Japan; 4. Tohoku University, CYRIC, Sendai, Japan; 5. Hokkaido University, Graduate School of Medicine, Sapporo, Japan

**Objectives:** Microreactor is a synthesizer that generates reactions inside microchannel in nano/micro litters. With the characteristics of effective heat conduction due to the large specific surface area and reduced mixed time due to short diffussion distance, reaction is possible in a short amount of time, with low volume and good yield, which is attracting a high level of interest in the field of radiochemistry. In this study, N-succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB), famous as an indirect labeling reagent for protein and/or peptide used for PET, was synthesized in 3 steps using microreactor, and such effective have been evaluated.

**Methods:** Each reaction of [$^{18}$F]SFB synthesis shown in Scheme 1 was optimized by using batch method and microreactor for 1-step-reaction (channel width: 150 $\mu$m, depth: 150 $\mu$m, length: 250 mm, volume: 5.63 $\mu$l). After optimizing the reacting condition, microreactor for 3-step-reaction (Figure 1; channel width: 150 $\mu$m, depth: 150 $\mu$m, length: 500 mm, volume: 11.3$\mu$l) and [$^{18}$F]SFB sythesis aiming for One-flow synthesis as well as reaction stage were designed/created. The One-flow synthesis method for [$^{18}$F]SFB was optimized by using the developed chip. Radiochemical yield was measured by radio HPLC and gamma counter.

**Results:** First, as we investigated each reaction condition, radiochemical yield did not decrease under low water condition in 3$^{rd}$ step reaction (3 → [$^{18}$F]SFB); therefore, we discovered that dewatering operation can be skipped in this synthesis after the 3 synthesis. Also, we recognized that evaporation of solvent can be prevented and reaction for 2$^{nd}$ step would progress under a milder condition by changing the solvent from MeCN to DMSO. Thus, we chose DMSO as the solvent. Next, we compared the yield at each reaction time between microreactor using the chip for 1-step-reaction and macro scale. As a result, both the yield constant and the reaction time improved considerably for microreactor (15 min, 67% → 5 min, 81%) compared to macro scale for the reaction in 1$^{st}$ step (1 → 2). Also, the reaction of microreactor and macro scale progressed quantitatively in a short period of time (< 30 sec) in 2$^{nd}$ step (2 → 3). We designed a microreactor for 3-step-reaction that performs reaction continuously on one chip at the optimal reaction time for each step (5 min, 30 sec, 1min). As a result of synthesizing [$^{18}$F]SFB with this chip, synthesis of [$^{18}$F]SFB was completed with reaction time 6.5 min and radiochemical yield 62% (n = 6), substantially improving the yield rate and reaction time compared to the original synthesis method (reaction time 18 min, radiochemical yield 44%).

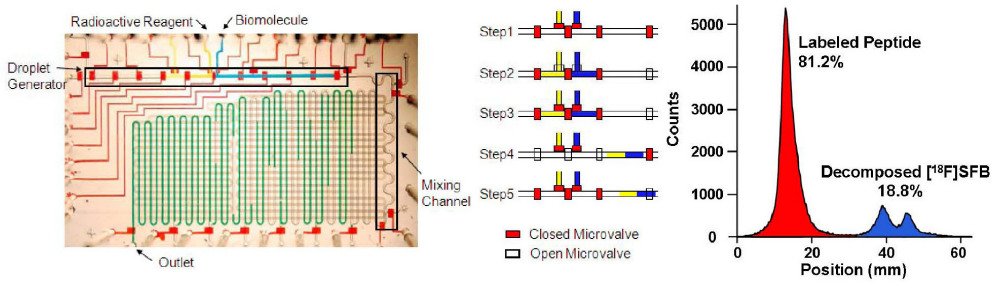
**Conclusions:** From the above results, we have succeeded in One-flow synthesis of [$^{18}$F]SFB with a higher yield rate and a shorter time period compared to the original method by using the microreactor for 3-step reaction designed and developed in this study.

