Supplemental Data

Optimizing radiolabeling yield of [^{18}F]-Alfatide

We developed a simple lyophilized kit for ^{18}F-labeling with RGD peptide which would provide a useful platform for commercial development. The kit consisted of NOTA-PRGD2 and $AlCl_3$, and their amounts were optimized. Using kits prepared with the optimal amounts of NOTA-PRGD2 peptide and $AlCl_3$, additional studies were conducted to assess the necessary temperature, pH of buffer solution, timing for the labeling procedure, and how the ^{18}F activity would affect labeling yields. The kit contains NOTA conjugated PRGD2 (NOTA-PRGD2) and $AlCl_3$, and the formulation was optimized with regard to reaction temperature, pH of the buffer, reaction time and ^{18}F activity.

Effect of Temperature

The temperature is a key factor in the radiolabeling procedure. The kits did not react with ^{18}F when the temperature hadn't reached 80°C. The radiolabeling yield significantly increased when it was higher than 100°C. Indeed, extending the temperature to 120°C did not improve the yield appreciably, and the optimum reaction temperature was 100°C (Supplemental Fig. 1).

Effect of pH

The radiolabeling is pH sensitive. When the pH was lower than 4, the radiolabel yield kept nearly 40%. The optimal pH for labeling was between 2 and 4. The yield fell rapidly outside the ideal pH value range (Supplemental Fig. 2).

Effect of Amounts of NOTA-PRGD2

The effect of amounts of NOTA-PRGD2 on the labeling efficiency was also investigated. The kits with 6 nmol $AlCl_3$ and 8, 16, 24 or 32 nmol NOTA-PRGD2 were tested when 1,110 Mq of ^{18}F (100 μL) and acetate acetonitrile buffer solution with same volume (pH = 4), respectively,

were added. The reaction solution was heated to 100 °C for 15 min. The radiolabeling yield increased with increasing amounts of peptide. The optimal amount of NOTA-PRGD2 in the kits was 16 nmol. Excessive amounts of peptide will decrease the yield and the specific activity of product (Supplemental Fig. 3).

Effect of Amounts of $AlCl_3$

The added amount of $AlCl_3$ is also critical in the labeling procedure. The kits with 2, 4, 6, 12 or 16 nmol $AlCl_3$ was applied to labeling. Radiolabeling yield reached a plateau as 40.1 ± 2.5% after incubation with 6 nmol or more $AlCl_3$. Reducing the $AlCl_3$ concentration resulted in lower yields, ranging from 18.4 ±2.8% at 1.5 nmol to 31.6 ±3.1% at 3 nmol of $AlCl_3$. Increasing the amount of $AlCl_3$ did not further improve the yield. Incubation with 60 nmol of $AlCl_3$ resulted in a radiolabeling yield of only 14.8 ± 3.0% (Supplemental Fig. 4).

Effect of Reaction Time

The effect of reaction time was studied with 6 nmol $AlCl_3$ and 16 nmol NOTA-PRGD2 in kits labeled with ~1,110 MBq $^{18}F^-$ in 100 μL of 1:1 DI H_2O/ethanol at 100°C for 5 - 30 min. Maximum of yields were obtained after 10 min of heating. Indeed, extending the incubation period to 30 min did not improve the yield appreciably (Supplemental Fig. 5).

Effect of ^{18}F Activity

The labeling yield kept consistent when the amount of ^{18}F was 20-50 mCi. Higher amounts of ^{18}F (greater than 50 mCi) decreased the labeling yield since excessive product that stayed in the C18 cartridges could not be eluted by additional 10 mM HCl ethanol solution (Supplemental Fig. 6).

