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1   **TITLE**:
2   Optimization of Radiochemical Reactions Using Droplet Arrays
3
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6
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13 **KEYWORDS:**
14 high-throughput, radiochemistry, synthesis optimization, microfluidics, nanomole chemistry,
15 green chemistry
16
17 **SUMMARY:**
18 This method describes the use of a novel high-throughput methodology, based on droplet
19 chemical reactions, for the rapid and economical optimization of radiopharmaceuticals using
20 nanomole amounts of reagents.
21
22 **ABSTRACT:**
23 Current automated radiosynthesizers are designed to produce large clinical batches of
24 radiopharmaceuticals. They are not well suited for reaction optimization or novel
25 radiopharmaceutical development since each data point involves significant reagent
26 consumption, and contamination of the apparatus requires time for radioactive decay before the
27 next use. To address these limitations, a platform for performing arrays of miniature droplet-
28 based reactions in parallel, each confined within a surface-tension trap on a patterned
29 polytetrafluoroethylene-coated silicon "chip", was developed. These chips enable rapid and
30 convenient studies of reaction parameters including reagent concentrations, reaction solvent,
31 reaction temperature and time. This platform permits the completion of hundreds of reactions
32 in a few days with minimal reagent consumption, instead of taking months using a conventional
33 radiosynthesizer.
34
35 **INTRODUCTION:**
36 Positron-emission tomography (PET) radiopharmaceuticals are widely used as research tools to
37 monitor specific in vivo biochemical processes and study diseases, and for the development of
38 new drugs and therapies. Moreover, PET is a critical tool for diagnosing or staging disease and
39 monitoring a patient's response to therapy[1–3]. Due to the short half-life of PET radioisotopes (e.g.,
40 110 min for fluorine-18-labeled radiopharmaceuticals) and radiation hazard, these compounds
41 are prepared using specialized automated systems operating behind radiation shielding and must
42 be prepared just before use.
43
44 Current systems used to synthesize radiopharmaceuticals are designed to produce large batches

45　that are divided up into many individual doses to share the production cost. While current
46　systems are suitable for the production of widely used radiotracers like [^{18}F]FDG (because
47　multiple patient scans and research experiments can be scheduled in a single day), these systems
48　can be wasteful for the production of novel radiotracers during early-stage development, or less
49　commonly used radiotracers. Volumes that conventional systems use are typically in the 1-5 mL
50　range, and the reactions require precursor amounts in the 1-10 mg range. Furthermore, using
51　conventional radiosynthesizers is generally cumbersome during optimization studies since the
52　apparatus becomes contaminated after use and the user must wait for radioactivity to decay
53　before performing the next experiment. Aside from equipment cost, the cost of the radioisotope
54　and reagents can, therefore, become very substantial for studies requiring production of multiple
55　batches. This can occur, for example, during the optimization of synthesis protocols for novel
56　radiotracers to achieve sufficient yield and reliability for initial in vivo imaging studies.
57
58　Microfluidic technologies have been increasingly used in radiochemistry to capitalize on several
59　advantages over conventional systems[4–6]. Microfluidic platforms, including those based on 1-10
60　µL reaction volumes[7–9], have shown a significant reduction of reagent volumes and consumption
61　of expensive precursors, as well as short reaction times. These reductions lead to lower costs,
62　faster heating and evaporation steps, shorter and more straightforward downstream
63　purification, an overall "greener" chemistry process[10], and higher molar activity of the produced
64　radiotracers[11]. These improvements make it more practical to perform more detailed
65　optimization studies by lowering the reagent cost of each synthesis. Further benefits can be
66　achieved by performing multiple experiments from a single batch of radioisotope in a single day.
67　For example, microfluidic flow chemistry radiosynthesizers operating in "discovery mode" can
68　sequentially perform dozens of reactions, each using only 10s of µL reaction volume[12].
69
70　Inspired by these advantages, a multi-reaction droplet array chip in which microvolume reactions
71　are confined to an array of surface-tension traps on a silicon surface, created using a patterned
72　Teflon coating, was developed. These chips enable multiple reactions at the 1-20 µL scale to be
73　performed simultaneously, opening the possibility to explore 10s of different reaction conditions
74　per day, each with multiple replicates. In this paper, the utility of this new high-throughput
75　approach for performing rapid and low-cost radiochemistry optimizations is demonstrated. Using
76　multi-reaction droplet chips allows for convenient exploration of the impact of reagent
77　concentrations and reaction solvent, and the use of multiple chips could enable the study of
78　reaction temperature and time, all while consuming very low amounts of precursor.
79
80　**PROTOCOL:**
81
82　CAUTION: This protocol involves the handling of radioactive materials. Experiments should not
83　be undertaken without the necessary training and personal protective equipment and approval
84　from the radiation safety office at your organization. Experiments should be performed behind
85　radiation shielding, preferably in a ventilated hot cell
86
87　**1. Fabrication of multi-reaction chips**
88

89   NOTE: Batches of multi-reaction microdroplet chips are fabricated from 4" silicon wafers using
90   standard photolithography techniques, as previously decribed[10] (**Figure 1**). This procedure will
91   produce 7 chips each with 4 x 4 array of reaction sites.
92
93   1.1.   Place silicon wafer on the spin-coater chuck, ensuring that it is centered. Deposit 3 mL of
94   polytetrafluoroethylene solution at the center of the wafer with a transfer pipette and coat wafer
95   at a 1000 rpm for 30 s (500 rpm/s ramp).
96
97   1.2.   To solidify the coating, place the wafer on a 160 °C hotplate for 10 min and then transfer
98   to a 245 °C hotplate for 10 min.
99
100 1.3.   Anneal the coating in a high-temperature oven at 340 °C for 3.5 h under nitrogen
101 atmosphere, followed by cooling to 70 °C at a 10 °C/min ramp.
102
103 1.4.   Place the silicon wafer on the spin-coater chuck, ensuring that it is centered. Pour 2 mL
104 of positive photoresist at the center of the wafer using a transfer pipette, and then perform
105 coating at 3000 rpm for 30 s (1000 rpm/s ramp).
106
107 1.5.   Solidify the photoresist by performing a soft bake of the wafer on a 115 °C hotplate for 3
108 min.
109
110 1.6.   Install the wafer and photomask in a mask aligner and perform a 14 s exposure at 12
111 mW/cm^2 lamp intensity and 356 nm wavelength in hard contact mode. This step uses a
112 transparency mask containing the negative final polytetrafluoroethylene pattern, i.e., a 4"
113 diameter pattern of 4 copies of the 16-reaction chip, with reaction sites transparent and all other
114 regions in opaque color.
115
116 1.7.   Submerge the wafer using 20 mL of photoresist developer solution in a glass container for
117 3 min with slight agitation to develop the exposed pattern.
118
119 1.8.   Rinse away the developing solution by submerging the wafer in a glass container with 20
120 mL of DI water for 3 min with slight agitation. Dry the wafer with a nitrogen gun.
121
122 1.9.   Remove the exposed polytetrafluoroethylene regions via reactive-ion etching (RIE) with
123 oxygen plasma under the following conditions: 30 s exposure, 100 mTorr pressure, 200 W power,
124 and 50 sccm oxygen flow.
125
126 1.10.   Dice the wafer into individual chips (7 total per wafer) using a silicon wafer cutter.
127
128 1.11.   Submerge each chip in acetone for 1 min to remove the photoresist, then isopropanol for
129 1 min. Finally, dry each chip with a nitrogen gun.
130
131 1.12.   Place dry chips in a glass container and cover with aluminum foil for storage until use.
132

2. Planning of the optimization study

NOTE: <mark>In this protocol, synthesis of the radiopharmaceutical [^{18}F]fallypride is used as an example to illustrate high-throughput optimization (**Figure 2**). With a single chip, 16 simultaneous reactions can be performed, for example, with varied precursor concentration (8 different concentrations, n=2 replicates each). The conditions are mapped to reaction sites in **Figure 3A**.</mark> Adjustments can be made to this protocol to optimize other reaction parameters (e.g. reaction solvent, reaction volume, amount of TBAHCO$_3$, etc.) or other radiopharmaceuticals.