# MICROFLUIDIC DROPLET MIXER FOR FLUORINE-18 LABELING OF BIOMOLECULES

**S. OLMA, K. LIU, Y. CHEN, R. VAN DAM and C. K. SHEN***
UCLA, Crump Institute for Molecular Imaging, Los Angeles, CA

**Objectives:** Fluorine-18-labeled biomolecules such as peptides and proteins have emerged as an important tool for targeted, in vivo molecular imaging. The high sensitivity of labeling reactions to pH, concentrations, and temperature, and the inherent batch-to-batch variability of the radiolabeling tag and biomolecule make it difficult to consistently achieve high radiolabeling yield. With conventional bench-scale methods, it is impractical to determine the optimal reaction conditions for a particular sample by screening, due to the large amount of biomolecule consumed for each test. A new platform that can significantly reduce reagent consumption and enable real-time optimization of labeling conditions is urgently needed. We have developed a novel microfluidic device that can automatically manipulate and mix reagents into small-volume (sub-microliter) droplets. Labeling conditions can be varied from droplet to droplet at the analytical scale and the optimal parameters can be obtained. The same chip can then perform a larger scale labeling reaction for production of $^{18}$F-labeled PET probes.

**Methods:** Short peptide sequences and diabodies specific for oncological targets were labeled with N-succinimidyl-4-[$^{18}$F] fluorobenzoate ([$^{18}$F]SFB) using a microfluidic chip. The chip was fabricated from PDMS by the multilayer soft lithography process. The valves were integrated into the chip to manipulate and mix the reagents. [$^{18}$F]SFB (in PBS buffer solution at pH 7.4) and biomolecule (in sodium borate buffer at pH 8.5) were loaded into the chip via separate inlets. They were measured into adjacent chambers. After the dividing valve is opened, two liquids merge into a droplet that is then rapidly mixed during flow through a reaction channel. This sequence is repeated, and the droplets can be collected separately in tubing (for analysis) or as a batch.




**Results:** The labeling of several peptides sequences and different diabodies using [$^{18}$F]SFB was accomplished in the microfluidic device. RCYs were analyzed by radio-TLC and -HPLC and were better or comparable to macroscopic experiments. However, the volume of batches for biological evaluation of the resulting radiotracer could be minimized. A few mL of reaction mixture can be produced and purified to give sufficient amounts of radioactivity for in vitro experiments. The second key feature of the device was the ability to instantaneously adjust the ratio of [$^{18}$F]SFB to biomolecule solutions by adjustment of microfluidic chamber sizes using valves. For each batch of [$^{18}$F]SFB and biomolecule, we performed several analytical-scale reactions with reagent ratio adjusted on the fly, and after radio-TLC analysis, performed preparative-scale labeling at the optimal conditions. Data from in vitro cell binding studies and preclinical in vivo imaging will be presented.

**Conclusions:** The feasibility of a novel microfluidic chip for $^{18}$F-labeling of biomolecules was demonstrated. Reaction conditions could be screened by sequential formation of droplets (analytical-scale "batches") with different composition. Batches of products could be scaled from a single droplet to mouse-dose scale to match the demand of radioactivity for radiotracer evaluation.

## MINI-FLUIDIC CHIP FOR THE TOTAL SYNTHESIS OF PET TRACERS

**S. VOCCIA[1], J. MORELLE[*1], J. AERTS[2], C. LEMAIRE[2], A. LUXEN[2] and G. PHILLIPART[1]**
1. Trasis SA, Liege, Belgium; 2. Universite de Liege, Centre de recherches du cyclotron, Liege, Belgium

**Objectives:** PET radiopharmaceuticals synthesis involves nano-molar quantities of active ingredients, which make the current radio-synthetic methods and device mostly inadequate to produce them in optimal conditions. The availability of instruments scaled down to dimensions closer to these quantities will be a major breakthrough. The phase transfer of the [18F]-fluorides from target water to a dry organic solution remains the most challenging step for the implementation of these radio-syntheses onto miniaturized devices.