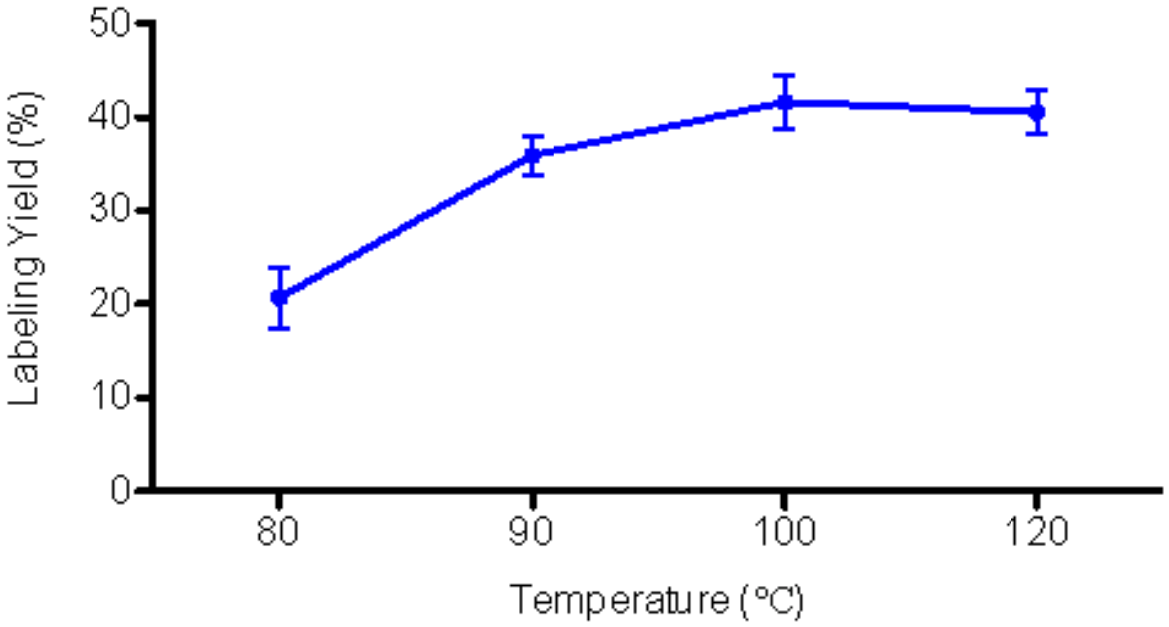
In summary, the optimal kits were made with 8 nmol NOTA-PRGD2 and 6 nmol $AlCl_3$•$6H_2O$ stock solutions, and the optimal reaction procedure was the following: $^{18}F^-$ (20-50

mCi) in 100 to 200 μL DI water and the same volume of sodium acetate ethanol solution (pH 4) were added to the crimp-sealed vial, and then heating to 100 °C for 10 min.

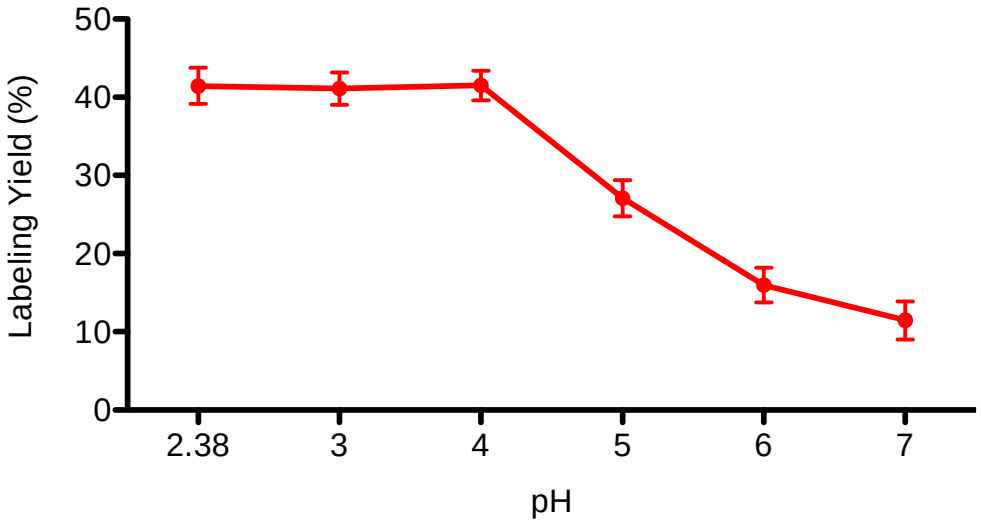
Under the optimal condition, the whole radiosynthesis including purification was accomplished within 20 min with a decay-corrected yield of 42.1 ± 2.0% and radiochemical purity of more than 95% (Supplemental Fig. 7). The specific activity of [^{18}F]-Alfatide was calculated to be at least 1000 mCi (37GBq)/μmol.

