Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.


# Optimization, automation and validation of $Al^{18}F$ tracer in a custom-made automatic platform with high yield for large scale radiosynthesis

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## 1. Materials and methods

All chemicals were purchased from Sigma-Aldrich (Bornem, Belgium) unless stated otherwise. Glu-urea-Lys (Ahx)-HBED-CC (PSMA-11) was purchased from ABX (Radeberg, Germany). NOTA-E[$PEG_4$-c(RGDfK)]2 (Alfatide II) and NOTA-Octreotide was purchased from Jangyuan (Ningbo, China). Aluminum chloride heptahydrate ($AlCl_3.6H_2O$), Sodium acetate trihydrate, and acetic acid were all trace metal analysis grade. HPLC eluents, acetonitrile, and trifluoroacetic acid (TFA) were of high-grade purity. Ultrapure water was prepared using a Seralpur pro 90 CN system (Belgolabo, Overijse, Belgium). Saline for injections was purchased from B. Braun Medical N.V. (Jinan, China). μQMA cartridge (Chromabond PS-$HCO_3$) was purchased from Huayi (Changshu, China), Oasis HLB Light and Sep-Pak $C_{18}$-Light cartridges were obtained from Waters (Milford, MA). Sterile filtration was performed by using 0.22-mm membrane filters (SRP25 17575-ACK, Minisart®).

0.5 M or 0.1 M Sodium acetate/acetic acid buffers with a pH of 4.0 were prepared by the means of mixing the 0.5 M or 0.1 M solutions of each component in suitable ratios, and final pH was verified by pH meter (pH 4).[1] A solution of 5mM $AlCl_3.6H_2O$ was prepared in a 0.1 M acetate buffer of pH 4.0 and stored at 4 ℃. 75% ethanol (V%/V%) was used for cleaning the pipeline of the platform, and 1 mM HCl ethanol was employed for eluting the $^{18}F$-tracers for the SPE cartridges.[2]

Aqueous (aq.) [$^{18}F$]fluoride was produced by an $^{18}O$ (p,n) $^{18}F$ nuclear reaction with Mini-trace cyclotron (9.6 MeV, GE Healthcare) using a 2.5 mL Niobium target. The enriched [$^{18}O$] water (97%) for irradiation was obtained from Cambridge Isotope

Laboratories (Tewksbury MA, USA). Finally, radioactivity measurements were performed using a dose calibrator Capintec CRC 15R (Capintec, Florham Park, NJ, USA). 3. The lyophilized NOTA-ligands in 1.5 mL Eppendorf tube was prepared by the lyophilizer LGJ-12A (JP Electronics, Shanghai). Liquid handling was performed by pipettes Pipet-Lite XLS+ (Metter Toledo, UK).

**2. Assembly of the platform**

The platform was mainly consisted of five electronic devices, Cavro XCalibur syringe pump (with 5 mL glass syringe (SP) and 9-port distribution vale (DV)), 3-port 2-position switch vale (SV2), 4-port 2-position switch vale (SV3) , 10-port 2-position switch vale (SV1) and heating block. As shown in Fig. S1, the ports of the devices were connected by PTEE or PEEK tube with different internal diameter (0.8 mm for PTEE, and 0.2 mm for PEEK). The solution of reagents and vial were connected to the distribution vale (DV) by pipeline. The μQMA cartridge was placed between port 5 and 2, and the $C_{18}$ cartridge was placed between port 10 and 7 of the SV1 by using luer taper for connecting. Port 6 in SV1 was connected to the line of cyclotron for $^{18}F$-$^{18}H_2O$ transfer by passing the SV2 (state 1). Port 8 in SV1 was connected to the tracer vial by using PTEE tube. Port 1 in SV1 was connected to the waste bottle. Port 3 and 9 in SV1 were connected to the port 1 and 2 in DV, respectively.

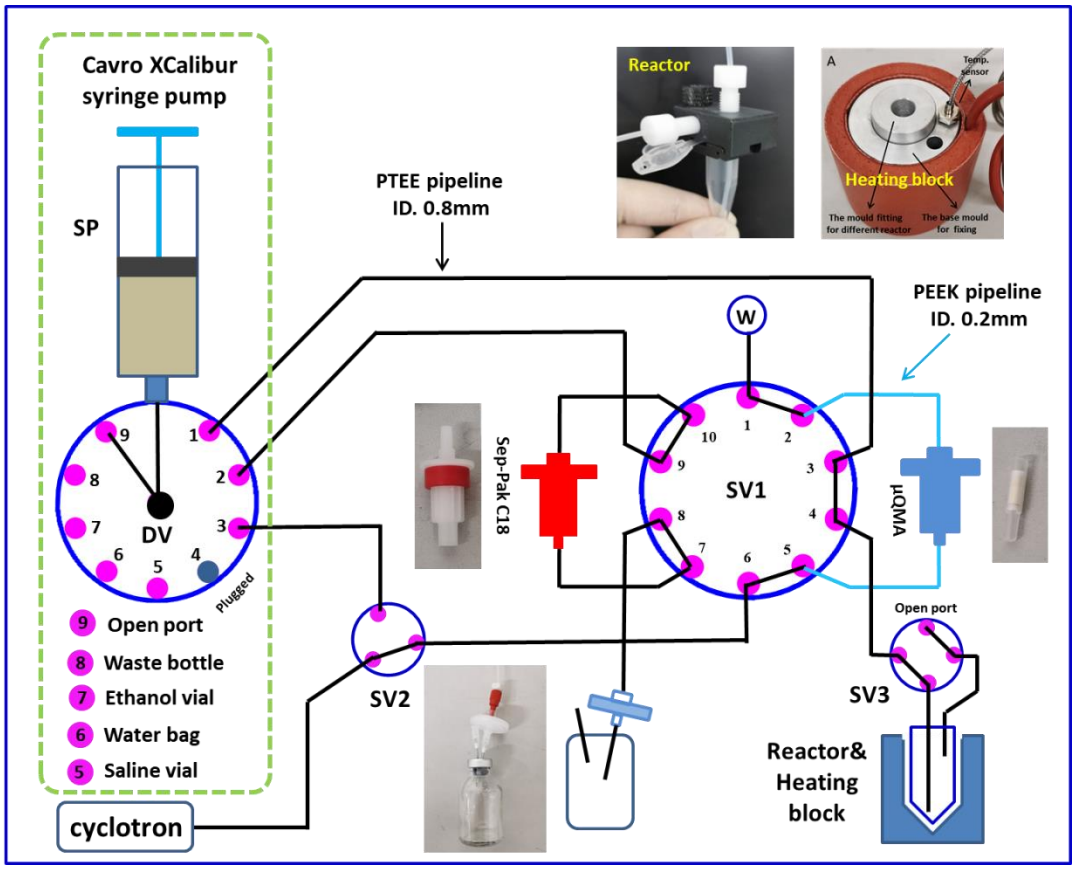



Fig. S1. The schematic representation of the fluid path for the custom-made platform and the photograph of the heating block, small-volume reactor, μQMA cartridge, Sep-Pak $C_{18}$-Light cartridges and the tracer vial.

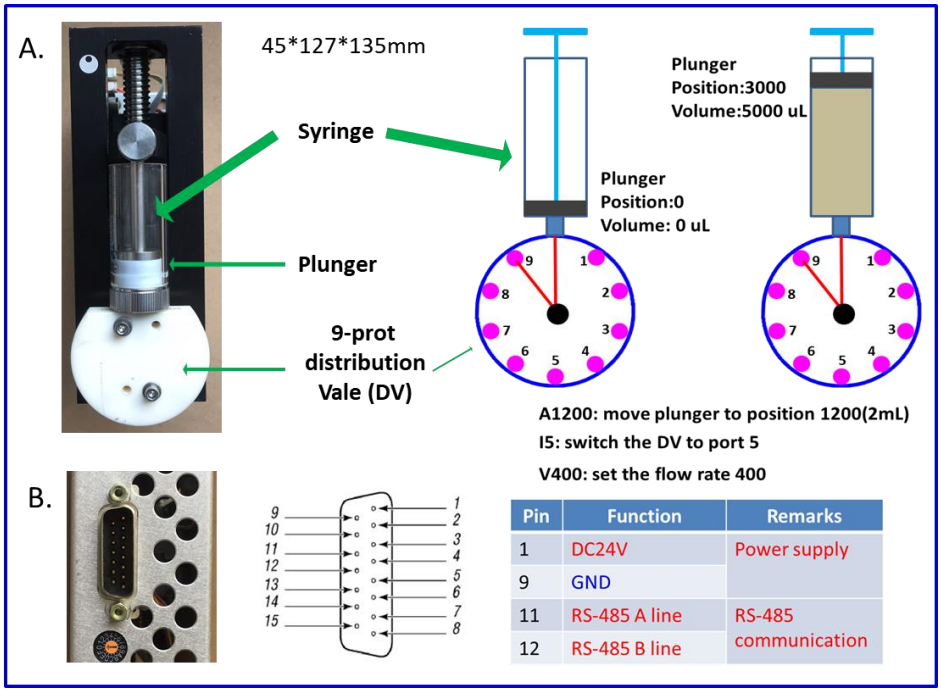



Fig. S2. (A) The photograph of 9 port Cavro® Xcalibur Pump and the scheme of the plunger movement and the 9 port distribution vale. (B) DB-15 cable connector and the pins' function.

