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Fast and Accurate Diffusion NMR Acquisition in Continuous Flow

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Supplementary Information

Contents

1. Materials and methods

All NMR data were acquired on a Bruker AVIII 500 MHz spectrometer equipped with a Prodigy cryoprobe or a Bruker BBO probe. FlowNMR measurements were performed using an InsightMR flow tube.

Chloroform (Fisher, stabilized with amylene or VWR, stabilized with ~0.6% ethanol), mesitylene (Acros), 18-crown-6 (Aldrich), adamantanecarboxylic acid (Aldrich), cyclohexane (VWR) and hexamethyldisiloxane (Alfa Aesar) were used without purification.

2. Experimental details

a. Flow DOSY method development: Δ, δ, and flow rate

FlowDOSY test solution: A single solution of five molecules with different diffusion coefficients was prepared in $CHCl_3$ (7.50 mL) with each species at 0.02 M concentration; quantities are shown in Table S1. The solution was syringe filtered prior to use.

Table S1. Components of flow DOSY test solution, in $CHCl_3$ (7.50 mL). Volumes are given for liquids.

Component	Mass / mg	Volume / µL
Mesitylene	18.0	21.0
18-crown-6	39.6	
Adamantanecarboxylic acid	27.0	
Cyclohexane	12.6	16.2
Hexamethyldisiloxane	21.6	

The heat exchangers controlling the temperature of the flow tube and flow path were set to 298 K, to match the temperature of the probe. A Bruker InsightMR flow tube with entirely PEEK tubing was used for these experiments. The test solution was circulated to fill the flow system using a Vapourtec SF-10 V-3 peristaltic pump.

For each experiment, the pump was set to selected flow rates between 0.0 and 10.0 mL min^{-1}. DOSY spectra were acquired at each flow rate using a convection compensated dstebpgp3s pulse sequence with a smooth rectangle gradient pulse. For each DOSY spectrum, 16 diffusion experiments each with 16 scans were performed, in a linear ramp. The diffusion delay, Δ or d20, was set to a value between 0.010 and 0.128 s, and the gradient pulse length, p30, was set between 500 and 1250 µs (δ = 1.0 – 2.5 ms). Each DOSY acquisition took approximately 10 min 40 s. After phase and baseline correction in TopSpin, diffusion data were processed in Dynamics Center to obtain diffusion coefficients for all molecules present in solution.

These experiments were repeated with different values of the DOSY acquisition parameters d20 and p30, as shown in Table S2.

Table S2: DOSY acquisition parameters chosen to map out the effects of flow on diffusion data.

Experiment	**d20**	**p30**
A	0.05	1000
B	0.10	707
C	0.025	1000
D	0.05	707
E	0.10	500
F	0.064	1250
G	0.128	884
H	0.010	1250
J	0.015	1250