In Vitro Stability of [^{18}F]-Alfatide

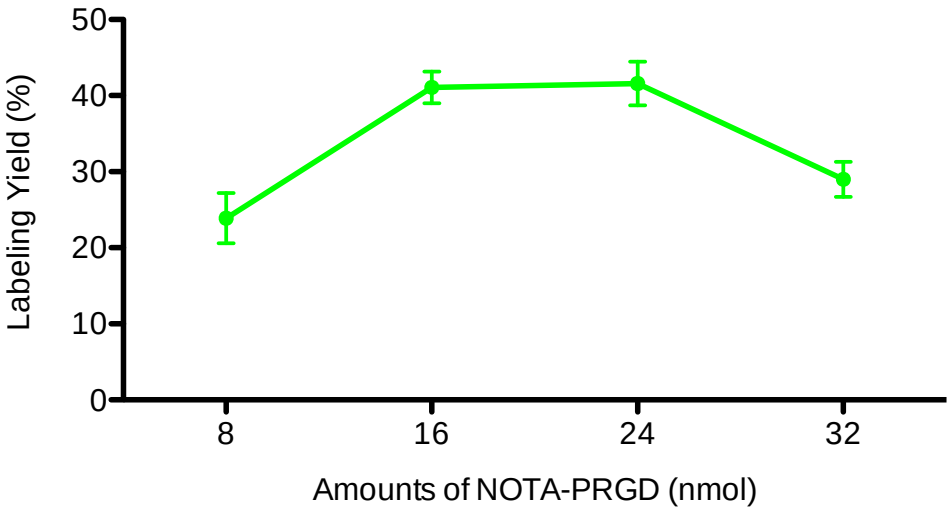
[^{18}F]-Alfatide was stable in saline both at 37 °C and 4°C. After two half-lives, the radiochemical purities were still greater than 95% as evidenced by HPLC analysis (Supplemental Fig. 8).



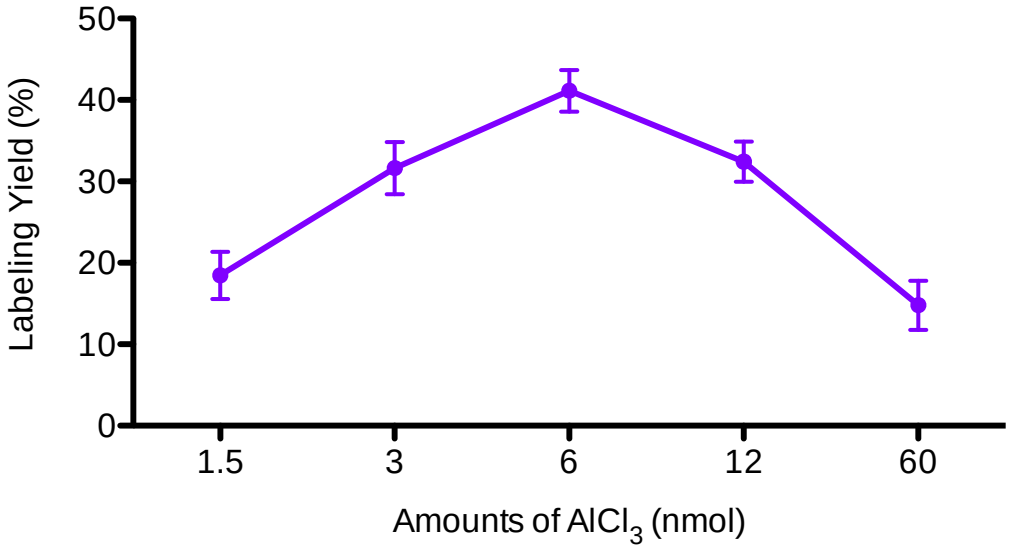
SUPPLEMENTAL FIGURE 1. Effect of reaction temperature on the labeling yield (n = 5).