**Methods:** A mini-fluidic chip has been designed for multiple steps radiochemical synthesis of [18F]-2-fluorodeoxyglucose (FDG), including [18F]-fluorides extraction and transfer to dry acetonitrile (ACN), labelling, hydrolysis, final purifications and formulation. The dimensions of the mini-fluidic chip are 90mmX80mm with 5mm thickness which makes the actuation set-up (synthesizer) very compact. It integrates and combines fluidic functions, i.e. valves, pumps, solid phase extraction (SPE) and built-in reagents, with new and specific methods for the phase transfer of the [18F] fluorides from target water to a dry organic solution without the time consuming azeotropic evaporation step. Reagents and SPE phases were loaded in the mini-fluidic chip prior to the synthesis while the solvents (ACN, water, buffer) are stored in vials outside the chip. FDG has been synthesized along this method on a single use miniaturized instruments.

**Results:** The phase transfer of [18F] fluorides, as produced from the irradiation of [18O]-enriched water, from target water (3ml) to ACN has been performed with reproducible yield of 95+% within 2 minutes without evaporation. The labelling, the hydrolysis and the final purifications have all been integrated on a single mini-fluidic chip. The total synthesis time, from cyclotron to final injectable solution is below 15 minutes. The radiochemical yield and the radiochemical purity are very promissing.

**Conclusions:** The mini-fluidic chip allows the complete implementation nuceophilic substitution based radiopharmaceutical synthesis process with high radiochemical yield. This new set of consumables, appropriate for cost effective mass production, will allow a facility to produce several tracers on any given day using the same synthesizer, thereby increasing productivity while reducing tracer production cost.

**Research Support**: Research was supported by a grant from the European Union, STRP 516984

## MODIFIED NON-IONIC SOLID SUPPORTS: A WAY TO HIGH ACTIVITY FLUORINE-18 RADIOCHEMISTRY IN MICROFLUIDIC DEVICES

**J. AERTS*, S. VOCCIA, C. LEMAIRE, F. GIACOMELLI, D. GOBLET, D. THONON, A. PLENEVAUX and A. LUXEN**
Liege University, Cyclotron Research Center, Liege, Belgium

**Objectives:** The most used procedure to recover [$^{18}$F]fluoride before nucleophilic substitution of an organic precursor consists in its evaporation in presence of potassium carbonate and kryptofix K222[1] after concentration on an anion exchange support. Although the concept of using integrated microfluidic devices was demonstrated for [$^{18}$F]FDG synthesis[2], the set-up of evaporation of a large amount of aqueous solvent is not feasible in small closed channels. Quaternary ammoniums on solid support were used to concentrate [$^{18}$F]fluoride before evaporation[3]. In this work, we present the use of a long alkyl chain quaternary ammonium adsorbed on a polymeric support, in order to trap [$^{18}$F]F from a large volume of water, to recover it in a small volume of non protic solvent and to perform [$^{18}$F]radiofluorination without evaporation.

**Methods:** N,N,N-trimethyltetradecan-1-ammonium bicarbonate was adsorbed on N-vinyl lactame/divinylbenzene copolymer sorbent, i.e. Waters Oasis™ HLB. The resulting solid was introduced in a solid phase extraction cartridge between two frits. [$^{18}$F]F water solution was passed through the solid. The cartridge was then purged with a small volume of hexane and/or a nitrogen flow. Acetonitrile (ACN) or dimethylsulfoxide (DMSO) was used to elute the radioactivity from the cartridge. Aliquots of the solution were added to solutions of different convenient precursors in the same solvent. The mixture was heated at convenient temperature to obtain the [$^{18}$F]F incorporation. Karl Fischer titrations were performed to evaluate the water content at different stages of the procedure.

**Results:** Quantitative [$^{18}$F]F trapping from more than 5 ml of water was obtained with about 100 mg of solid support. After purging, the eluted percentage in 1 ml of solvent was higher than 85% in ACN or 60% in DMSO. The recovery process took about 10 minutes manually. The water content in the eluted solutions was less than 7000 ppm. Elutions in ACN were used for the labeling of different triflated or tosylated aliphatic precursors at 100°C, while those in DMSO were used for nitro- or trimethylammonium-aromatic precursors at higher temperatures. The yields of [$^{18}$F]F incorporation, corrected for the losses on the reactor surfaces, were above 55% in all cases.