2.1.   Select the reaction parameter(s) to be varied, the specific values to be used, and the number of replicates.

2.2.   Compute the number of chips needed to perform the experiment.

2.3.   For each chip, prepare a map of which reaction conditions will be used at each reaction site to assist with reagent preparation and performing the droplet reactions.

3.   <mark>**Preparation of reagents and materials for optimizing the radiosynthesis of [^{18}F]fallypride**</mark>

NOTE: The droplet-based radiosynthesis of [^{18}F]fallypride (**Figure 2**) begins with the addition of [^{18}F]fluoride and phase transfer catalyst (TBAHCO$_3$) to the reaction site, followed by heating to evaporate water and leave a dried residue. Next, a droplet of precursor (tosyl-fallypride) in reaction solvent (thexyl alcohol and acetonitrile) is added and heated to perform the radiofluorination reaction. Finally, the crude product is collected from the chip for analysis. The reagent preparation and synthesis procedures should be adapted if performing optimization of a different tracer.

3.1.   <mark>Prepare a 30 µL stock solution of precursor (tosyl-fallypride) in the reaction solvent with the maximum concentration to be explored (77 mM).</mark> Ensure that the volume is enough to perform the planned experiment. In this example optimization, ~30 µL is sufficient.

3.2.   <mark>Prepare a stock solution of the reaction solvent, consisting of thexyl alcohol and acetonitrile in a 1:1 by volume mixture. Ensure that the volume is enough to create the planned dilution series.</mark> In this example optimization, ~30 µL is sufficient.

3.3.   <mark>From the precursor stock solution and reaction solvent, perform 2x serial dilutions to prepare the different concentrations of the precursor solution.</mark> Ensure that the volume of each dilution is enough to perform the desired number of replicates for each condition. In this example optimization, ~15 µL of each concentration is sufficient.

3.4.   <mark>Prepare microcentrifuge tubes to collect each crude reaction product using a permanent marker to label each tube with a unique number. Ensure that the total number of microcentrifuge tubes matches the number of conditions multiplied by the number of replicates (8 x 2 = 16).</mark>

177

3.5.   Prepare a stock of collection solution (10 mL) comprising 9:1 methanol:DI water (v/v). Aliquot 50 µL into each of 16 additional labeled microcentrifuge tubes (one per reaction site on the chip).

3.6.   Prepare a [^{18}F]fluoride stock solution in a 500 µL microcentrifuge tube by mixing [^{18}F]fluoride/[^{18}O]H$_2$O (~260 MBq [7 mCi]) with 75 mM TBAHCO$_3$ solution (56 µL) and diluting with DI water up to 140 µL. 8 µL of this solution will be loaded to each reaction site (containing ~15 MBq [0.40 mCi] of activity, and 240 nmol of TBAHCO$_3$).

4.   **Parallel synthesis of [^{18}F]fallypride with different precursor concentrations**

NOTE: The chip is operated atop a heating platform (constructed as previously described[13]) consisting of a 25 mm x 25 mm ceramic heater, controlled using an on-off temperature controller using the internal thermocouple signal for feedback. Heater surface temperatures were calibrated using thermal imaging. If such a platform is not available, a pair of hot plates can be used (one at 105 °C and one at 110 °C).

4.1.   Load [^{18}F]fluoride stock solution (with phase transfer catalyst).

4.1.1. Using a micropipette, load an 8 µL droplet of [^{18}F]fluoride stock solution on the first reaction spot of a multi-reaction chip. Measure the activity of the chip by placing it in a dose calibrator and record the time at which measurement is conducted.

4.2.   Remove the chip from dose calibrator and then load an 8 µL droplet of [^{18}F]fluoride stock solution on the second reaction spot. Measure the activity on the chip by placing it once again in the dose calibrator and record the time at which measurement is conducted.

4.3.   Repeat for all other reaction sites on the chip.

4.4.   Calculate the activity loaded per reaction spot by taking the activity measurement after loading the radioisotope and subtracting the previous measurement (decay-corrected) before that site was loaded.

4.2.   Align the multi-reaction chip on the heater.

4.2.1. Add a thin layer of thermal paste on top of the ceramic heater.

4.2.2. Carefully place the chip on top of the heater using tweezers to avoid the spill of the droplets, aligning the reference corner of the chip with the reference corner of the heater (as shown in **Figure 3B**). The chip will overhang the heater by a small amount.

4.3.   Dry the [^{18}F]fluoride and phase transfer catalyst.

221   4.3.1.   Heat the chip for 1 min by setting the heater to 105 °C in the control program to evaporate
222   the droplets to dryness leaving a dried residue of [^{18}F]fluoride and TBHACO$_3$. After 1 min, cool
223   the chip by setting the heater to 30 °C and turning on the cooling fan with the control program.

225   **4.4.**   Add the precursor solution.

227   4.4.1.   Using a micropipette, add a 6 µL solution of fallypride precursor on top of the dried
228   residue on the first reaction site.

230   4.4.2.   Repeat for all other reaction sites on the chip. Use the optimization plan to determine
231   which concentration of the dilution series is used for each reaction site.

233   **4.5.**   Perform fluorination reaction.

235   4.5.1.   Heat each chip to 110 °C for 7 min using the control program to perform radiofluorination
236   reaction. Afterwards, cool the chip by setting the heater to 30 °C and turning on cooling fan with
237   the control program.

239   **4.6.**   Collect the crude products from the reaction sites.

241   4.6.1.   Collect the crude product at the first reaction site by adding 10 µL of collection solution
242   from the designated microcentrifuge tube via micropipette. After waiting for 5 s, use the
243   micropipette (with the same tip installed) to aspirate the diluted crude product and transfer to
244   its corresponding labeled collection microcentrifuge tube.

246   4.6.2.   Repeat this process a total of 4 times using the same pipette tip for all operations.

248   4.6.3.   Repeat the collection process for all other reaction sites on the chip.

250   **5.**   **Synthesis analysis to determine reaction performance and optimal conditions**

252   **5.1.**   Determine the "collection efficiency" for the first reaction on the chip.

254   5.1.1.   Place the microcentrifuge tube with the collected crude product of the first reaction spot
255   in the dose calibrator to measure the activity. Record the measurement and time of the
256   measurement. Repeat this process for each of the collected crude products.