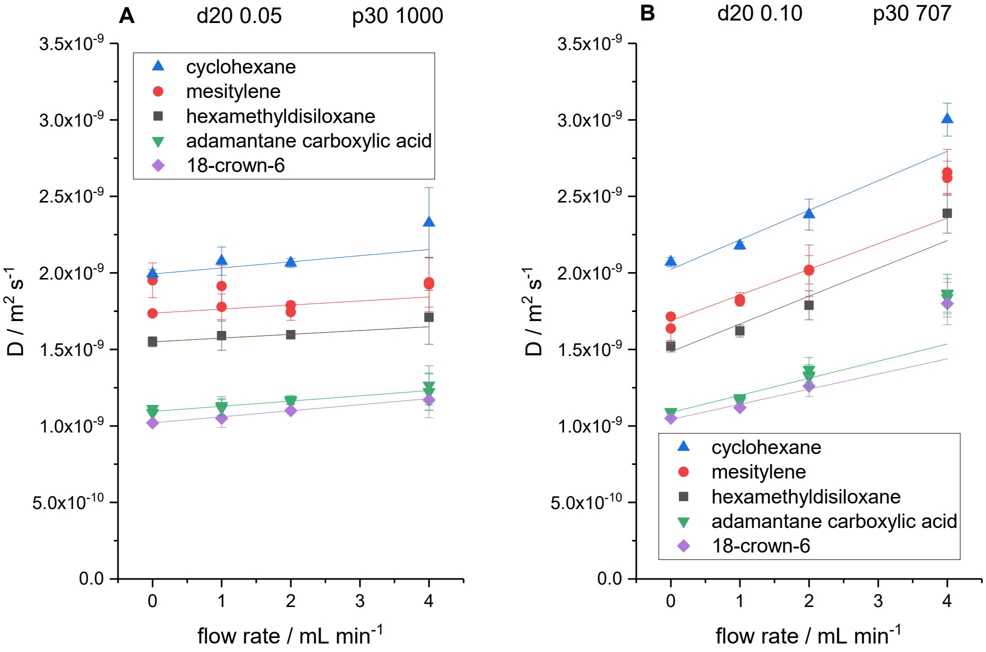


Figure S1: Flow diffusion coefficients obtained in experiments A and B.