**Conclusions:** Non-ionic polymeric solid supports modified with a long alkyl chain quaternary ammonium allow the rapid and efficient recovery of [$^{18}$F]F from [$^{18}$O]water to a low water content organic solution compatible with the nucleophilic labeling of most precursors for PET radiopharmaceuticals without the use of an evaporation step.

**Research Support:** This research was supported by a grant from European Union's FP6 STRP 516984 MI-Lab-On-Chip. A. Plenevaux is a research associate from FRS-F.N.R.S. Belgium.

**References:** [1] K. Hamacher, H. Coenen, G. Stcklin. J. Nucl. Med.27 (1986) 235-238. [2] C. Lee, G. Sui, A. Elizarov, C. Shu, Y. Shin, A. Dooley, J. Huang, A. Daridon, P. Wyatt, D. Stout, H. Kolb, O. Witte, N. Satyamurthy, J. Heath, M. Phelps, S. Quake, H. Tseng. Science 310 (2005) 1793-1796. [3] D. Jewett, S. Toorongian, G. Mulholland, G. Watkins, M. Kilbourn, Appl. Radiat. Isot. 11 (1988) 1109-1111.

# NOVEL MICROREACTOR TECHNOLOGIES FOR 11C RADIOLABELLING

**P. MILLER*[1], N. LONG[1], R. VILAR[1], A. DE MELLO[1], A. GEE[1], D. BENDER[2], H. AUDRAIN[2], L. GOLONKA[3] and K. MALECHA[3]**

1. Imperial College London, Department of Chemistry, London, United Kingdom; 2. PET Imaging Division, Aarhus University Hospital, Aarhus, Denmark; 3. Wroclaw University of Technology, Faculty of Microsystem Electronics and Photonics, Wroclaw, Poland

**Objectives:** Microfluidic technologies continue to attract significant interest for performing chemical reactions because of associated improvements in reaction rates and yields, however, they are only begining to be exploited for radiolabelling. Our objectives are: 1. To assess new glass and ceramic microfluidic devices (MFDs) and packed tube reactors (PTRs) for Pd mediated $^{11}C$ radiolabelling reactions. 2. To develop a microfluidic reactor system for screening catalysts over a range of reaction conditions. 3. To identify and select the best catalysts for Pd mediated $^{11}C$ reactions.

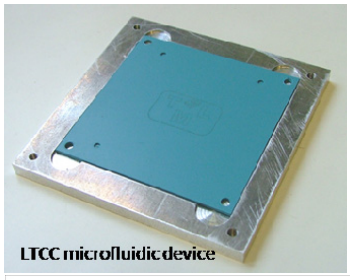
**Methods:** Glass MFDs were prepared by wet etching and ceramic devices using low temperature co-fired ceramic (LTCC) technology. PTRs were prepared by packing a silica supported catalyst into commercially available polymeric tubing. We have applied these devices to a range of chemical reactions including Pd catalysed carbonylation and methylation reactions which are relevant for $^{11}C$ labelling. We have focused on the Pd catalysed carbonylation reaction of arylhalides with primary amines to give amides and investigated a range of Pd catalysts for enhancing this reaction.