258   5.1.2.   Calculate the collection efficiency by dividing the activity of the collected crude product
259   by the starting activity measured for the same reaction site (decay-correcting the activity values
260   to the same timepoint).

262   5.1.3.   Repeat for all other reaction sites on the chip.

265   5.2.   Analyze the composition (fluorination efficiency) of each collected crude product.

266

267   NOTE: To make practical the analysis of all samples in a short time, fluorination efficiency is
268   analyzed using a previously described high-throughput radio-thin layer chromatography (radio-
269   TLC) approach[14]. This technique allows up to eight samples to be processed in parallel by spotting
270   then side by side (5 mm pitch, 0.5 µL per spot) on a single TLC plate, then developing together,
271   and performing readout together using Cerenkov imaging[14, 15]. For the example optimization with
272   16 parallel reactions, 2 TLC plates are needed. Another option is to use radio- high-performance
273   liquid chromatography (radio-HPLC) for analysis, though the time for separation, cleaning, and
274   equilibration may limit the number of samples that can be analyzed.

275

276   5.2.1.   For each TLC plate (50 mm x 60 mm), with a pencil, draw a line at 15 mm away from one
277   50 mm edge (bottom), and another line 50 mm away from the same edge. The first line is the
278   origin line; the second is the solvent front line. Draw 8 small "X"s along the origin line at 5 mm
279   spacing to define the sample spotting position for each of 8 "lanes".

280

281   5.2.2.   Using a micropipette, transfer 0.5 µL of the first crude product onto the TLC plate at the
282   "X" for the first lane. Repeat for additional crude products (up to 8 per TLC plate). Wait for the
283   crude product spots to dry on the TLC plate.

284

285   5.2.3.   For each TLC plate, develop using a mobile phase of 60% MeCN in 25 mM NH_4HCO_2 with
286   1% TEA (v/v) until the solvent front reaches the solvent front line. Wait for the solvent on the TLC
287   plate to dry and then cover with a glass microscope slide (76.2 mm x 50.8 mm, 1 mm thick).

288

289   5.2.4.   Obtain a radioactivity image of each TLC plate by placing the plate in a Cerenkov imaging
290   system for a 5 min exposure. Perform standard image corrections (dark current subtraction, flat
291   field correction, median filtering, and background subtraction).

292

293   5.2.5.   Use region of interest (ROI) analysis for the first lane of the first TLC plate. Draw regions
294   around each band visible in the lane. The software will compute the fraction of integrated
295   intensity of each region (band) compared to the total integrated intensity of all regions (bands).

296

297   5.2.6.   With this mobile phase, the following bands are expected at the indicated retention
298   factors: Rf = 0.0: Unreacted [^{18}F]fluoride; Rf = 0.9: [^{18}F]fallypride; Rf = 0.94: Side product.
299   Determine the fluorination efficiency as the fraction of activity in the [^{18}F]fallypride band.

300

301   5.2.7.   Repeat this analysis for all other lanes on all TLC plates.

302

303   NOTE: If a Cerenkov imaging chamber is not available, a small animal (preclinical) *in vivo* optical
304   imaging system can be used to image the TLC plates. Alternatively, a 2-dimensional TLC scanner
305   can be used. Alternatively, if only a 1-dimensional TLC scanner is available, the TLC plates can be
306   analyzed by cutting into strips with scissors (1 per lane), and scanning each strip individually.

307

308   5.3.   Determine the crude radiochemical yield (crude RCY) for each reaction site.

309

5.3.2. Repeat for all other reaction sites.

5.4.2. Plot the collection efficiency, fluorination efficiency, and crude RCY as a function of the parameter that was varied (precursor concentration in this example).

5.4.3. Select the optimal conditions based on the desired criteria. Typically, this is the maximum crude RCY. Additionally, the point is often chosen in a region where the slope of the graph is relatively flat, indicating it is insensitive to small changes in the parameter, providing a more robust protocol.

REPRESENTATIVE RESULTS:
A representative experiment was performed to illustrate this method. Using 16 reactions, optimization studies of the radiopharmaceutical [18F]fallypride were performed by varying precursor concentration (77, 39, 19, 9.6, 4.8, 2.4, 1.2, and 0.6 mM) in thexyl alcohol:MeCN (1:1, v/v) as the reaction solvent. Reactions were performed at 110 °C for 7 min. Collection efficiency, sample composition (i.e., proportions of [18F]fallypride product, unreacted [18F]fluoride, and side product) are tabulated in **Table 1** and are summarized graphically in **Figure 4**.

The study showed that the fluorination efficiency (proportion of [18F]fallypride) increases with increasing precursor concentration, and that the remaining unreacted [18F]fluoride varied inversely (**Figure 4A**). There was a small amount of a radioactive side product at low precursor concentrations, but the proportion decreased to near zero at the higher precursor concentrations (**Figure 4A**). The collection efficiency was nearly quantitative for most conditions, though it dropped slightly at low precursor concentrations.

From these results, the highest RCY can be achieved with ~230 nmol of precursor (i.e., 39 mM concentration in a 6 μL droplet). At this condition, the fluorination efficiency was 96.0 ± 0.5% (n=2) and the crude RCY was 87.0 ± 2.7 (n=2), and there was no observed radioactive side product formation. While the use of 77 mM precursor showed similar results, in general it is desirable to use a lower amount of precursor to reduce cost and simplify downstream purification steps.

FIGURE AND TABLE LEGENDS:

Figure 1: Fabrication of multi-reaction microdroplet chips via photolithography. (**A**) Photograph of multi-reaction microdroplet chip with 4 x 4 array of reaction sites. The chip consists of polytetrafluoroethylene -coated silicon with circular regions of polytetrafluoroethylene etched

353 away to create the hydrophilic reaction sites. (**B**) Schematic of the fabrication procedure. A silicon
354 wafer is spin-coated with Teflon solution and baked to solidify the coating. Next, the photoresist
355 is spin-coated and patterned via photolithography to produce an etch mask. The photoresist is
356 developed with a photoresist developing solution. The exposed Teflon is then removed via dry
357 etching with oxygen plasma. The wafer is diced into individual chips, and the photoresist is
358 stripped.

360 **Figure 2: Procedure for parallel reactions.** Experimental procedure for performing 16 parallel
361 syntheses of the radiopharmaceutical [^{18}F]fallypride on a multi-reaction chip. In this example, the
362 precursor concentration is varied for each reaction.

364 **Figure 3: Map of conditions at reaction sites.** (**A**) Experimental design to explore the influence
365 of precursor concentration on the radiofluorination of tosyl fallypride using a single 16-reaction
366 chip (top view). Eight different concentrations were explored, each with n=2 replicates. Other
367 reaction conditions were held constant (temperature: 110 °C; time: 7 min; solvent: thexyl
368 alcohol:MeCN; the amount of TBAHCO$_3$: 240 nmol). Each reaction was performed with ~14 MBq
369 of activity. (**B**) Photograph of a 16-reaction chip installed on the heater platform during the
370 experiment. Red lines represent the reference corner of the chip used for alignment with the
371 reference corner of the heater.