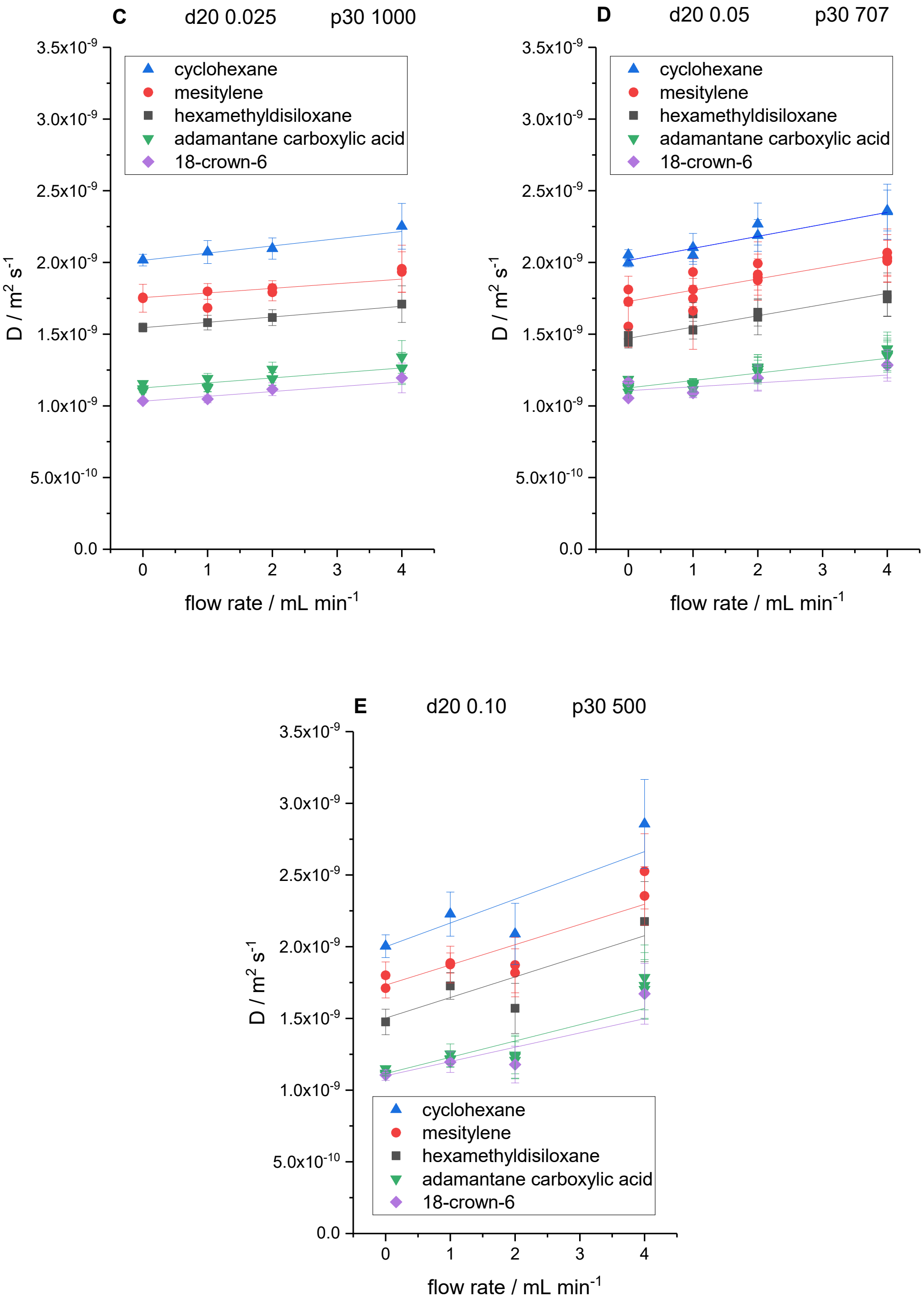


Figure S2: Flow diffusion coefficients obtained in experiments C, D and E.

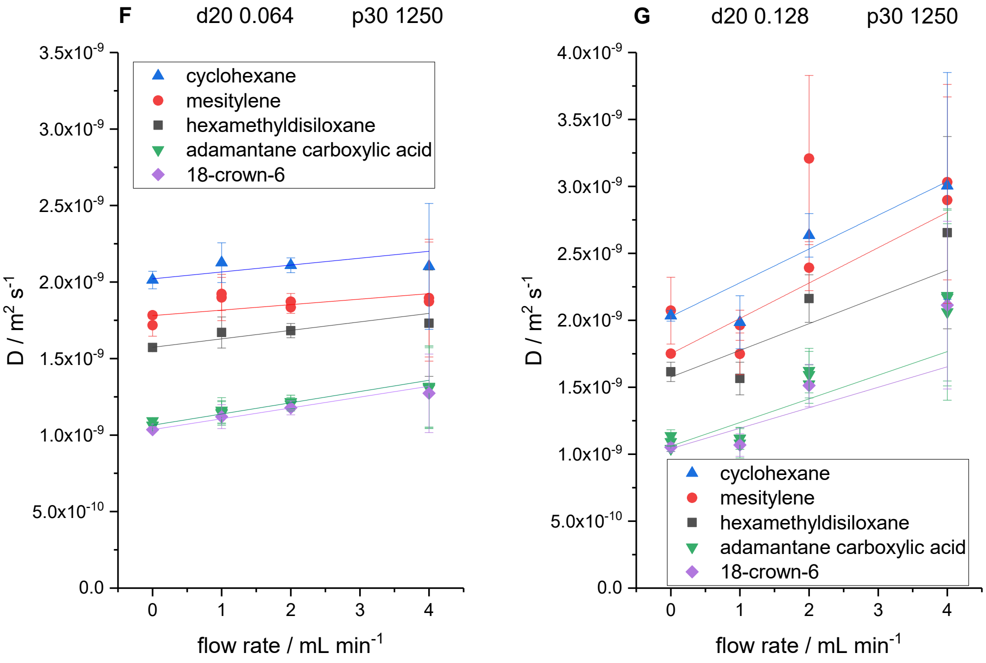


Figure S3: Flow diffusion coefficients obtained in experiments F and G.

