Electronic supplementary material

UHPLC Gradient

Table 1: UHPLC Gradient.

Time (min)	Flow (mL/min)	0.1%TFA (A %)	Methanol (B %)
0	0.5	100	0
0.14	0.5	100	0
0.2	0.5	55	45
2.36	0.5	40	60
2.50	0.5	0	100
2.80	0.5	0	100
2.90	0.5	100	0
3.50	0.5	100	0

Data analysis

The kinetic data was fitted non-linearly using a second-order kinetic model (neglecting the different protonated species of DOTA-TATE)^{1, 2}. The kinetic association rate k for such a model is expressed as given in Equation 1^3 :

$$k = \frac{ln\left(\frac{[A] \cdot [B_0]}{[A_0] \cdot [B]}\right)}{([A_0] - [B_0])t} \quad (1)$$

with $[A_0]$ and $[B_0]$ being the starting concentrations of both reactants, and [A] and [B] the concentrations at time t. Equation 1 can be reformulated to give the concentration of the product C at any given time point:

$$[C] = 1 - \frac{[A_0] - [B_0]}{[A_0] - [B_0] \cdot e^{-kt([A_0] - [B_0])}} \quad (2)$$

The kinetic association rate constants obtained for different temperatures were fitted non-linearly using the Arrhenius equation³:

$$k = A \cdot e^{-\frac{E_A}{R \cdot T}} \quad (3)$$

in which T is the absolute temperature (K), A is the pre-exponential factor ($M^{-1} s^{-1}$), E_A is the activation energy (J mol⁻¹), and R is the universal gas constant (R = 8.3145 J mol⁻¹ K⁻¹).

Determination of diffusion coefficients

To simulate the microfluidic synthesis setups, diffusion coefficients were required as input parameters. The diffusion coefficient of Lu^{3+} ($D = 2.14 \times 10^{-9}$ m² s⁻¹ at 20°C) was calculated based on the Stokes-Einstein equation⁴. However, the diffusion coefficients for DOTA-TATE or Lu-DOTA-TATE were also not available, and calculations based on known equations to estimate the diffusion coefficient gave a too large range of possible values ($10^{-10}-10^{-12}$ m² s⁻¹)⁵⁻⁷. The diffusion of [¹⁷⁷Lu]Lu-DOTA-TATE was, therefore, measured using a method described by Miložič et al⁸: two solutions (0.43 M sodium ascorbate and 0.11 M gentisic acid in 0.05 M HCl), of which only one contained 0.26 mM [¹⁷⁷Lu]Lu-DOTA-TATE, were pushed at flowrates of 1-20 uL/min through an H-channel chip (TOPAS, 75/150, Microfluidic ChipShop). The final concentrations, measured at the two outlets using a shielded well type Nal(Tl) counter coupled to a 2048 analyser (Wallac Wizard, PerkinElmer), depend on the diffusion of the compound and the applied flowrates. A diffusion coefficient of $1.9\pm0.4\times10^{-10}$ m² s⁻¹ at 20±1°C was determined for [¹⁷⁷Lu]Lu-DOTA-TATE by fitting the data to a computational model made by COMSOL Multiphysics[®]. The diffusion coefficient of DOTA-TATE was

approximated to be the same as the one for [¹⁷⁷Lu]Lu-DOTA-TATE. All diffusion coefficients were adjusted to the required temperatures by using the Stokes-Einstein equation⁴.

Considerations regarding reaction rate determination

Several studies have shown that it is possible to calculate reaction times in continuous flow systems with laminar flow regime based on the total flow rate and the tubing length in which the reaction takes place⁹⁻¹⁴. However, since this conclusion is reaction- and setup-dependent, a few considerations have to be made before using the here studied microfluidic setup to determine the formation rate of [¹⁷⁷Lu]Lu-DOTA-TATE:

Formation of [177Lu]Lu-DOTA-TATE. The formation of metal-DOTA complexes is a process involving several species (depending on the protonation status of the carboxylic groups on the DOTA molecule) and also reversible intermediate forms^{2, 15}. The overall reaction is considered a second order reaction², and the second order kinetic association rate constant for Lu-DOTA at 25°C and pH 4.2 has been determined previously by capillary electrophoresis¹. Although the formation of [¹⁷⁷Lu]Lu-DOTA-TATE has been studied extensively before^{16, 17}, to the best of our knowledge no kinetic rate constants are known.

The reaction taking place in the microfluidic setup can be fully attributed to the formation of [¹⁷⁷Lu]Lu-DOTA-TATE. Any dissociation during the experiments can be neglected due to the inert character of the complex¹⁸. Additionally, all experiments in this study have been done with non-radioactive lutetium or activities of \leq 1 MBq per reaction, which means that decomposition of the final complex due to radiolysis is not expected.

Another point to consider is that the applied reaction time and pH can determine which Lu-H_nDOTA-TATE (n=0-2) species is formed. This is relevant due to different stabilities of these species¹⁸. The microfluidic setup allows for very short reaction times which could lead to the formation of different species than in the conventional process. To investigate this, we tested the stability of the formed complex in the reaction solution over several days. No dissociation of the Lu-DOTA-TATE complex was detected. Based on dissociation kinetics reported previously for the different species¹⁸, we concluded that the same species are formed as in the conventional radiolabelling process.