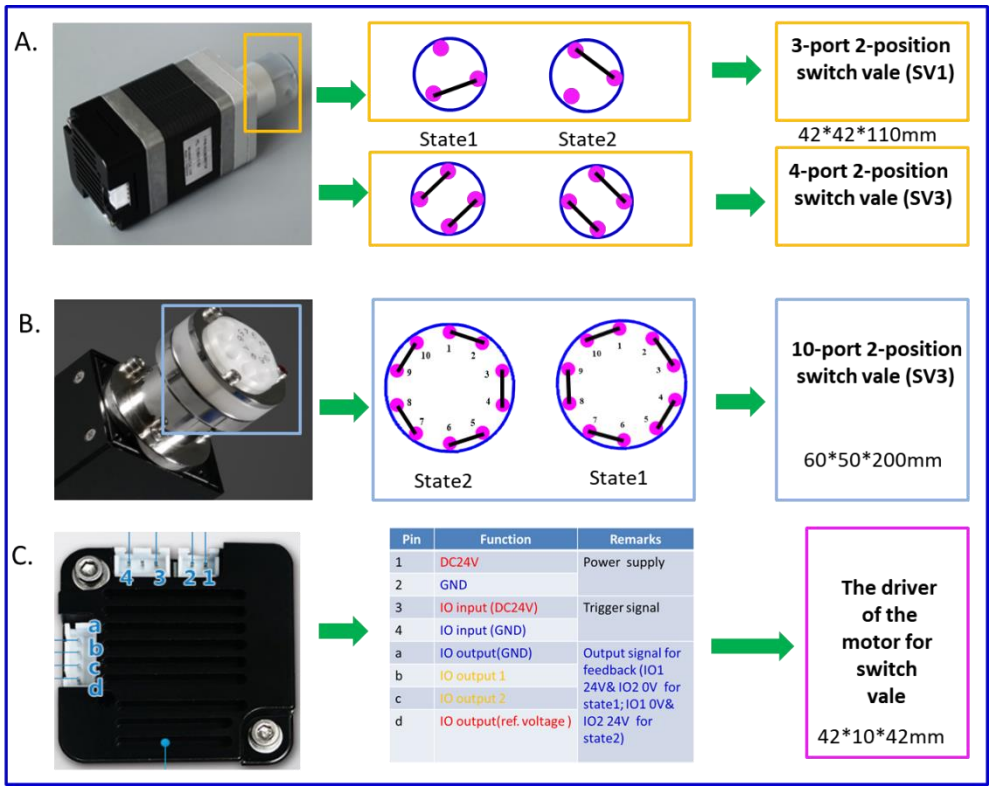



Fig. S3. (A) The photograph of 3 and 4 port switch vales and the flow path for the two

state. (B) The photograph of 10 port switch vale and the flow path for the two state. (C) The photograph of the driver of the switch vale's motor and the functions of the pins.

A microliter-scaled labeling subsystem, which was consisted of a 1.5 mL Eppendorf tube as the reactor and one corresponding adapter for tube fixing and pipeline connection, was custom-made. Together with the 4 port 2 position switch vale (SV3), the flow path for the labeling system could be switched in two states, one for sealed heating and the other for liquid transfer from the tube (Fig. 4). Temperature of the subsystem, which was consisted of a custom manufactured aluminum block and heating jacket, is maintained by a closed-loop temperature controller (Fig. S4). Within the range from 50 to 150℃, the temperature can be precisely controlled.

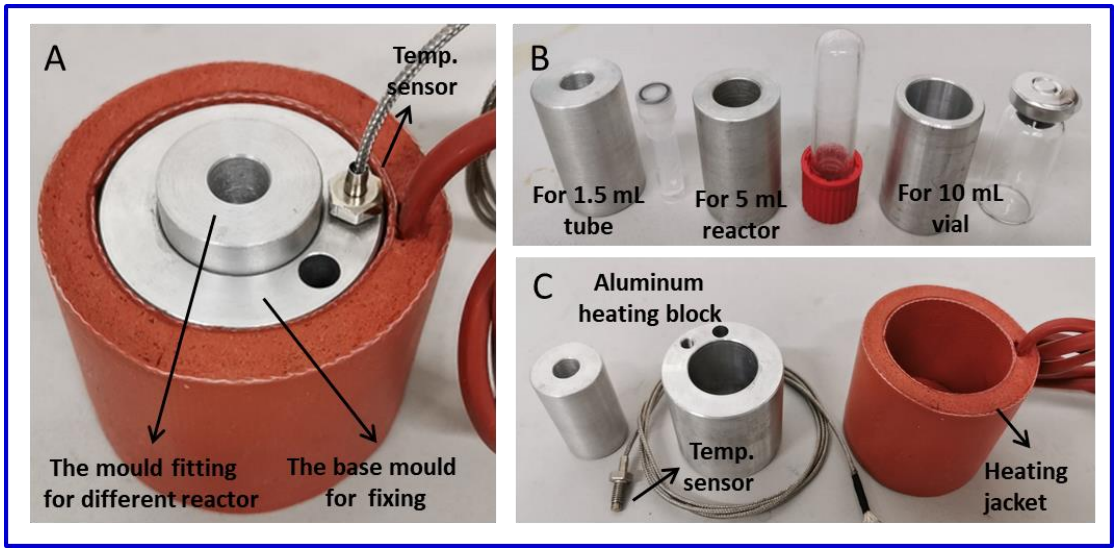



Fig. S4. (A) The photograph of heating block, which include the different sub-block matching with the corresponding reators showed in B. (C) The photograph of the sub-block, basic block, the heating jacket and the *K*-type thermocouple temperature sensor used in this reaserch.

All electronic components (e.g. one heating block, one syringe pump and three 2-position switch vales) were connected to a CCU hub based on ARM microcontroller, which was designed for the hardware control (Fig. S5). Power for platform, including valves, syringe pumps, heating block and CCU hub, was provided by a DC power supply (24V, 120W). Software was developed basing on Qt creator (Version 4.10.1) environment. The communication between software (PC desktop) and CCU hub was realized by RS485 bus. There were two interfaces for the remote control. One was for parameter setting & manual operation (Fig. S6A) and the other for time list programming automation (Fig. S6B).