373 **Figure 4: Influence of precursor concentration on the microdroplet synthesis of [^{18}F]fallypride.**
374 (**A**) Proportion of radioactive species present in the collected crude reaction product, i.e.
375 [^{18}F]fallypride, side product, or unreacted [^{18}F]fluoride. (**B**) Synthesis performance. Collection
376 efficiency, fluorination efficiency, and crude RCY are plotted as a function of precursor
377 concentration. In both graphs, data points represent the average of n=2 replicates, and error bars
378 represent the standard deviation.

380 **Table 1: Data obtained from study of precursor concentration.** All values are averages ±
381 standard deviations computed from n=2 replicates.

383 **DISCUSSION:**
384 Due to limitations of conventional radiochemistry systems that allow only one or a small number
385 of reactions per day and consume a significant quantity of reagents per data point, only a tiny
386 portion of the overall reaction parameter space can be explored in practice, and many times
387 results are reported with no repeats (n=1). Compared to conventional systems, this multi-
388 reaction droplet radiosynthesis platform makes it practical to accomplish more comprehensive
389 and rigorous studies of radiosynthesis conditions while consuming very little time and amount of
390 precursor, potentially enabling new insights on parameters that impact product yield and side-
391 product formation. The information can be used to choose the conditions that result in the
392 highest product yield or the most robust synthesis. The low precursor consumption may be
393 especially useful in the early development of novel radiotracers when only a small amount of
394 precursor may be available or when the precursor is expensive. While the open nature of the
395 chips contributes to rapid synthesis time and ease of access via pipette, it can lead to substantial
396 losses of volatile molecules and may not be practical when optimizing the synthesis of

397 radiopharmaceuticals that have volatile precursors, intermediates, or products.
398
399 Due to the hazard of radiation exposure, it should be reiterated that these experiments should
400 be performed only with suitable training and approvals and should be conducted behind
401 radiation shielding, preferably in a ventilated hot cell. Due to the short half-life of the
402 radioisotopes, it is important to perform the experiments quickly and efficiently. Pipetting
403 reagents to the chip and collecting products from the chip should be practiced under non-
404 radioactive conditions to become familiar with the reduced access and visibility in a hot cell.
405 Similarly, installing and removing the chip, and making measurements of the chip with the dose
406 calibrator should also be practiced. In addition, it is critical to be organized, with a detailed
407 experiment map (i.e., specific reaction conditions at each site on the chip). It is also helpful to
408 prepare in advance a table of results to be filled in as measurements are made. To ensure
409 reproducibility, especially with the possibility of human error, multiple replicates of each set of
410 conditions should be performed. It is important to be especially careful during the step of
411 collecting the crude samples from the chip to avoid spilling liquid outside the reaction site and
412 causing cross-contamination with adjacent reaction sites. If any errors are noticed, it is important
413 to flag these reaction sites so the data can be excluded from the eventual analysis.
414
415 In this example study, the amount precursor consumed for 16 data points was 1.1 mg (~70 µg
416 each), compared to 4 mg per data point using a conventional radiosynthesizer. Furthermore, all
417 16 reactions were completed in 25 min all in a single experiment. In comparison, the synthesis of
418 crude [18F]fallypride on a conventional radiosynthesizer requires ~15-20 min per reaction[16, 17].
419
420 This representative experiment demonstrated the utility of a multi-reaction microdroplet chip
421 with 16 reactions to optimize conditions for the radiosynthesis of the radiopharmaceutical
422 [18F]fallypride by exploring 8 different precursor concentrations (n=2 replicates for each
423 condition) in a fast and economical manner. Other variables that can be conveniently optimized
424 using a multi-reaction chip include the amount of radioactivity, type of phase transfer catalyst,
425 amount of phase transfer catalyst, evaporation/drying conditions (e.g., number of azeotropic
426 drying steps), reaction solvent, etc. By using multiple multi-reaction chips, it also is possible to
427 explore the influence of reaction temperature and reaction time, in addition to conditions such
428 as evaporation/drying temperature and time. Such studies would need to be performed
429 sequentially using the single heater or could be parallelized by operating multiple heaters at the
430 same time.
431
432 The underlying droplet synthesis method has been shown to be compatible with a wide range of
433 18F-labeled radiopharmaceuticals, such as[18F]fallypride[10], [18F]FET[18], [18F]FDOPA[19], [18F]FBB[20]
434 and it can be used for the optimization of the majority of other 18F-labeled compounds and
435 compounds labeled with other isotopes. Moreover, the resulting optimized droplet-based
436 reactions intrinsically leverage the advantages of microvolume radiochemistry, including reduced
437 precursor consumption, faster process times, and compact instrumentation, and can offer these
438 same advantages for routine production of large batches. Larger batches simply require scaling
439 up the amount of activity initially loaded at the start of the reaction. To prepare a tracer suitable
440 for use in in vitro or in vivo assays, the crude product must be purified (e.g., using analytical-scale

441 HPLC) and formulated (e.g. via evaporative or solid-phase solvent exchange[21]) Alternatively, it
442 may be possible to adapt the optimal conditions from droplet-scale to a conventional vial-based
443 radiosynthesizer. Investigation of this possibility is ongoing.
444