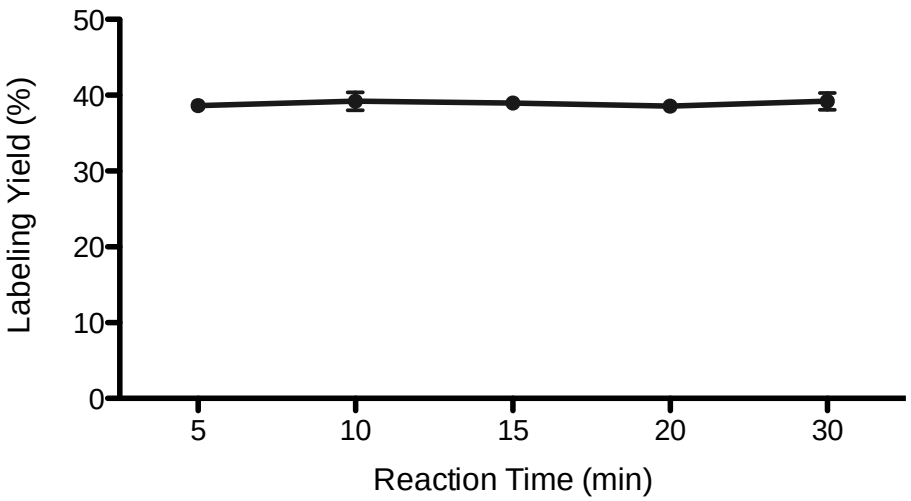
SUPPLEMENTAL FIGURE 2. Effect of pH value on the labeling yield (n = 5).



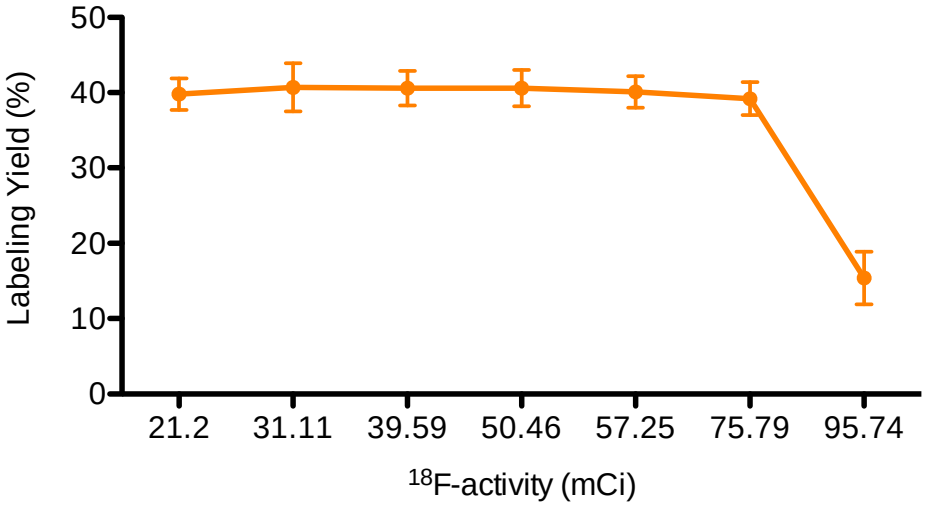
SUPPLEMENTAL FIGURE 3. Effect of amount of NOTA-PRGD2 (nmol) on the labeling yield (n = 5).