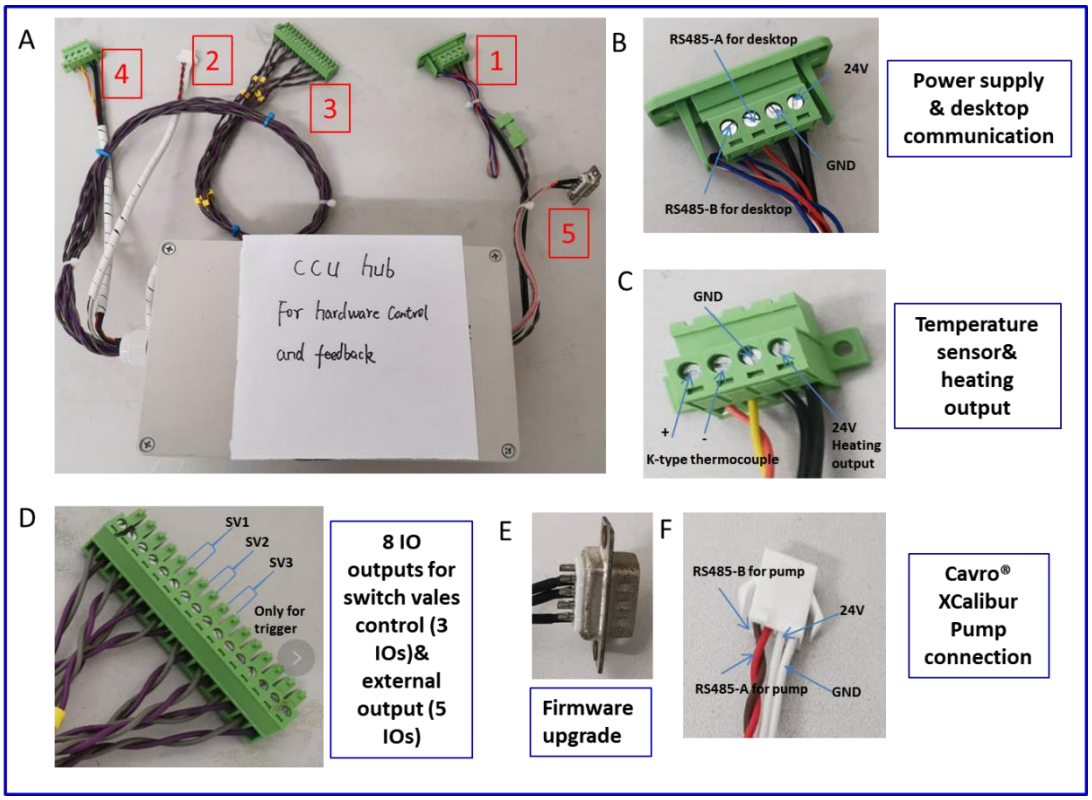
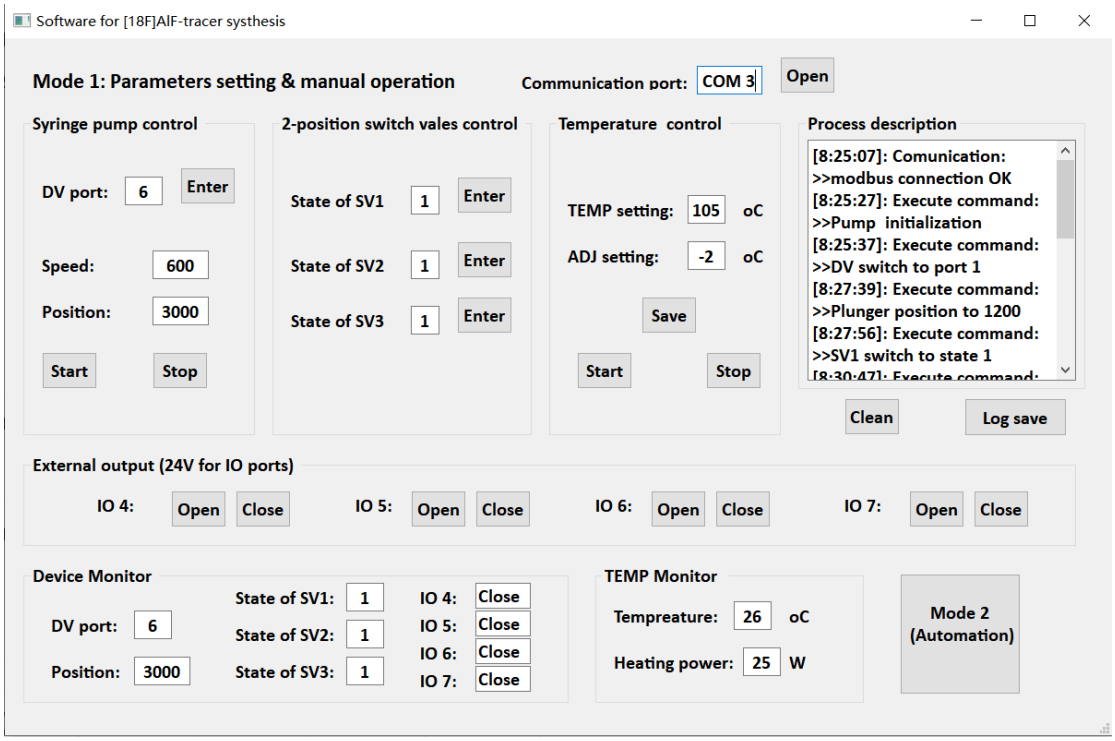



Fig. S5. (A). The photograph of the central control unit (CCU). (B) was the connector for power supply and the RS-485 bus comunicating to software (desktop). (C) was the connector for heating block including *K*-type sensor and PWM controlled output to heating jacket. (D) was the connector for IO output controling SVs (IO1 for SV1, IO2

for SV2 and IO3 for SV3) and externals. (E) was the DB9 serial connector for the CCU firmware upgrade. (F) was the connector for the Cavro® Xcalibur Pump including 24V power supply and RS-485 bus comunicating to CCU.

A



B

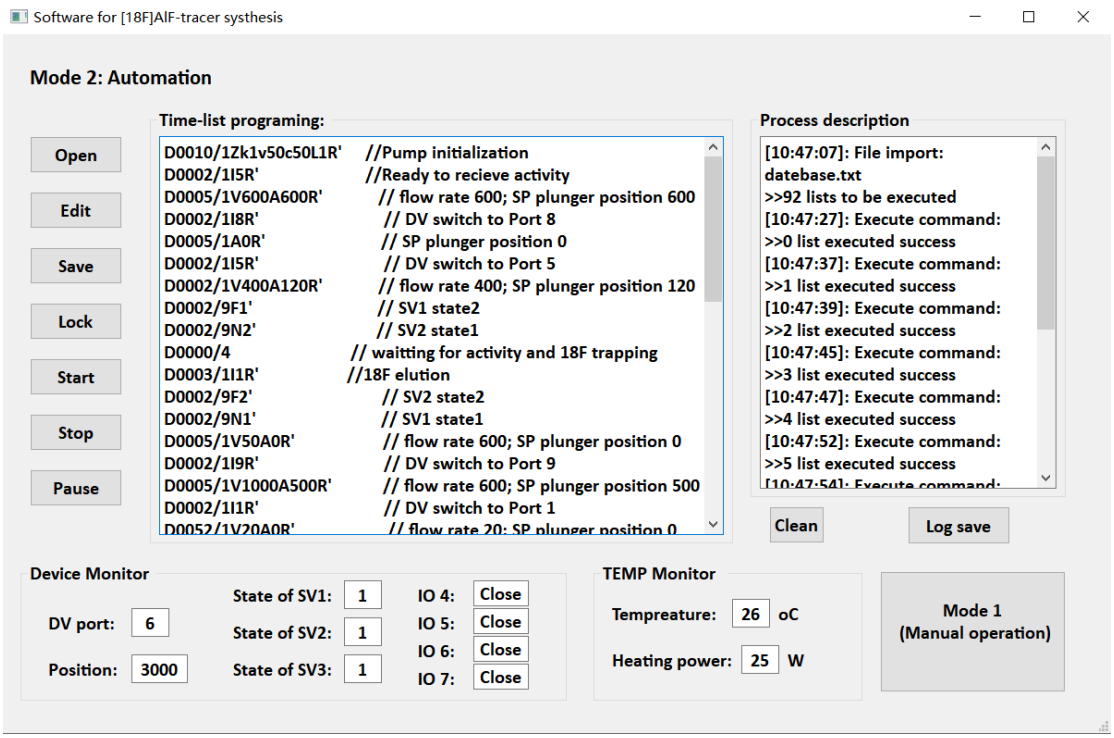



Fig. S6. (A) was the interface for manual operation and setting and (B) was for time list programing of automation. Both of the interface could monitor and feedback the state of the pump,vales and the tempreature.

## 3. The lyophilized NOTA-ligands in the disposable 1.5 mL Eppendorf tube as the reactor for radiofluorination

Firstly, 1 mmol precursor was dissolved in 1 mL DI water. Then, the fraction of 100 uL was transferred into 1.5 mL EP tube using pipette, making sure that each tube contains 100 nmol ligands. Subsequently, the tube were frozen and then transferred to the lyophilizer cooling to −16 °C, and the vacuum went below 100 mTorr. 16h later, the tubes were taken out from the lyophilizer and sealed by sealing film (Parafilm). In the preliminary steps before receiving activity, the tube was fixed on the adaptor as the reactor showed in Fig.4B for radiolabeling.[3]

## 4. Optimization for each unit operations

4.1 Isolation and concentration of [$^{18}$F]fluoride

No-carrier-added aqueous [$^{18}$F]fluoride (typically 2.2 mL, 1.5–2.0 GBq) in enriched $^{18}$O water was delivered to the platform and trapped on the μQMA cartridge (Fig. S7A). After trapping of the activity, the μQMA column was purged to dryness. In order to calculate the trapping efficiency of the μQMA, the activities in the $^{18}$O $H_2O$ recovery bottle and μQMA cartridge were measured by dose calibrator, respectively. Then, the activity was eluted using 150 uL saline with varied flow rate (5, 10, 15, 30, 50 and 75uL/s). The eluant was collected and measured in a dose calibrator (Fig. S7B). The efficiency of the elution was calculated to optimize the best flow rate for [$^{18}$F]Fluoride releasing from the μQMA column. Detailed description of the operation and the programing control was described clearly in Table S2.