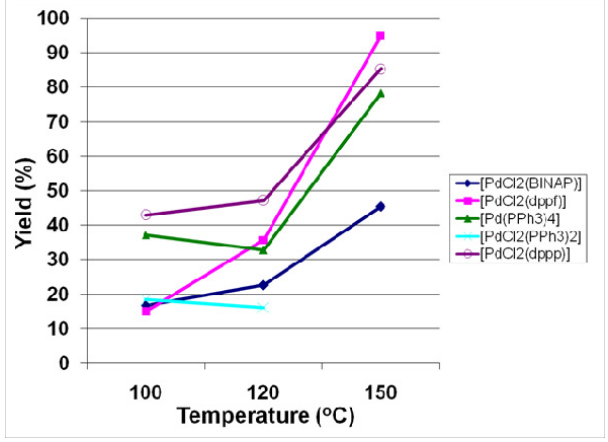
**Results:** Glass and ceramic MFDs significantly enhanced Pd catalysed carbonylation reactions compared to corresponding batch reactions. We have also found this reaction to be highly dependent on the catalyst and reaction temperature. Catalyst screening results over a range of temperatures showed [$PdCl_2$(dppf)] to be the most effective catalyst giving highest yields at 150°C (see supporting data) within 2 min. Results of translating this optimised microfluidic system to $^{11}CO$ labelling will be presented. PTRs provide a simple and cost effective alternative to fabricated microreactors. We have developed a silica supported Pd catalyst PTR that has proven convenient for performing $^{11}CO$ labelling reactions of amides at low pressures. After initial trapping and release of $^{11}CO$, coupling reactions of a range of aryl halides with benzyl amine to produce $^{11}C$ amides gave good radiochemial yields (45-79%) and purities (70-96%) within a short time frame (10 min).

**Conclusions:** Fabricated MFDs and PTRs are proving to be practical technologies for enhancing chemical and radiolabelling reactions. We have obtained high yields of amides within short reaction times via Pd catalysed carbonylation reactions using fabricated devices and are currently applying these optimised systems to $^{11}C$ labelling. Additionally, we are currently expanding the scope of our PTR-$^{11}CO$ based labelling reactions.

**Research Support:** P. W. Miller is grateful to the EPSRC for a Life Sciences Interface Fellowship.

**References:** [1] P. W. Miller, Radiolabelling with short-lived PET (Positron Emission Tomography) isotopes using microfluidic reactors, Journal of Chemical Technology and Biotechnology, in press, 2009. [2] P. W. Miller, N. J. Long, R. Vilar and A. Gee, Synthetic radiolabelling strategies for radiotracers in Positron Emission Tomography (PET) imaging, Angew., Chem., Int. Ed.,8998-9033, 2008. [3] P. W. Miller, N. J. Long, A. J. de Mello, R. Vilar, H. Audrain, D. Bender, J. Passchier and A. Gee, Rapid multiphase carbonylation reactions by using a microtube reactor: Applications in positron emission tomography C-11-radiolabeling, Angew. Chem., Int. Ed., 2007, 46, 2875-2878. [4] P. W. Miller, N. J. Long, A. J. de Mello, R. Vilar, J. Passchier and A. Gee, Rapid formation of amides via carbonylative coupling reactions using a microfluidic device, Chem. Commun., 2006, 546-548.



# KINETICS AND ENERGETICS OF THE RADIOFLUORINATION OF DIARYLIODONIUM CHLORIDES STUDIED WITH A MICROFLUIDIC REACTOR

**J. CHUN*, S. LU and V. W. PIKE**
National Institutes of Health, National Institute of Mental Health, Molecular Imaging Branch, Bethesda, MD

**Objectives:** The kinetics and energetics of radiofluorination reactions have seldom been studied in detail. We were interested in the kinetics and energetics of the radiofluorination of diaryliodonium salts [Pike and Aigbirhio, J. Chem. Soc., Chem. Commun., 1995, 2215.] in order to gain greater insight into the mechanisms and outcomes of these reactions, which are increasingly useful for radiotracer synthesis [Cai et al., Eur. J. Org. Chem., 2008, 17, 2853]. For this purpose we decided to use a microfluidic apparatus as a reaction platform since it offers the possibility to control reaction conditions closely and allows rapid sequential reactions [Lu et al., Curr. Radiopharm., 2009, 2, 49].