Heating of reaction channel. All calculations done within this study are based on the assumption that the only place where the reaction occurs is within the heated part of the reaction capillary. This means that no reaction should happen at room temperature, which is not expected for [¹⁷⁷Lu]Lu-DOTA-TATE based on previous experiments in conventional systems¹⁷ and was verified by pushing both reactant solutions through the non-heated microfluidic setup and analysing the resulting reaction mixture. No formation of [¹⁷⁷Lu]Lu-DOTA-TATE could be detected, even if the reaction mixture was left standing for several hours.

Another important aspect is the heat transfer process. Since the kinetic association rate constant depends on the temperature, the reactant solutions should reach the desired temperature as soon as they reach the heated part of the reaction capillary. The length of capillary required can be calculated by Equation 10¹⁹:

$$ln\left(\frac{T_W - T_0}{T_W - T_x}\right) = \frac{4hx}{\rho\langle v \rangle C_p D_H} \quad (10)$$

In Equation 10, the hydraulic diameter (D_H) of the heated capillary is 100 µm, the density (ρ) of the solution is 1000 kg m⁻³ and the specific heat capacity (c_p) of the solution is 4.2×10³ J kg⁻¹ K⁻¹ (assumed as pure water). T_w, T₀, and T_x represent the temperature of the capillary wall (59.5-88.5°C), the temperature of the solution before it enters the heated part of the reaction capillary (regarded as room temperature of 20°C), and the temperature of the reaction solution with respect to the position x along the heated part of the capillary, respectively. T_x equals T₀ at the beginning of the heated capillary (x=0). The heat transfer coefficient (h) can be calculated from Equation 11, where *Nu* is the dimensionless Nusselt number, and the thermal conductivity (λ) of the solution is 0.6 J m⁻¹ s⁻¹ K⁻¹.

$$h = Nu \frac{\lambda}{D_H} \quad (11)$$

In the case of fully developed laminar flow in a circular tube, for simplification, *Nu* can be regarded as a constant with a value of 3.66.

As the solution flows through the heated part of the capillary, its temperature rises from T_0 to T_w . Since the length needed to achieve the desired temperature, T_w , increases with the average velocity, we only calculated the required length for the highest applied velocity (0.25 m/s, equaling 120 µL/min in a capillary with 100 µm ID) and the highest applied temperature (88.5°C). In this case, the required length for the solution to reach the temperature of the capillary walls is 0.59 cm. Compared with the total length of the reaction channel (0.96-1.16 m), this is negligible. However, we added an uncertainty of ±2 cm to the length of the heated capillary when analysing the experimental data obtained in this study.

Mixing performance. To investigate the mixing performance, we simulated two systems: one where the reactants are statically mixed at the beginning of the reaction channel with the help of a frit, and a normal tee where the reactants reach the reaction channel through two separate inlets. For a channel of 100 μ m inner diameter, the simulations gave similar results for the achieved yield at each flow rate, with the largest deviation being 1.1% between both systems. To confirm the simulations, we experimentally studied the use of the normal tee instead of a static mixing tee with a frit in our setup. The only difference to the simulations was a distance of 3.5 cm between the exit of the tees and the inlet of the heated part of the reaction capillary. The experimental results agreed with the simulations and showed only small differences at higher flowrates between the normal and mixing tee setup (data not shown). These differences were smaller than the experimental uncertainty. Based on both the simulation and the experimental results, we could conclude that the use of a mixing tee has no significant influence on the obtained results (constraints: inner diameter of 100 μ m, flowrates 15-120 μ L/min).

Diffusion limitation. When the two solutions enter the reaction channel, diffusion is the only mechanism that can mix both since the flow is laminar. To be able to measure the reaction constant properly, we therefore must ensure that the diffusion time scale is negligible compared to the one of the reaction. To this purpose, we calculated the Damköhler number $(Da)^{20}$. This is a dimensionless number, which denotes if a reaction is limited by the reaction rate or by diffusion. It can be represented as follows:

$$Da = \frac{\tau_D}{\tau_r} = \frac{kcD_H^2}{D} \quad (12)$$

where k is the kinetic rate constant of the reaction, and c the initial concentration of the reactant with the lower

 $\tau_D = \frac{D_H^2}{D}$ represents a diffusion time scale, and $\tau_r = (k \cdot c)^{-1}$ a time scale for the chemical reaction. Generally, if Da < 1, then the overall reaction is limited by the reaction rate, and if Da > 1, then the reaction is in the diffusion-limited regime. The Damköhler number for our setup (100 µm ID, 80°C) is 0.12, which means that the reaction is dominated by the reaction rate, and diffusion of the reagents is negligible when analysing the experimental data.

Konermann et al. not only demonstrated that it is possible to measure the reaction kinetics under continuous laminar flow conditions^{9, 11-13}, but considered theoretically both the influence of diffusion and of the variation of flow velocity across the channel on the observed kinetics⁹. Konermann proposed that laminar flow does not bring a noticeable influence on the measured reaction kinetics, if the following equation holds true:

$$t > \frac{D_H^2}{144\text{D}} \quad (13)$$

where t is the residence time. For our setup (diffusion coefficient for DOTA-TATE, 100 μ m ID, 80°C), this would mean that the effect of laminar flow can be ignored as long as the residence time is longer than 0.1s.

Arrhenius plot



Based on the linear line fitted to the data points (Y = -(14498±617.5) * X + (47.69±1.778), R²=0.9946), the Arrhenius parameters can be calculated: $E_A = -$ slope * R = 120.5±5.1 kJ/mol; A = e^{y-intercept} = 5.15±9.15x10²⁰ M⁻¹s⁻¹.

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