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497 microreactor: application   to   O-(2-[18F]fluoroethyl)-L-tyrosine   ([18F]FET).   *EJNMMI*
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500 radiopharmaceutical [18F]FDOPA using a microdroplet reactor. *Reaction Chemistry &*
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503 Florbetaben ([F-18] FBB) radiosynthesis to a microdroplet reactor. *Journal of Labelled*
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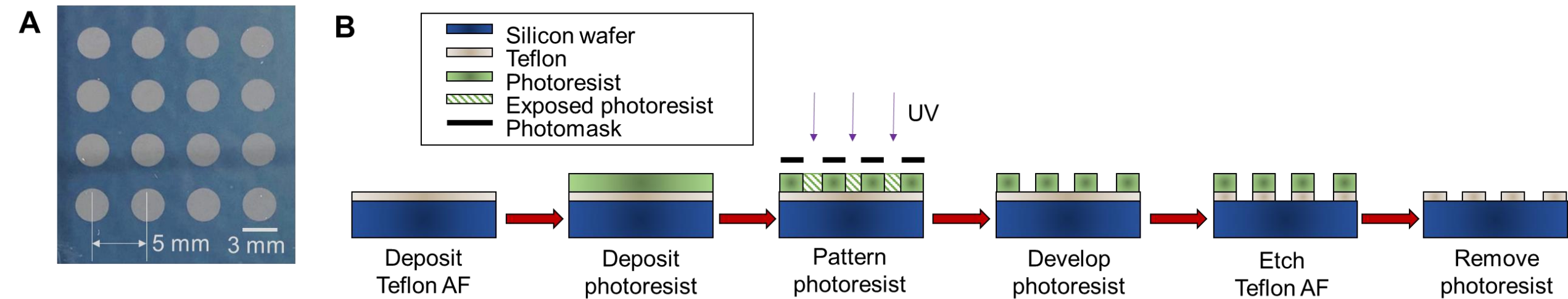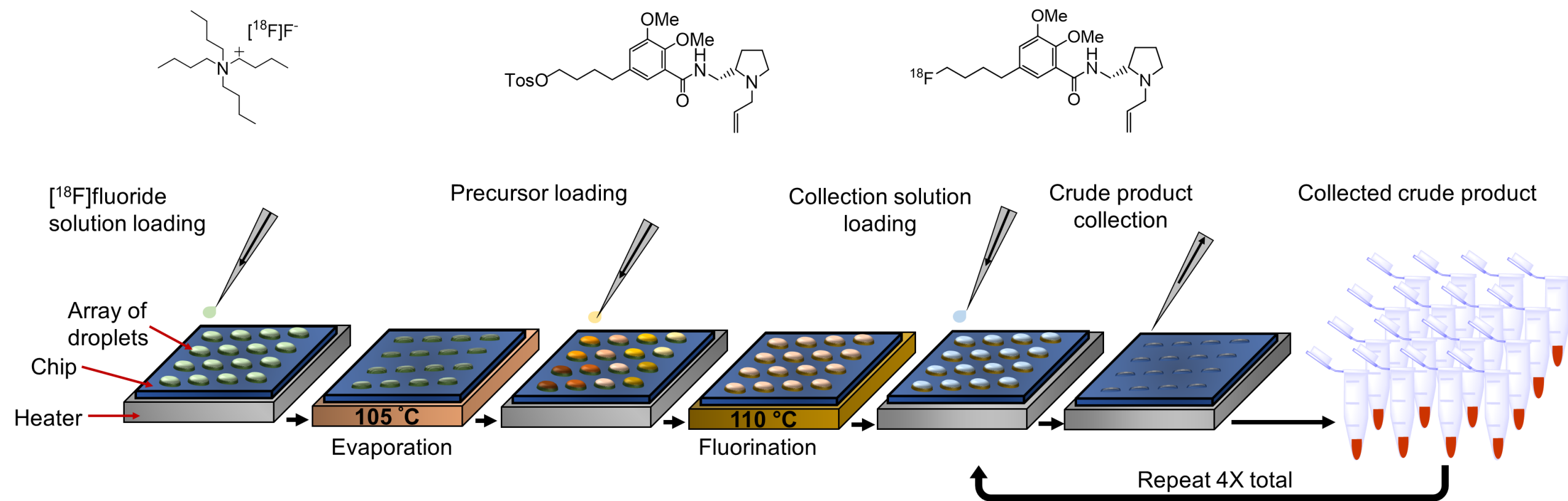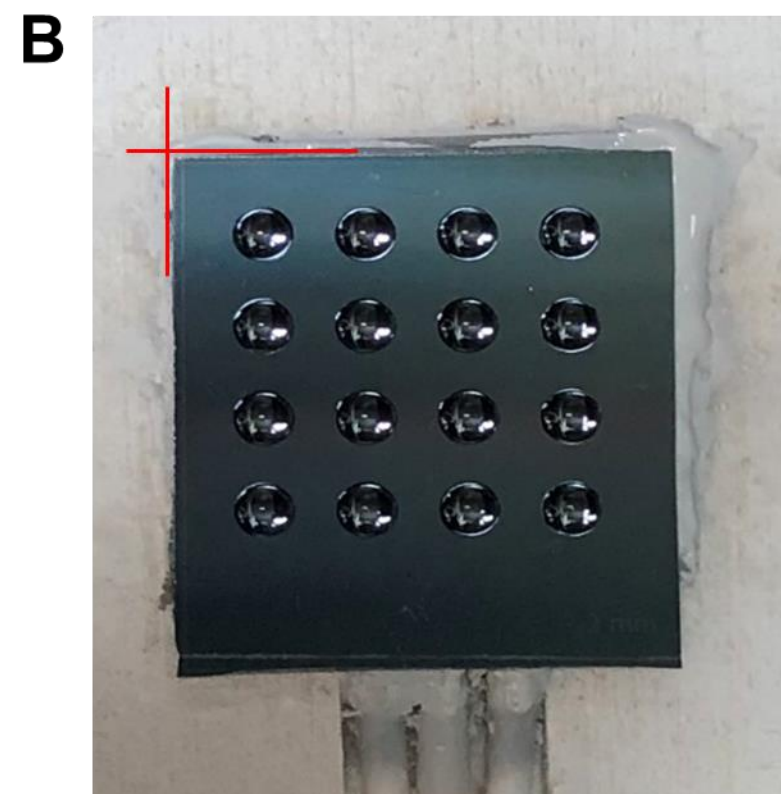506 [18F]fallypride in a droplet microreactor. *RSC Advances*. **10** (13), 7828–7838 (2020).