The efficiency of the trapping (%) = Activity (trapped on cartridge)/[Activity ($^{18}$O $H_2O$ recovery bottle)+ Activity (trapped on cartridge)]*100%

The efficiency of the elution (%) = Activity (in eluant)/ Activity (trapped on cartridge)]*100%

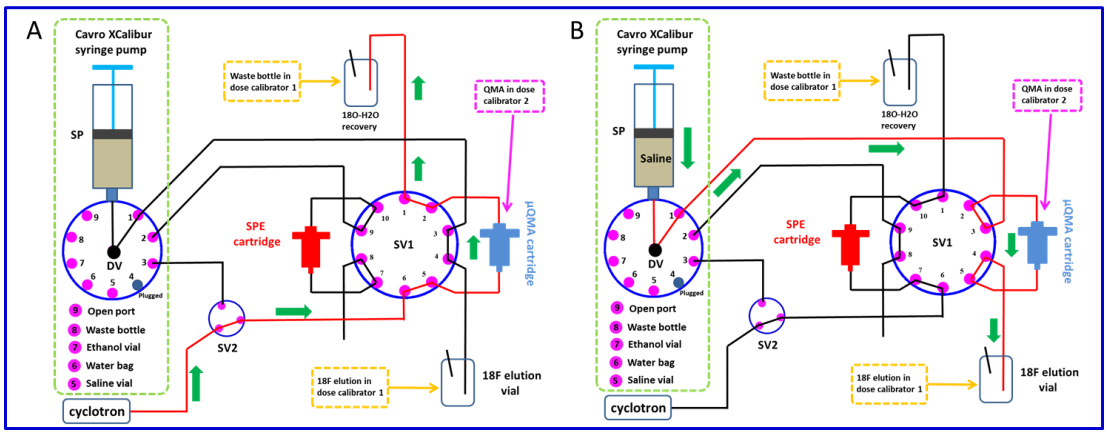



Fig. S7. Schematic representation of the [$^{18}$F]Fluoride trapping and elution unit

operation. (A) The fluid path of irradiated [$^{18}$O]$H_2O$ for [$^{18}$F]Fluoride trapping using a μQMA anion exchange cartridge. (B) The fluid path of eluent for [$^{18}$F]Fluoride releasing.

**Table S1. The Efficiency of [$^{18}$F]fluoride trapping and eluting by using μQMA cartridge with varying amounts of activity [(a) (b)].**

| Entry | Activity trapped on μQMA(mCi) | Activity in recovery vial (mCi) | Activity for $^{18}$F elution (mCi) | Efficiency of $^{18}$F trapping | Efficiency of $^{18}$F elution |
|---|---|---|---|---|---|
| 1 | 5.8 | 0.1 | 4.2 | 98% | 71% |
| 2 | 23.4 | 0.4 | 20.8 | 98% | 87% |
| 3 | 43.1 | 0.3 | 37.2 | 99% | 86% |
| 4 | 107.4 | 1.4 | 97.5 | 98% | 89% |
| 5 | 297.2 | 2.3 | 276.2 | 99% | 95% |
| 6 | 520.7 | 4.7 | 494.2 | 99% | 94% |
| 7 | 734.2 | 5.8 | 711.3 | 99% | 96% |
| 8 | 920.0 | 6.3 | 874.5 | 99% | 94% |
| 9 | 1126.0 | 9.1 | 1045.2 | 99% | 92% |

(a) It took about 100 s for [$^{18}$F]Fluoride releasing form the cartridge after the [$^{18}$F]Fluoride trapped on the cartridge, including 60 s for the [$^{18}$F]Fluoride elution with flow rate 15 uL/s and 40 s for purging the pipeline making the activity transferred into [$^{18}$F]Fluoride elution vial efficiently.

(b) The efficiency for [$^{18}$F]Fluoride trapping was calculated with non-decay corrected for all

entry.

**Table S2. The detailed synthesis programs for [$^{18}$F]fluoride trapping and elution.**

| Steps | Operation | SV1 | SV2 | SV3 | Channel port | Plunger position | Media in syringe | Flow rate | Duration (s) | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Initialization | 1 | 1 | 1 | 9 | →0 | \ | \ | 10 | |
| 2 | Withdraw $^{18}$F eluent | 2 | 1 | 1 | 5 | 0→120 | Saline | 400 | 2 | |
| 3 | Waiting for target water | 2 | 1 | 1 | 1 | 120 | Saline | \ | …… | Cyclotron bombard-ment |
| 4 | Receiving the target water | 2 | 1 | 1 | 1 | 120 | Saline | \ | 180 | $^{18}$F trapping |
| 5 | Transfer the elute | 1 | 2 | 1 | 1 | 120→0 | \ | 50 | 5 | $^{18}$F elution |
| 6 | Withdraw air | 1 | 2 | 1 | 9 | 0→500 | Air | 1000 | 2 | |
| 7 | Eluent was passed though the QMA slowly | 1 | 2 | 1 | 1 | 500→0 | \ | 20 | 52 | |
| 8 | Withdraw air | 1 | 2 | 1 | 9 | 0→1000 | Air | 1000 | 2 | |
| 9 | Dry the pipeline | 1 | 2 | 1 | 1 | 1000→0 | \ | 100 | 20 | |

4.2 Optimization of the chelation reaction

An amount of 100 nmol precursor preloaded in 1.5 mL Eppendorf tube, 10 μL $AlCl_3$ solution (5 mM in 0.1 M acetate buffer of pH 4.0), 50 uL acetate buffer (0.5M, pH 4.0) and 100 μL ethanol were mixed.[4] Then, the adapter for tube sealing and pipeline connection was fixed for receiving the [$^{18}$F]Fluoride elution. After the [$^{18}$F]fluoride was trapped on a preconditioned μQMA and eluted with 150 uL saline in the reactor, the SV3 was switched in the state 2 for sealed heating the mixture for 10 min at 105℃. After cooling down, the mixture was diluted with 10 mL water

manually. 10 μL of the mixture was taken out and analyzed for the RCY by radio-HPLC. In order to investigate the impact of reactor volume on the RCYs, two different reaction vessels (the small volume one, 1.5mL, in our platform and the conventional one, 10mL, for GE TRACERlab FXFN) were tested under the same condition.

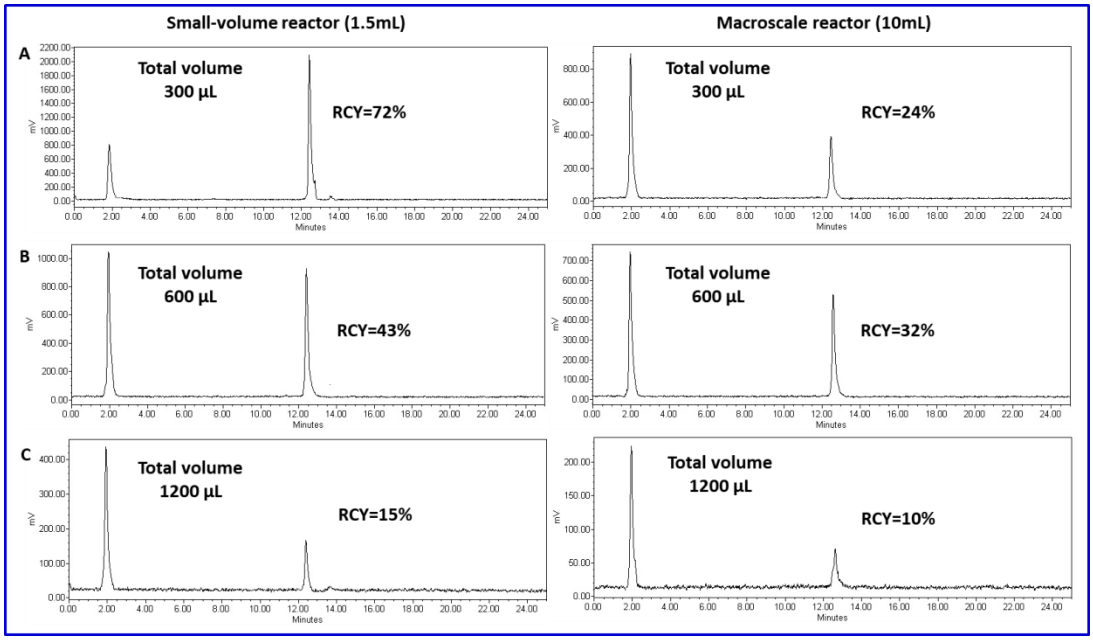



Fig. S8. HPLC chromatograms: Radio-HPLC profile of the reaction for crude mixture volume 300 μL (A), 600 μL (B) and 1200 μL (C) by using the small-volume reactor and macroscale one, respectively.