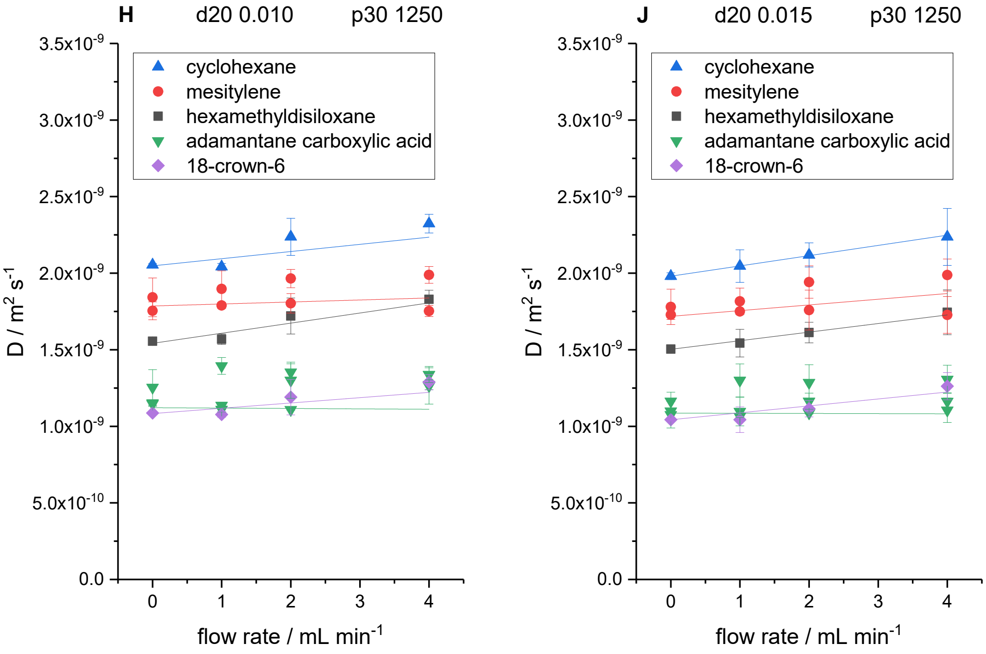


Figure S4: Flow diffusion coefficients obtained in experiments H and J.

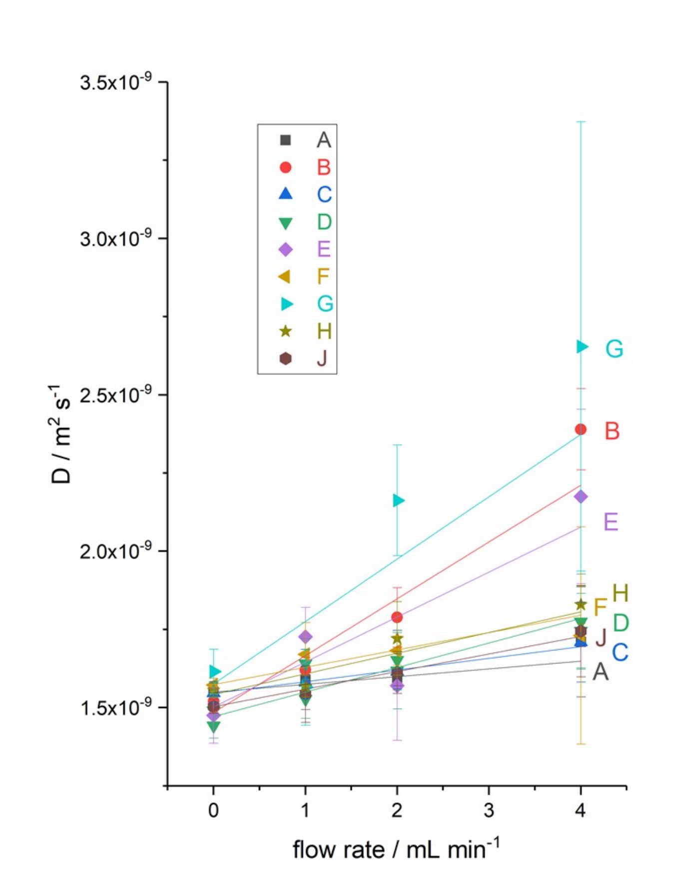


Figure S5: Observed diffusion coefficients for hexamethyldisiloxane as measured with DOSY parameters A – J.