507

508

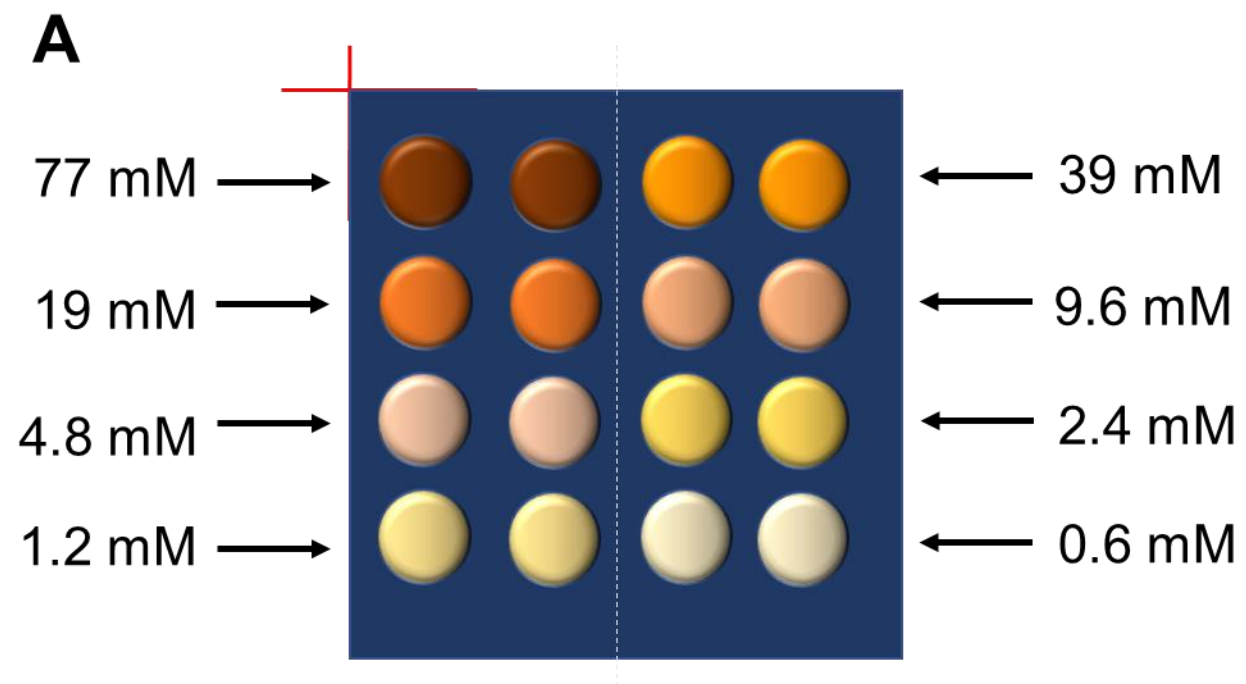Figure 1

Figure 3

A

77 mM →

19 mM →

4.8 mM →

1.2 mM →

← 39 mM

← 9.6 mM

← 2.4 mM

← 0.6 mM

B

Figure 4

Click here to access/download;Figure;Figure 4.pdf ±

Table 1
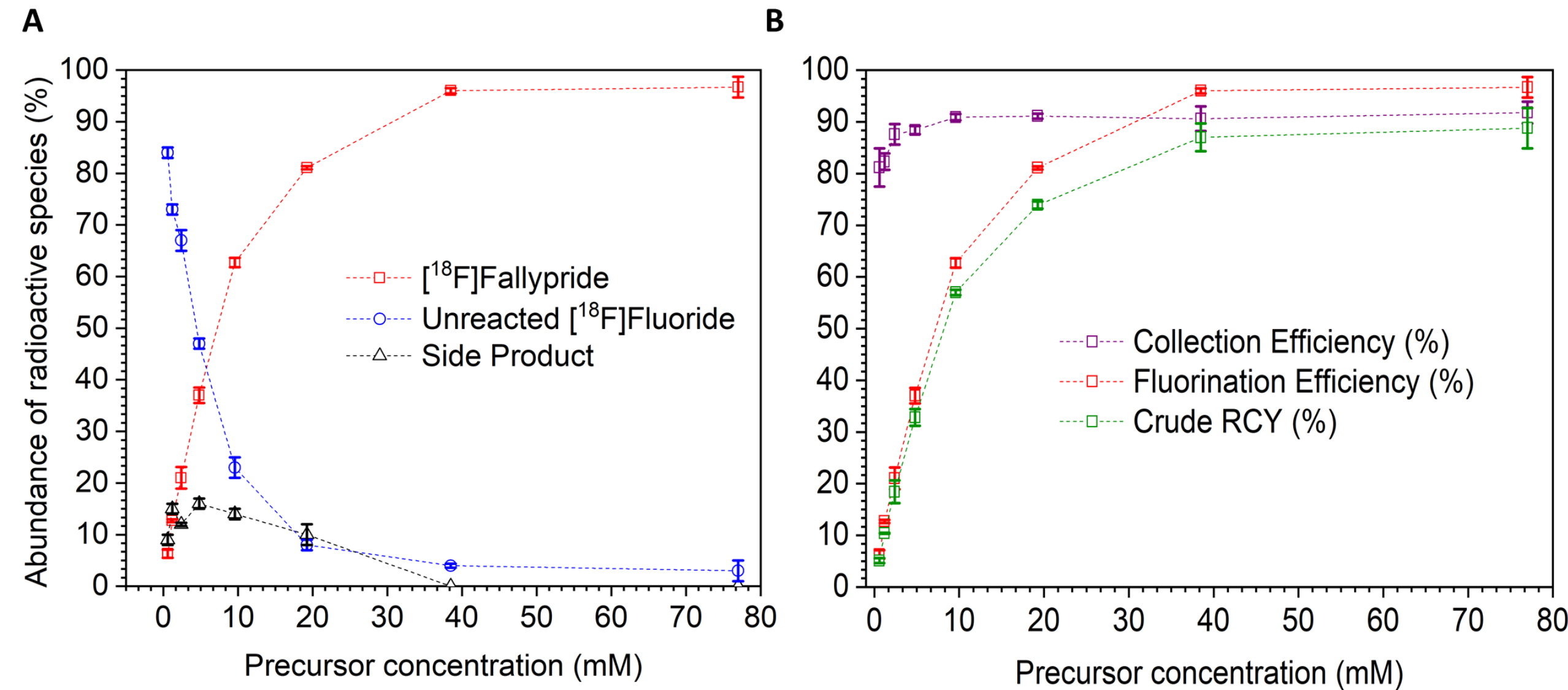
Precursor concertation (mM)	Collection efficiency (%)	Fluorination efficiency (%)	Crude RCY (%)
77	91.8 ± 2.1	96.7 ± 2.0	88.8 ± 3.9
39	90.6 ± 2.4	96.0 ± 0.5	87.0 ± 2.7
19	91.1 ± 0.5	81.1 ± 0.3	73.9 ± 0.7
9.6	90.9 ± 0.6	62.7 ± 0.9	57.0 ± 0.5
4.8	88.4 ± 0.8	37.0 ± 1.5	32.8 ± 1.6
2.4	87.6 ± 2.0	21.0 ± 2.1	18.4 ± 2.2
1.2	82.3 ± 1.6	12.7 ± 0.3	10.4 ± 0.1
0.6	81.2 ± 3.7	6.3 ± 0.8	5.1 ± 0.5

Unreacted [18F]fluoride (%)	Side product (%)
3.3 ± 2.0	0.0 ± 0.0
4.0 ± 0.5	0.0 ± 0.0
8.4 ± 1.2	10.5 ± 2.0
23.3 ± 2.1	14.0 ± 0.9
47.3 ± 0.8	15.7 ± 1.0
67.4 ± 2.1	11.6 ± 1.0
72.8 ± 0.7	14.5 ± 1.0
84.3 ± 0.2	9.4 ± 1.0

Name of Material/ Equipment	Company	Catalog Number
2,3-dimethyl-2-butanol (thexyl alcohol)	Sigma-Aldrich	594-60-5
Acetone	KMG Chemicals	
Ammonium formate (NH_4HCO_2)	Sigma-Aldrich	540-69-2
Anhydrous acetonitrile (MeCN)	Sigma-Aldrich	75-05-8
Ceramic heater	Watlow	Utramic CER-1-01-0093
Cerenkov imaging chamber	Custom built	
DI water	Sigma-Aldrich	7732-18-5
Disposable transfer pipets, 3 mL	Falcon	13-680-50
Dose calibrator	Capintec, Inc.	CRC-25 PET
Fallypride	ABX Advanced Biochemical Compounds	1560.0010.000
$[^{18}F]$fluoride in $[^{18}O]H_2O$	UCLA Ahmanson Biomedical Cyclotron Facility	
Glass cover plates (76.2 mm x 50.8 mm x 1 mm thick)	C&A Scientific	6101
Headway spin coater	Headway Research, Inc.	PWM50-PS-R790 Sipinner system
High temperature oven	Carbolite	HTCR 6 28
Hot plate	Thermo Scientific	Super-Nuova HP133425
Isopropanol (IPA)	KMG Chemicals	
Mask aligner	Karl Suss	MA/BA6
Methanol (MeOH)	Sigma-Aldrich	67-56-1
Microcentrifuge tube	Eppendorf	0030 123.301
Micropipette (0.5-10 µL)	Labnet	BioPette P3940-10