**Table S3. The detailed synthesis programs for radiolabeling.**

| Steps | Operation | SV1 | SV2 | SV3 | Channel port | Plunger position | Media in syringe | Flow rate | Duration (s) | script |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sealed the tube | 1 | 2 | 2 | 9 | 0 | \ | \ | 2 | |
| 2 | Heating to 105°C | 1 | 2 | 2 | 6 | 0 | \ | \ | 720-900 | |

4.3 Purification based on SPE cartridge

After cooling down the mixture, the SV3 was switched in the state 1, the mixture

was transferred into syringe and then diluted with 4 mL water. The DV switched the channel to port 3, and the SV1 was switched in the state 2, the SV1 was switched in the state 2. The mixture in the syringe was transferred into flow path, leading to the tracer trapped on the $C_{18}$ cartridge with precise flow rates (ca. 100 μL/s). The reactor vessel was washed with an additional aliquot of water (2 mL×2), and finally the cartridge was dried for product elution. Detailed description of the operation and the programming control was described clearly in Table S4.

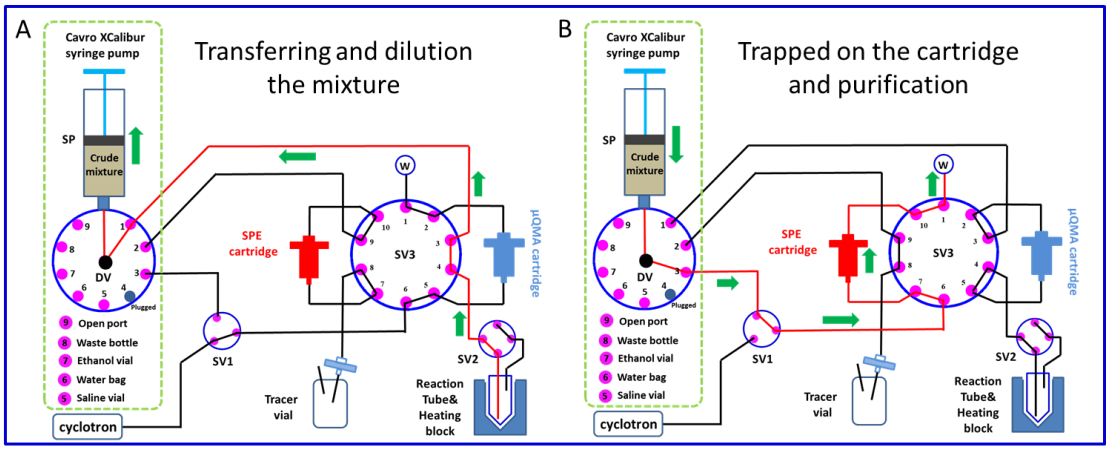



Fig. S9. Schematic representation of the tracer purification unit operation in the platform. The fluid path (A) was for transferring the mixture and dilution with water, and the fluid path (B) was for tracer trapping using a Waters t$C_{18}$ Light cartridge.

**Table S4. The detailed synthesis programs for purification based on SPE cartridge.**

| Steps | Operation | SV1 | SV2 | SV3 | Channel port | Plunger position | Media in syringe | Flow rate | Duration (s) | script |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Withdraw water | 1 | 2 | 2 | 6 | 0→2000 | Water | 1000 | 6 | |
| 2 | Cooling down the tube | 1 | 2 | 2 | 6 | 2000 | Water | \ | 180 | |
| 3 | Switch the | 1 | 2 | 1 | 6 | 2000 | Water | \ | 1 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SV3 | | | | | | | | | |
| 4 | Transfer the mixture into syringe | 2 | 2 | 1 | 1 | 2000→3000 | Diluted mixture | 400 | 6 | $^{18}$F-Tracer trapping |
| 5 | Load on the cartridge | 1 | 2 | 1 | 3 | 3000→0 | \ | 100 | 60 | |
| 6 | Withdraw water | 1 | 2 | 1 | 6 | 0→600 | Water | 1000 | 2 | Wash the reactor |
| 7 | Wash the reactor (1st) | 1 | 2 | 1 | 1 | 600→0 | \ | 400 | 5 | |
| 8 | Withdraw the mixture | 1 | 2 | 1 | 1 | 0→1000 | Washed mixture | 400 | 6 | |
| 9 | Load on the cartridge | 1 | 2 | 1 | 3 | 1000→0 | \ | 100 | 20 | |
| 10 | Withdraw water | 1 | 2 | 1 | 6 | 0→600 | Water | 1000 | 3 | Wash the reactor |
| 11 | Wash the reactor(2nd) | 1 | 2 | 1 | 1 | 600→0 | \ | 400 | 5 | |
| 12 | Withdraw the mixture | 1 | 2 | 1 | 1 | 0→1000 | Washed mixture | 400 | 6 | |
| 13 | Load on the cartridge | 1 | 2 | 1 | 3 | 1000→0 | \ | 100 | 20 | |
| 14 | Withdraw water | 1 | 2 | 1 | 6 | 0→1200 | Water | 400 | 8 | Wash the cartridge |
| 15 | washing the cartridge(1st) | 1 | 2 | 1 | 3 | 1200→0 | \ | 100 | 25 | |
| 16 | Withdraw Air | 1 | 2 | 1 | 9 | 0→1500 | Air | 1000 | 4 | Dry the cartridge |
| 17 | dry the cartridge(1st) | 1 | 2 | 1 | 3 | 1500→0 | \ | 400 | 9 | |
| 18 | Withdraw Air | 1 | 2 | 1 | 9 | 0→1500 | Air | 1000 | 4 | |
| 19 | dry the cartridge (2nd) | 1 | 2 | 1 | 3 | 1500→0 | \ | 20 | 9 | |

In order to validate if any activity ($^{18}$F-tracer) breakthrough occurred during purification, the analysis for filtrate in the waste bottle was measured by radio-HPLC. As shown in Fig. S10, there was only 3.2% of the $^{18}$F tracer observed ($t_R$=12.4 min) in the waste bottle. Compared with the crude mixture, there was about 72% of the tracer

seen at the same retention time.