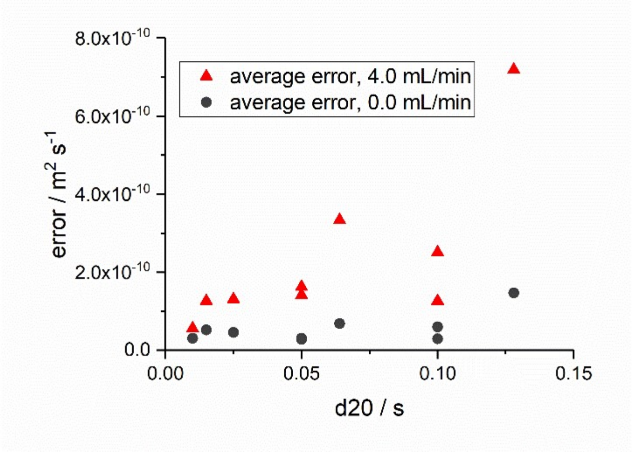
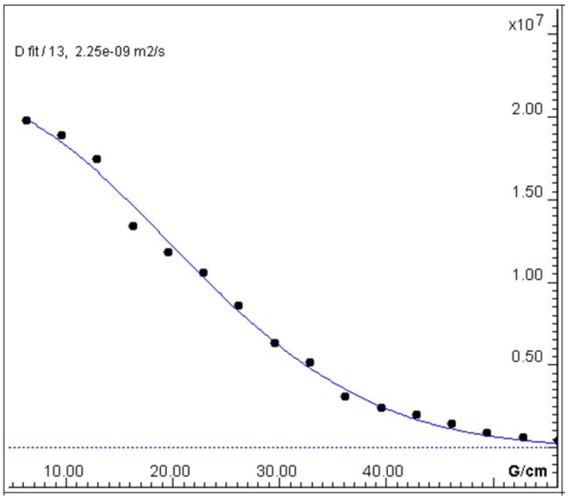
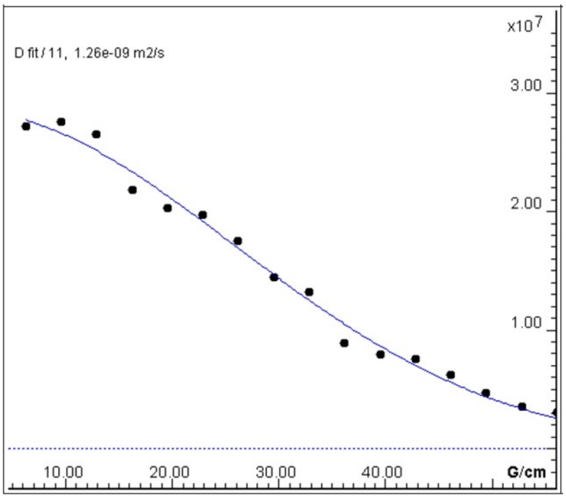
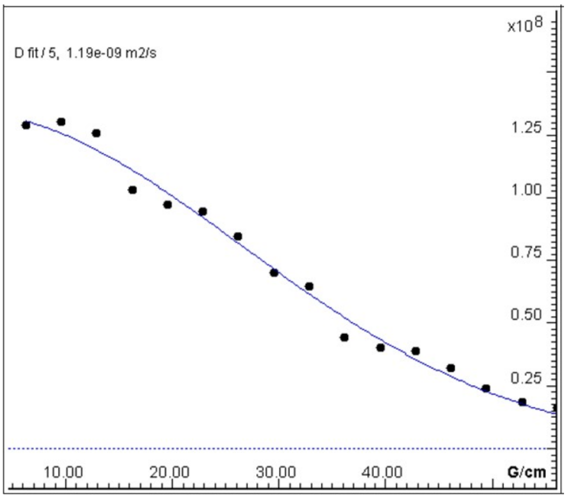
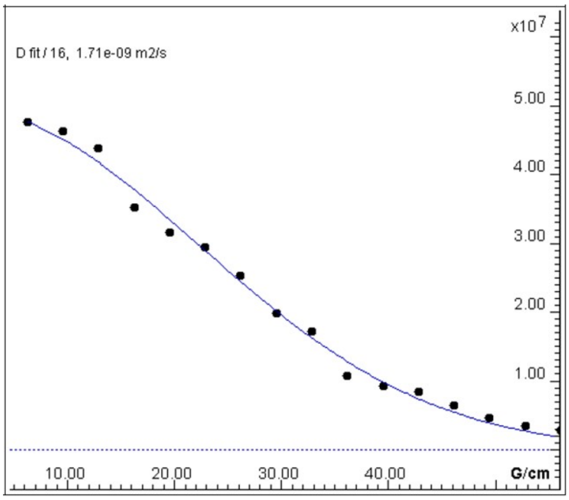
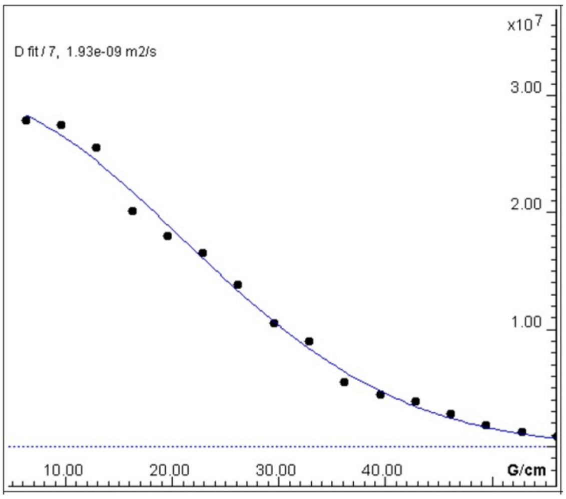