Micropipette (100-1000 µL)	Labnet	BioPette P3940-1000
Micropipette (10-100 µL)	Labnet	BioPette P3940-100
Micropipette tips (0.1-10 µL)	USA Scientific Inc Tips	11113810
Micropipette tips (2-200 µL)	BrandTech	13-889-143
Micropipette tips (50-1000 µL)	BrandTech	13-889-145
Photoresist developer solution	MicroChem	MEGAPOSIT MF-26A
Positive photoresist	MicroChem	MEGAPOSIT 220-7.0
Reactive-ion etcher (RIE)	Oxford Instruments	Plasma Lab 80 Plus
Silicon wafer cutter	Euro Tool	CSCB-431.00
Silicon wafer; 4" diameter	Silicon Valley Microelectronics Inc.	0017227-048
Teflon AF 2400	Chemours	D14896765
Tetrabutylammonium bicarbonate (TBAHCO$_3$)	ABX Advanced Biochemical Compounds	808
Themal conducting paste	OMEGA	OT-201-2
TLC plates	Merck KGaA	1.05554.0001
Tosyl-fallypride	ABX Advanced Biochemical Compounds	1550.004.000
Trimethylamine (TEA)	Sigma-Aldrich	75-50-3
Tweezers	Cole-Parmer	UX-07387-08

Comments/Description

98%

Cleanroom LP grade

97%

99.80%

25 mm x 25 mm

Other instruments can be used for TLC plate readout including: small animal *in vivo* optical imaging system, 2D radio-TLC scanner, 1D radio-TLC scanner

Fallypride reference standard, >95%
Due to short half-life this must be obtained from local radiochemistry lab or commercial radiopharmacy

PWM50-control box, PS-motor, R790-bowl

Cleanroom LP grade

≥99.9%
500 µL, colorless, polypropylene

P type, boron doped, thickness 525 ± 25 μm
1% solids

Aqueous solution stabilized with ethanol, 0.075 M

Silica gel 60 F$_{254}$, 50 mm x 60 mm, aluminum back
Fallypride precursor, >90%
≥ 99%
Stainless steel, fine tip

Response to Reviewer Comments

We appreciate the time and effort of the editor and reviewers to review our manuscript. The helpful comments and suggestions have led to important improvements in the manuscript. We provide below a point by point response to each comment. The original comments are in black text and our responses are in green text.

Editorial comments:

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.
We have carefully checked spelling, grammar, and abbreviations.

Please provide an email address for each author.
We have provided an email address for each author in the online submission system.

Unfortunately, there are sections of the manuscript that show overlap with previously published work. Please revise the following lines: Discussion lines 364-368 (…formation)
We have revised this portion of the discussion to differentiate from previously published work.

JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: Teflon AF 2400, HTCR 6 28, Carbolite; SPR 220-7, MA6, Karl Suss, MF-26A, Oxford80 Plus RIE, Eppendorf tube, etc
We have removed the trademark etc. symbols, and have remove the product names/models from the main paper and moved into the materials list. We have left "Teflon" in the paper due to its ubiquitous use; if this is not acceptable, it can be changed to "perfluoropolymer"

Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
We have removed all personal pronouns.

Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.
We have checked the protocol section and corrected to ensure we are using imperative tense everywhere.

Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should

be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
We have added more details to each step.

Line 154: Are you using glass vials or Eppendorf tubes?
We are using Eppendorf tubes. We have removed mention of vials and use the term microcentrifuge tubes for clarity.

Please check all chemical formulae, e.g., line 242: do you mean NH_4HCO_3?
We checked this and the original version is correct, NH_4HCO_2.

==After including a one-line space between each protocol step==, highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. This will clarify what needs to be filmed.
We checked the highlighted portions of the manuscript and made few changes to fit the highlighted protocol text in 3 pages with a one-line space in between each protocol step.

As we are a methods journal, please add to the discussion any critical steps within the protocol, modifications and troubleshooting of the technique, limitations of the technique.
We have added some additional elements to the discussion.

Please submit each figure individually as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps.). Please remove the embedded figure(s) from the manuscript. Figure legends (titles and description) should appear after the Representative Results of the manuscript text.
We removed the figures and consolidate captions in the place indicated.

Please remove the embedded Table from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Table captions (title and description) should after the Representative Results of the manuscript text.
We removed the table and consolidated the caption in the place indicated.

Please sort the Materials Table alphabetically by the name of the material.
We have sorted it.

Reviewer 1 Comments:

Please make sure to use the latest nomenclature guided from SRS.

We have checked carefully and believe we are using the correct nomenclature.

In order to ensure the safest process, is there an indication on the need (or not) of active ventilation/extraction during the execution of these experiments?

We appreciate the reviewer's question; we have clarified this at the beginning of the Protocol section.

In order to gauge robustness of the process, how does the data compare among different days or different chips?

We thank the reviewer for the insightful question. To assess robustness of the process when we perform experiments in different days and different chips, we include a few data points with identical conditions to the previous day as a reference point. From our recent work in **RSC Adv.**, 2019,**9**, 20370-20374, the following are some examples of these replicated conditions to give an idea of repeatability:

Set 1 (Reaction conditions: 240 nmol of $TBAHCO_3$ with a 4 µL droplet solution of 77 mM of precursor):

Performance measurement	Day 1 (n=2)	Day 2 (n=2)
Collection efficiency (%)	94 ± 1	92 ± 1
Fluorination efficiency (%)	98.9 ± 0.2	96 ± 1

Set 2 (Reaction conditions: 240 nmol of $TBAHCO_3$ with a 6 µL droplet solution of 77 mM of precursor):

Performance measurement	Day 2 (n=2)	Day 3 (n=2)
Collection efficiency (%)	92 ± 1	92 ± 2
Fluorination efficiency (%)	98 ± 1	97 ± 2

Can the authors comment whether or how this data can be used to transfer the process into a "full-batch" synthesis, comprising purification, formulation and higher starting activities?

We thank the reviewer for the interesting question. One approach is to scale up the activity in a droplet and do a 'full-batch' synthesis under the same droplet conditions as found by the optimization study, and perform purification by analytical-scale radio-HPLC and formulation using evaporation or cartridge methods. We've previously reported scale-up of [^{18}F]fallypride to 41 GBq starting activity **RSC Adv.**, 2020,**10**, 7828-7838, followed by purification, formulation, and quality control testing. We have added a note to this effect at the end of the Discussion section.

We plan to study in the future the possibility to adapt the conditions to the macroscale. After choosing a suitable reaction volume (e.g. 1 mL), a good starting point may be to preserve the same precursor concentration and calculate the amount of base to preserve the base:precursor ratio. Temperature can be kept the same, but likely a longer reaction time will be needed, as it can take many minutes for the reactor contents to reach the desired temperature.

It took that all additions/handing is done manually (e.g. pipette). Is it possible to automate these tasks? Can the authors comment on what would be difference in using multi-well automated synthesizer robots? Even based on new concepts, https://pubs.rsc.org/en/content/articlelanding/2019/lc/c9lc00622b#!divAbstract

To increase safety and reduce tedious work, we are currently working on automation. We have developed a custom system (to fit in a mini-cell and using very fast motors to minimize decay) to dispensing various reagents, collecting products, etc. Publication of the system will be forthcoming, but some details can be found in the following abstract from ISRS 2019:

Jones, J.; Rios, A.; Chao, P.; Wang, J.; van Dam, R. M. High-Throughput Microdroplet Radiochemistry Platform to Accelerate Radiotracer Development. In *Journal of Labelled Compounds & Radiopharmaceuticals*; 2019; Vol. 62, pp S350–S351.