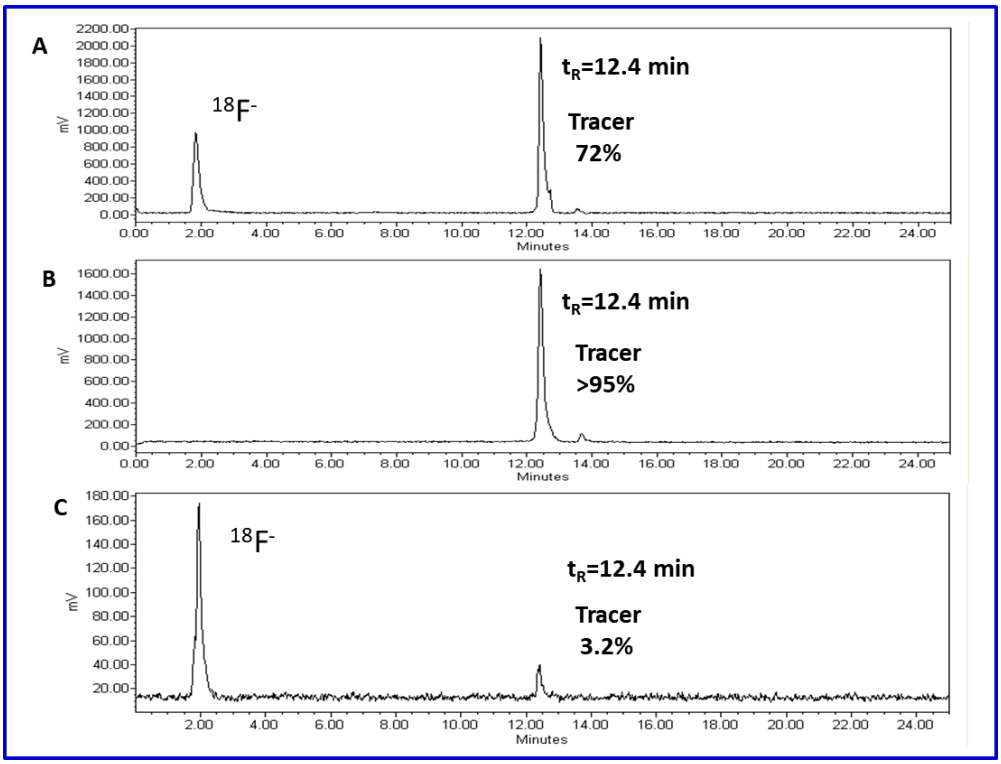



Fig. S10. HPLC chromatograms: (A). Radio channel HPLC profile of labeling reaction crude; (B). Radio channel HPLC profile of $C_{18}$-purified [$^{18}$F]-Alfatide II; (C). Radio channel HPLC profile of the waste after passing through $C_{18}$ column.

4.4 Formulation of sterile injectable solutions

After the tracer trapped on the $C_{18}$ cartridge and dryness, the channel of DV was switched to port 2, the SV1 in the state 2. The eluate (1 mM HCl, 400 uL) was pulled into flow path making the tracer released from the $C_{18}$ cartridge with flow rates (ca. 100 uL/s). Finally, the pipeline was dried to make sure that the elution was transferred into tracer vial completely (Fig. S11A). The final sterile Injectable solution was prepared by adding appropriate volume of saline, in which way the ethanol concentration was limited lower than 10%, and the concentration of the final formulation was no more than 20 mCi/mL (Fig. S11B). Detailed description of the

operation and the programming control was described clearly in Table S5.

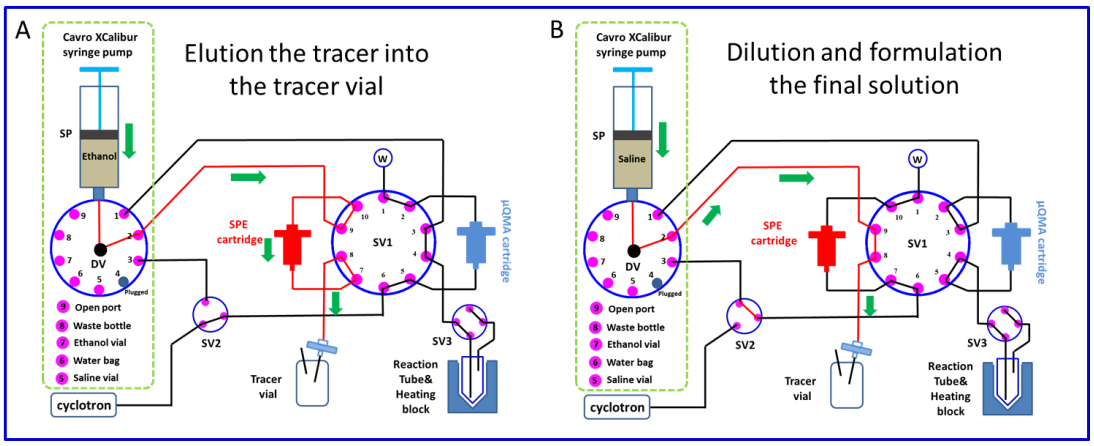



Fig. S11. Schematic representation of the tracer purification unit operation in the platform. The fluid path (A) was for eluting the tracer into the sterile vial with ethanol, and the fluid path (B) was for diluting and forming the final solution with saline.

**Table S5. The detailed synthesis programs for formulation of sterile injectable solutions.**

| Steps | Operation | SV1 | SV2 | SV3 | Channel port | Plunger position | Media in syringe | Flow rate | Duration (s) | script |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Withdraw EtOH | 2 | 2 | 1 | 7 | 0→280 | EtOH | 400 | 3 | $^{18}$F tracer elution |
| 2 | Transfer the EtOH | 2 | 2 | 1 | 2 | 280→0 | EtOH | 50 | 15 | |
| 3 | Withdraw air | 2 | 2 | 1 | 9 | 0→1000 | Air | 1000 | 3 | |
| 4 | EtOH was pass though the tC18 slowly | 2 | 2 | 1 | 3 | 1000→0 | \ | 50 | 42 | |
| 5 | Withdraw saline | 2 | 2 | 1 | 5 | 0→600 | Saline | 400 | 5 | |
| 6 | Washing the cartridge | 2 | 2 | 1 | 2 | 600→0 | \ | 50 | 26 | |
| | | | | | | | | | | |
| 11 | Withdraw saline | 1 | 2 | 1 | 5 | 0→1800 | Saline | 400 | 11 | Dilution and formation |
| 12 | Transfer into the tracer | 1 | 2 | 1 | 2 | 1800→0 | \ | 200 | 24 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | vial | | | | | | | | | |
| 13 | Withdraw air | 1 | 2 | 1 | 9 | 0→1500 | Air | 1000 | 5 | |
| 14 | Dry the pipeline(1st) | 1 | 2 | 1 | 2 | 1500→0 | \ | 200 | 17 | |
| 15 | Withdraw air | 1 | 2 | 1 | 9 | 0→1500 | Air | 1000 | 5 | |
| 16 | Dry the pipeline (2nd) | 1 | 2 | 1 | 2 | 1500→0 | \ | 200 | 17 | |

## 5. Automated radiosynthesis for Al$^{18}$F tracers

5.1 Cleaning the platform

Briefly, the syringe, vales, and the pipeline were washed with ethanol (~5 mL×2) followed by the DI waters (~5 mL×3). Finally, the residue in the flow path was removed and purged to dryness. Detailed descriptions of the operation and the programming control were described clearly in Table S6.

**Table S6. The detailed programs for cleaning the platform.**

| Steps | Operation | SV1 | SV2 | SV3 | Channel port | Plunger position | Media in syringe | Flow rate | Duration (s) | script |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Initialization | 1 | 1 | 1 | 9 | →0 | \ | 1000 | 10 | |
| ***Washing the pipeline with ethanol*** | | | | | | | | | | |
| 2 | Withdraw EtOH | 1 | 1 | 1 | 5 | 0→1200 | EtOH | 400 | 8 | |
| 3 | Wash the pipeline ($^{18}$F trapping) | 1 | 1 | 2 | 3 | 1200→600 | EtOH | 400 | 4 | |
| 4 | Wash the pipeline (tracer trapping) | 1 | 1 | 1 | 3 | 600→0 | EtOH | 400 | 4 | |
| 5 | Withdraw EtOH | 1 | 1 | 1 | 5 | 0→2400 | EtOH | 400 | 14 | |
| 6 | Wash the pipeline (18F elution) | 1 | 1 | 1 | 1 | 2400→1800 | EtOH | 400 | 4 | |
| 6 | Wash the pipeline (tracer elution) | 1 | 1 | 2 | 2 | 1800→1200 | EtOH | 400 | 4 | |
| 7 | Wash the pipeline (tracer dilution) | 1 | 1 | 1 | 2 | 1200→600 | EtOH | 400 | 4 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 | Wash the pipeline (mixture transfer) | 1 | 1 | 2 | 1 | 600→0 | EtOH | 400 | 4 | |
| ***Washing the pipeline with water*** | | | | | | | | | | |
| 2 | Withdraw water | 1 | 1 | 1 | 6 | 0→1200 | Water | 400 | 8 | |
| 3 | Wash the pipeline(18F trapping) | 1 | 1 | 2 | 3 | 1200→600 | Water | 400 | 4 | |
| 4 | Wash the pipeline(tracer trapping) | 1 | 1 | 1 | 3 | 600→0 | Water | 400 | 4 | |
| 5 | Withdraw water | 1 | 1 | 1 | 6 | 0→2400 | Water | 400 | 14 | |
| 6 | Wash the pipeline(18F elution) | 1 | 1 | 1 | 1 | 2400→1800 | Water | 400 | 4 | |
| 6 | Wash the pipeline (tracer elution) | 1 | 1 | 2 | 2 | 1800→1200 | Water | 400 | 4 | |
| 7 | Wash the pipeline (tracer dilution) | 1 | 1 | 1 | 2 | 1200→600 | Water | 400 | 4 | |
| 8 | Wash the pipeline (mixture transfer) | 1 | 1 | 2 | 1 | 600→0 | Water | 400 | 4 | |
| ***Drying the pipeline*** | | | | | | | | | | |
| 2 | Withdraw air | 1 | 1 | 1 | 6 | 0→1200 | Air | 1000 | 4 | |
| 3 | Wash the pipeline(18F trapping) | 1 | 1 | 2 | 3 | 1200→600 | Air | 400 | 4 | |
| 4 | Wash the pipeline(tracer trapping) | 1 | 1 | 1 | 3 | 600→0 | Air | 400 | 4 | |
| 5 | Withdraw air | 1 | 1 | 1 | 6 | 0→2400 | Air | 1000 | 6 | |
| 6 | Wash the pipeline(18F elution) | 1 | 1 | 1 | 1 | 2400→1800 | Air | 400 | 4 | |
| 6 | Wash the pipeline(tracer elution) | 1 | 1 | 2 | 2 | 1800→1200 | Air | 400 | 4 | |
| 7 | Wash the pipeline(tracer dilution) | 1 | 1 | 1 | 2 | 1200→600 | Air | 400 | 4 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 | Wash the pipeline(mixture transfer) | 1 | 1 | 2 | 1 | 600→0 | Air | 400 | 4 | |