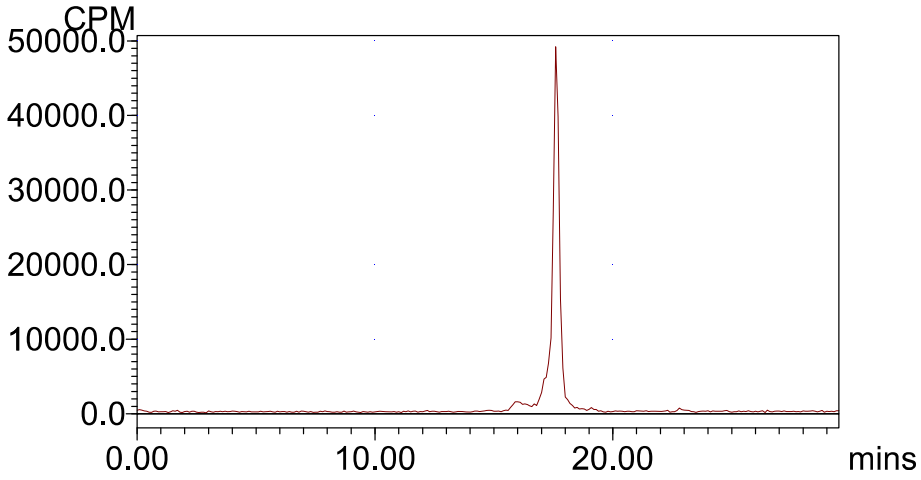
SUPPLEMENTAL FIGURE 4. Effect of amount of $AlCl_3$ (nmol) on the labeling yield (n = 5).



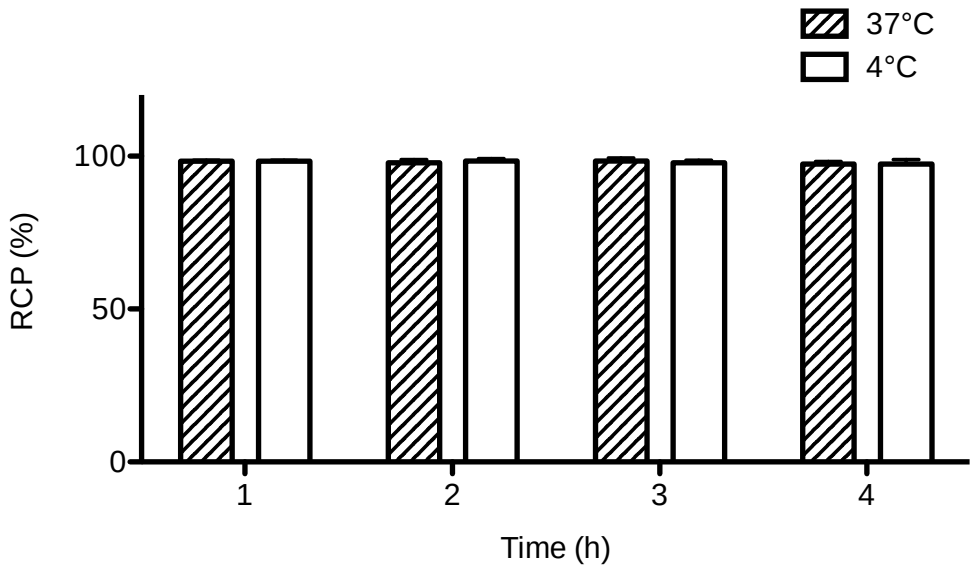
SUPPLEMENTAL FIGURE 5. Effect of reaction time on the labeling yield (n = 5).



SUPPLEMENTAL FIGURE 6. Effect of ^{18}F activity (mCi) on the labeling yield (n = 5).



SUPPLEMENTAL FIGURE 7. HPLC result of [^{18}F]-Alfatide.



SUPPLEMENTAL FIGURE 8. In vitro stability results of [^{18}F]-Alfatide.