We thank the reviewer for the interesting paper. Our automated platform uses non-contact piezoelectric dispensers, but we are very interested in switching to a lower-cost dispensable dispenser approach in the future.

Minor comments:

Please edit the text for grammar and syntax, there is sometimes a little tendency to repeat the same word at short distance.

We have checked the grammar and syntax, and also removed any duplicated words.

Why the yellow highlight?

The yellow highlight is requested by the journal to indicate the portions of the manuscript to be used for the narrative script in the protocol video.

In the text, it is not clear how to measure the starting activity with dose calibrator

We thank the reviewer for pointing out this issue. We first start by loading activity to the first reaction site on the chip and measure the chip with a dose calibrator and record the time at which the measurement was conducted. We then load activity to the second reaction site and perform another dose calibrator measurement, taking the difference between the new measurement and previous measurement as the amount of activity loaded for the second reaction (corrected for decay). The process is repeated for all sites. We have modified the text to clarify this.

Line242: "NH4HCO3"?

Our original text NH4HCO2 is the intended formula.

Line 258: or using HPLC? I believe such method cannot be forgotten to be proposed as an option

Yes, we agree! We have added a statement to the end of the note at the beginning of Protocol step 2.

What is the total time for each 16 reactions run, from radioactivity bulk delivery to obtaining all the TLC results? It is said 25 min, but it is not clear when the "stopwatch" is started and stopped.

We thank the reviewer for question. The stopwatch starts when we first load the [^{18}F]fluoride mixed with the base / phase transfer catalyst to the first reaction spot on the chip and the stopwatch is stopped when we measure the last crude product that is extracted from the chip. The TLC results can be obtained in 10 additional minutes.

Is it possible or suggestible to use DoE approaches to setup the conditions to be tested? This approach would allow for this; however, given the difficulty to have an "online" analysis method, it still would not be useful to achieve an automated optimization (e.g. using feedback algorithms).

We thank the reviewer for the useful comment. Certainly we believe the platform can be used in conjunction with DoE design approaches, though they might need to be somewhat constrained to efficiently make use of parallelism. (E.g. if all conditions had different temperature or reaction time, they would have to be performed sequentially.) We are very interested in exploring this in the future. Furthermore, we are currently working on a platform that can allow the performance of 64 simultaneous

reactions and adds parallelism in the temperature and time parameters to further facilitate parallel optimization.

The reviewer is correct that we lack an online analysis method and cannot currently doing automated optimization. We are not sure how to best combine some amount of parallelism with automated selection of the next set of conditions, but this is an interesting challenging for the future!

Reviewer 2 Comments:

In my idea it could be published without revision

We thank the reviewer for their very positive assessment of our manuscript.

Reviewer 3 Comments:

Abstract line 28-30. "These chips enable enables rapid and convenient studies of reaction parameters including reagent concentrations and reaction solvent, and the use of multiple chips enables the study of reaction temperature and time". The reaction parameters contain the reaction temperature and time. So, I think it. would be good to write the contents of multi-chips concisely in one sentence as follow. "These chips enable rapid and convenient studies of reaction parameters including reagent concentrations, reaction solvent, temperature and time."

We thank the reviewer for the useful comment. We had originally separate it because a single chip cannot efficiently explore different temperature or times, and it takes multiple chips to explore those variables with parallelism. However, we agree that this reads better in the suggested form.

Uniform the style. In vivo -> in vivo (italic), [18F]Fallypride -> [18F]fallypride

We thank the reviewer for the useful comment, and we changed the style as suggested in the manuscript.

Introduction line 38-41. Due to the short half-life of these molecules -> Due to the short half-life of radioisotopes.

We have modified as suggested.

Tracer -> radiotracer

We have made the suggested change.

"because multiple patient scans and research experiments can be scheduled at the same time" At the same time -> in a single day

Thank you for the suggestion, we have made this change.

Line 75-77. "Using multi-reaction droplet chips allows for convenient exploration of the impact of reagent concentrations and reaction solvent, and the use of multiple chips could enable the study of reaction temperature and time, all while consuming very low amounts of precursor" I want to change the sentence to be concise like the above my minor concern

We thank the reviewer for the comment. We have elected to leave the original text because we feel here the distinction between what can be done on one chip is distinct from what can be explored with multiple chips. Since the whole chip is heated at once, all reactions performed simultaneously must be performed at the same temperature and reaction time. Multiple different chips (and heaters) are needed to explore different temperatures or reaction times in parallel. If the reviewer feels strongly about this point, we can modify as suggested.

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August 12th, 2020

Dr. Rafaela Vasiliadou
Guest Editor, Journal of Visualized Experiments
School of Life, Health, and Chemical Sciences
Open University
UK

Dear Dr. Vasiliadou,

Please find attached our manuscript entitled "Rapid and economical optimization of radiochemical reactions using droplet arrays", which we respectfully submit for the upcoming special collection "Miniaturization of biochemical and chemical techniques" in the *Journal of Visualized Experiments*. The full author list includes: Alejandra Rios, Travis H. Holloway, Jia Wang, and R. Michael van Dam (corresponding author).

In this paper, we describe a new method for economical high-throughput synthesis optimization of positron-emission tomography (PET) radiopharmaceuticals. Due to the unique challenges in this field (radiation hazard, short half-life of radioisotopes), synthesis of these compounds is usually carried out in an automated fashion using "radiosynthesizers". These systems, however, are designed for producing large batches and can only be used on an occasional basis (i.e. typically one waits ~10 half-lives (i.e., 18 hours if using fluorine-18 with 109.8 min half-life) to allow residual radioactivity to decay to background levels before the system can be used again. As a result, optimization experiments are very costly and time-consuming, and only a tiny fraction of the available parameter space can be explored. Instead, we developed a multi-reaction microdroplet chip that contains an array of reaction sites for performing 16 simultaneous droplet-based radiochemical reactions. Each reaction uses minimal reagents and radioisotope, and the timeline and costs for optimization can be compressed 10-fold or more.

Using the synthesis of [^{18}F]Fallypride (a clinically-relevant PET tracer for visualizing D2/D3 receptors in the brain) as an example, we show how this technique is used to optimize one of several synthesis parameters, namely the precursor concentration. The ability to perform 16 reactions in 25 min allowed the optimization to be completed much more quickly than would be

possible using a conventional synthesizer. We believe this approach will be a valuable tool in the field of radiochemistry to accelerate synthesis optimization and to enable the study of reaction conditions in far greater detail than is currently practical. Moreover, the resulting optimized droplet-based reactions intrinsically leverage the advantages of microvolume radiochemistry, including reduced precursor consumption, faster process times, and compact instrumentation, and can offer these same advantages even for routine production of larger batches.

We believe that this work will be of broad interest to readers and viewers of *Journal of Visualized Experiments*, and will be of particular interest to those in the fields of radiochemistry and nuclear medicine (especially those developing new radiotracers, those developing new radiosynthesis/radiolabeling methods, and those developing, optimizing, and translating radiosynthesis protocols into the clinic).

Thank you for considering this manuscript and we look forward to your response.

Sincerely,

R. Michael van Dam, Ph.D.
Professor, Department of Molecular & Medical Pharmacology
Co-Associate Director, Crump Institute for Molecular Imaging
David Geffen School of Medicine at UCLA