5.2 High activity synthesis

Optimized parameters from the unit optimization were programmed as digital code (an editable text file), and further adjustments to the reactant solutions, plumbing and tracers vial were made to further optimize the whole process to allow for radiosynthesis.

After irradiation, the irradiated [$^{18}$O]$H_2O$ (generally between 3.7 and 37.0 GBq) was delivered to the platform and the [$^{18}$F]fluoride was trapped on an anion exchange cartridge (μQMA), which was pre-activated with saline (5 mL) and water (10 mL) and finally purged to dryness. The [$^{18}$F]fluoride was then eluted with 150 uL saline into the reactor which was preloaded with a mixture of 100-200 nmol precursor, 10-20 μL $AlCl_3$ solution (5 mM, in 0.1 M acetate buffer of pH 4.0), 50-100 μL acetate buffer solution (0.5 M, pH 4.0) and 100-200 μL ethanol. The reactor was sealed and the mixture was allowed to react for 10 minutes at 110℃. When the labeling was completed, the reaction mixture was diluted with 4 mL DI water and passed through SPE cartridge (HLB for PSMA and $C_{18}$ for Alfatide II and Octreotide), which was pre-activated with ethanol (5 mL) and water (10 mL) and finally purged to dryness, so as to trapping the Al$^{18}$F tracers. Then, the reactor was washed with 1 mL water for twice to remove the residue in the reactor. Subsequently, the trapped product was washed with 5 mL DI water and purged to dryness. The product was then eluted with 400 uL ethanol from the SPE cartridge, entering into the tracer vial though the filter. And the lines were purged to dryness. After completion of the elution, 3-5 mL saline

was added into the product vial to dilute the fraction of the ethanol. After filtration by one disposable filter, the formulation of a sterile injectable solution was ready for dispensing and direct use.

5.3 Low activity synthesis

The procedure was the same as the “High activity synthesis” except that the low activity of the $^{18}F$ (0.37-1.11 GBq) was used.

5.4 Dry run

The procedure was the same as the “High activity synthesis” except that the reactant solution was just solvent only.

## 6. Quality Control

Radiochemical purity and activity/peptide ratio (total activity in GBq divided by the total amount of peptide in μmoles) of $^{18}F$ tracers were determined by analytical high performance liquid chromatography (HPLC) using an Waters Breeze system (Milford, MA) which includes a waters 1525 binary pump, and a column oven. Ultraviolet (UV) absorption was detected with a Waters 2487 dual wavelength detector at the wavelength of 218 nm in series with a radio-detector (flow-count, Bioscan, Belgium) for radioactivity detection. As mobile phase a gradient system (Solvent A: water (0.1%) TFA; Solvent B: acetonitrile (0.1% TFA); Method A (for $^{18}F$-Alfatide II and $^{18}F$-NOTA- Octreotide analysis)：A reverse phase column Agilent ZORBAX Eclipse Plus $C_{18}$ 4.6×250 mm 5 μm column was used at room temperature (25 $^{o}C$). Between 0-2 min: 15% B, between 2-20 min: from 15% B to 70%, between 21-25 min from 70% B to 15% B) was used with a flow rate of 1 mL/min. Method B

(for $^{18}$F-PSMA-11 analysis)：A reverse phase column Waters XBridge BEH $C_{18}$ 4.6× 100 mm 2.5 μm column was used at room temperature (25$^{o}$C). Between 0-1 min: 10% B, between 1-12 min: from 10% B to 50%, between 13-14 min from 50% B to 10% B) was used with a flow rate of 1 mL/min. Endotoxins were determined using the Endosafe PTS system (Charles-River, Charleston, USA). The pH of injection was measured by pH Meter (SP-2500, SUNTEX, China). The analysis of sterility was entrusted to the tracer group of Department of clinical laboratory, Shandong Cancer Hospital and Institute.

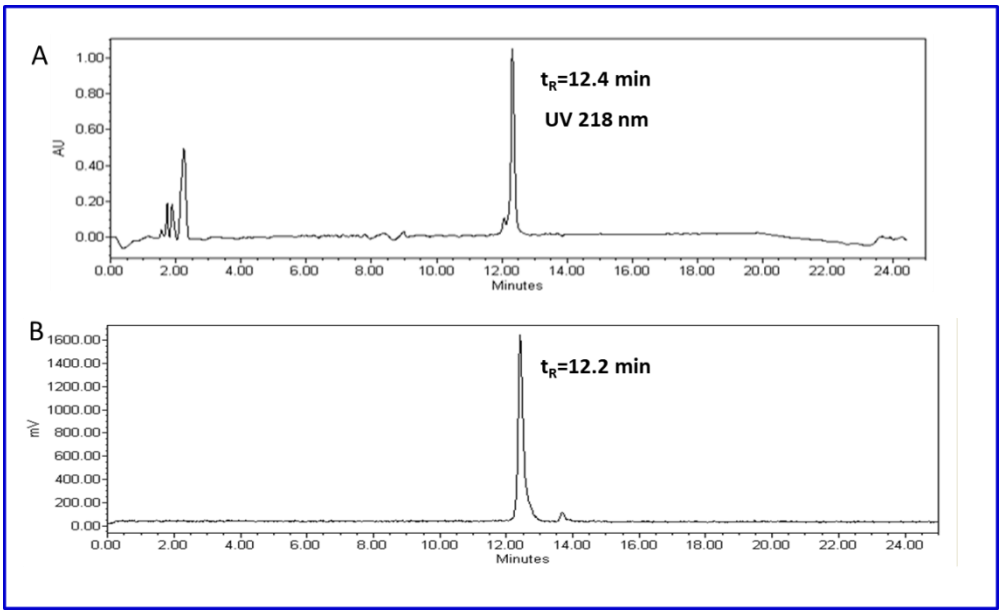



Fig. S12. Co-injection of purified [$^{18}$F]-Alfatide II and [$^{19}$F]-Alfatide II. A was UV channel HPLC profile and B was the radio channel HPLC profile.