**Methods:** Cyclotron-produced [$^{18}$F]fluoride ion (100–200 mCi) in [$^{18}$O]$H_2O$ with a solution of $K_2CO_3$ and Kryptofix 2.2.2 in MeCN-$H_2O$ (9: 1 v/v; 150 $\mu$L) was dried in a 1-mL V-vial by four consecutive cycles of MeCN addition-evaporation. Radiofluorinations were performed with a 10-fold molar ratio of diaryliodonium salt to carrier KF and K 2.2.2. in DMF containing 0.25 % (v/v) water. Reaction (residence times) times were varied in the range 10–400 s by setting the flow rates at which the [$^{18}$F]KF-K 2.2.2 solution and the diaryliodonium chloride solution (10 mM) were simultaneously infused into the micro-reactor from two separate reservoirs of the apparatus (Advion). Flow rates were set to be equal in the range, 5–100 $\mu$L/min. Reactions were run for at least six different temperatures (T). Reactor outputs were immediately quenched by dilution with MeCN-$H_2O$ (1: 1 v/v) at room temperature and analyzed with reverse phase radio-HPLC to determine decay-corrected radiochemical yield (RCY). For each of three different diaryliodonium chlorides, the estimated initial reaction rates (k; % radiochemical yield of product per second) (e.g., Figure, panel A) were plotted as lnk versus 1/T to estimate the Arrhenius activation energy ($E_a$) as negative slope of plot × R (e.g., Figure, panel B).

**Results:** Each symmetrical diaryliodonium chloride ($Ar_2I^+Cl^-$) gave a single radioactive product ($Ar^{18}F$) in moderate (Ar = 2-$MeOC_6H_4$) or high (Ar = Ph or 2-$MeC_6H_4$) maximal RCY (51–85%). Arrhenius plots were adequately linear for $E_a$ estimation. The estimated values were 21–28 kcal/mol.

**Conclusions:** The microfluidic device proved to be a convenient and effective platform for the study of the kinetics and energetics of the radiofluorination reactions. The ability to perform several reactions sequentially in this device allowed conditions for establishing initial rates and also for achieving maximal RCYs to be established rapidly. Reactions were consistent and reproducible, even in the absence of a radical scavenger such as TEMPO [Carroll et al., J. Fluorine Chem., 2007, 128, 127]. The determined $E_a$ values are now available for comparison with ongoing theoretical predictions from mechanistic models.

**Research Support:** Intramural Research Program of NIH, NIMH

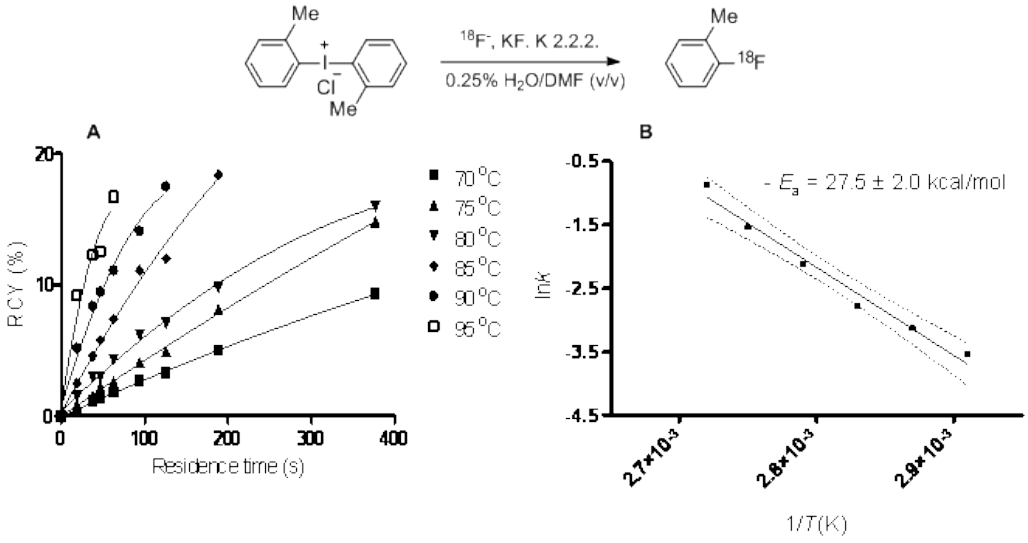



**Figure** **Panel A**: Kinetics of the radiofluorination of (*o*-MePh)$_2$I$^+$Cl$^-$ in DMF-$H_2O$ (99.75: 0.25, v/v) to give [$^{18}$F]2-fluorotoluene at different temperatures. **Panel B**. Arrhenius plot of the kinetic data in Panel A from which the $E_a$ value was derived.