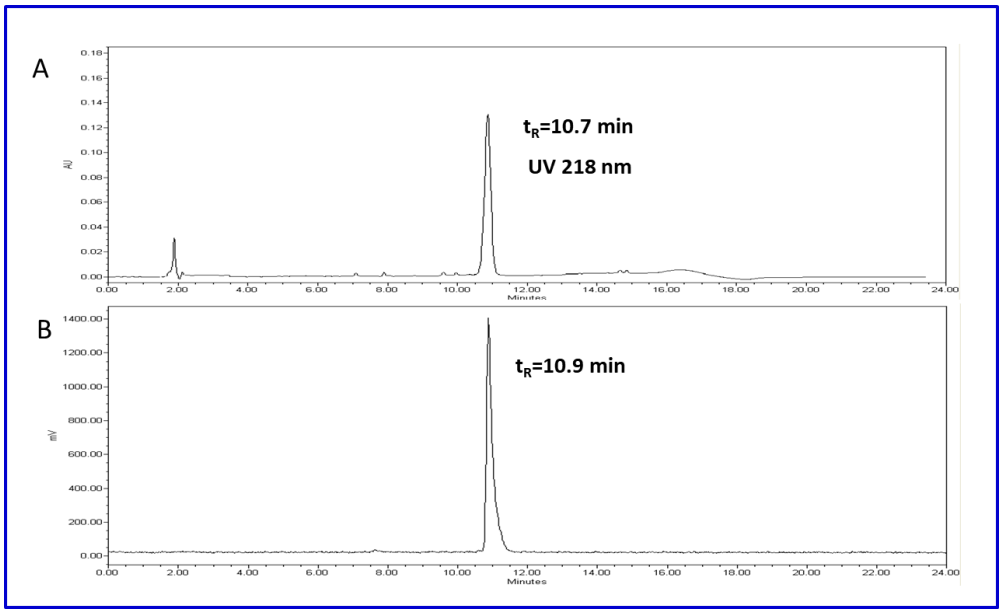



Fig. S13. Co-injection of purified [$^{18}$F]-NOTA-Octreotide and [$^{19}$F]-NOTA-Octreotide. A was UV channel HPLC profile and B was the radio channel HPLC profile.

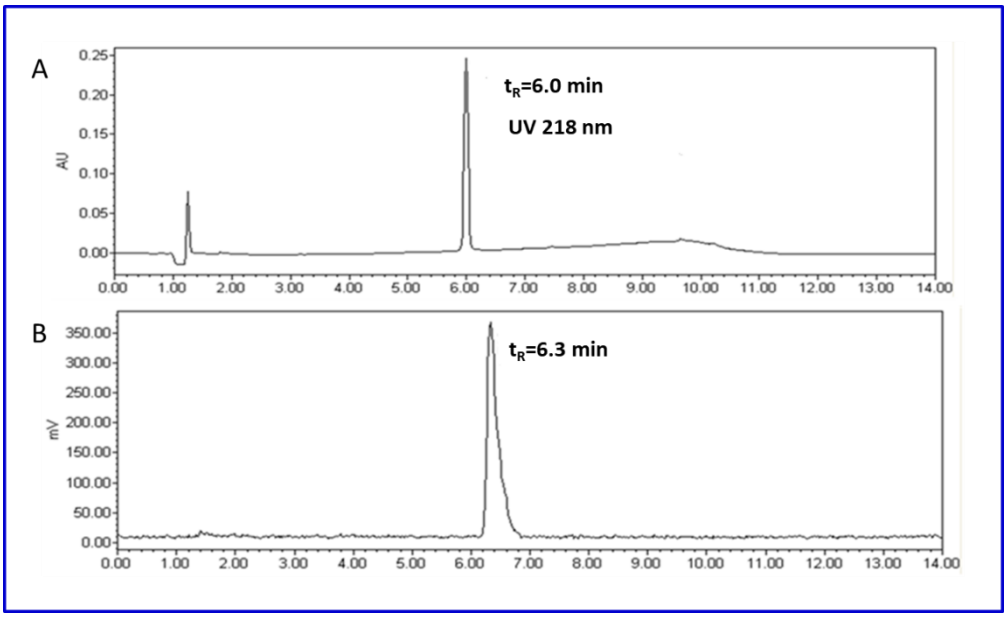



Fig. S14. Co-injection of purified [$^{18}$F]-PSMA-11 and [$^{19}$F]-PSMA-11. A was UV channel HPLC profile and B was the radio channel HPLC profile.

**7. Stability in final injectable solution and serum**

*In vitro* stability of three tracers (13.56-15.34 GBq/μmol) was determined both in saline and in 5% human serum albumin (HSA). For the stability in saline, at room

temperature at time points of 1 h, 2 h, 3 h and 4 h. 50 μL mixture was taken out and analyzed for the radiochemical purity by radio-HPLC. For the stability in HSA, at 37℃ at time points of 0.5 h, 1 h, 1.5 h and 2 h. 100 μL mixture was taken out and treated with 100 μL of acetonitrile, then centrifuged, after which the supernatant was analyzed by radio-HPLC. All tests were repeated in triplicates.

The stability of the product solution (<1.85±0.37 GBq/mL) was determined during 4 h post production (Fig.S15A), verifying a radiochemical purity greater than 90% in the final formulation vial, while in serum stability was kept in this value only during the first 2 h (Fig.S15B). For this reason, PET images should be acquired during this time after tracer administration, which was consistent with previous reports for the pilot clinical studies.

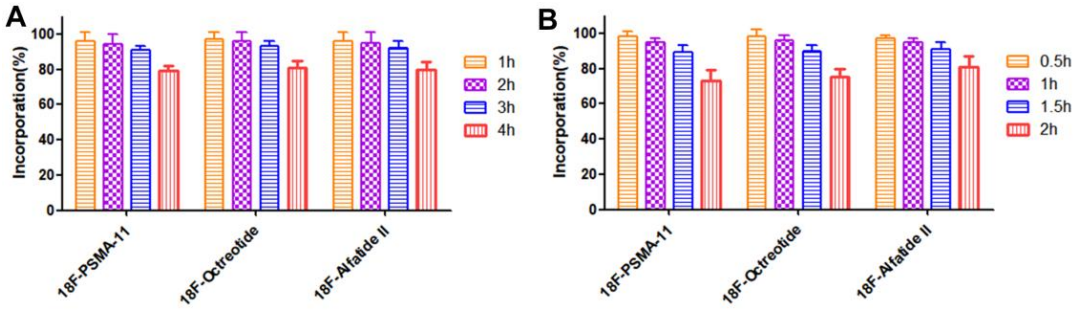



Fig. S15. In vitro stability of $Al^{18}F$ tracers. A: Radio-HPLC patterns of $Al^{18}F$ tracers in saline (1 h, 2 h, 3 h, 4 h); B: Radio-HPLC patterns of $Al^{18}F$ tracers in serum (0.5 h, 1 h, 1.5 h, 2 h). The mobile phase was a mixture of MeCN/0.1% TFA (A) and $H_2O$/0.1% TFA (B); 5%-40% B in 0-20 min; flow rate: 1 mL/min.

**Table S7.** The comparison of the $Al^{18}F$ tracers radiosynthesis using the commercially available module in report literature.

| $Al^{18}F$ Tracers | Amount of precursor | Synthesizer | Reaction volume(mL) | Radio-HPLC(%) | Isolate yield(%) | MA (GBq/μmol) | Duration (min) | Purification & formulation | Reference |
|---|---|---|---|---|---|---|---|---|---|
| NOTA-RGDfK | 60 nmol | GE[a] | 1.0-1.3 | 37.9±1.6 | 17.9±3.9[d] | 5.4±0.8 | 35 | HLB-SPE [f] | [5] |
| NOTA-Octreotide | 60 nmol | GE[a] | 1.0-1.3 | 40.1±6.5 | 29.4±4.6[d] | 6.2±0.5 | 35 | HLB-SPE [f] | [5] |
| NOTA-RGDfK | 60 nmol | AIO[b] | 1.0-1.3 | 48.3±10.6 | 15.3±6.5[d] | 7.4±1.1 | 26 | HLB-SPE [f] | [5] |
| NOTA-Octreotide | 60 nmol | AIO[b] | 1.0-1.3 | 75.0±1.8 | 56.2±4.2[d] | 12.7±0.14 | 26 | HLB-SPE [f] | [5] |
| [$^{18}$F]-PSMA-11 | 60 μg | GE[a] | ~1.7 | <30 | 15.0-22.2[e] | 58-544 | >40 | Semi-HPLC[g] | [6] |
| [$^{18}$F]-PSMA-11 | 200 μg | Synthra[c] | ~1.4 | ~90 | 21.0±3.0[e] | None | 35 | HLB-SPE[h] | [1] |

(a) The model of platform was GE TRACERlab FXFN, (b) was Trasis AIO, and (c) was Synthra FCHOL.

(d) The isolate RCYs for these tracers were calculated at start of synthesis (SOS), and (e) were calculated at end of synthesis (EOS).

(f) The cartridge of HLB was used for purification, while the elution and formation of $^{18}$F-tracer was implemented by hand.

(g) It was carried out the purification by semi preparative HPLC to achieve the final product with the automation during the whole process.

(h) The cartridge of HLB was used for purification, and the elution and formation of $^{18}$F-tracer was implemented automatically by Synthra FCHOL.

**Notes and references**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