Figure S6: Comparison of errors associated with diffusion coefficients measured on static and flowing (4.0 mL min^{-1}) samples, as a function of d20. Errors are averaged across all five molecules.

Table S3: Representative fit curves for analysis of data acquired at 4.0 mL min^{-1} with acquisition parameters C (Δ =0.025 s and $\delta/2$ =1000 µs).

Cyclohexane  	Adamantanecarboxylic acid 
18-crown-6  	Hexamethyldisiloxane 
Mesitylene  	

b. Optimising experiment time: relaxation delay, acquisition time and flip angle

Experiments to minimise acquisition time by reducing d1 and AQ were carried out on a static sample of the DOSY test solution (0.5 mL, Table S1) in a conventional 5 mm NMR tube. The convection compensated DOSY experiment dstebpgp3s was used, with 16 gradient steps and 16 scans. The relaxation delay, d1, was first reduced while keeping the acquisition time, AQ, constant (Table S4, entries 1 to 3), then AQ was reduced to 1 s while keeping the lowest value of d1 (entry 4). Finally, using the smallest value of d1 and AQ, the flip angle was changed by setting p0 to $\frac{x^\circ}{90}$ x 12 where x° is the desired flip angle (entries 4 to 7).

Table S4: Acquisition parameters used in experiments to determine effect of shortening d1 and AQ.

entry	d1 / s	AQ / s	Flip angle / °	Experiment time
1	1	1.3631488	90	10 min 41 s
2	0.5	1.3631488	90	8 min 28 s
3	0.0000005	1.3631488	90	6 min 15 s
4	0.0000005	1	90	4 min 39 s
5	0.0000005	1	80	4 min 39 s
6	0.0000005	1	70	4 min 39 s
7	0.0000005	1	60	4 min 39 s

Diffusion coefficients and associated errors were obtained for all spectra by curve fitting in Dynamics Center. Signal-to-noise values for entries 4 to 7 were measured using the sinocal command in TopSpin, with the noise region set to a 1 ppm width in the range 10 – 8 ppm, and the signal region set to a small, separate region containing each peak of interest.

The values of diffusion coefficient for entries 1–3 were found to remain the same, within error, for all molecules in the solution at all three d1 values (Table S5). This confirms the 1 second relaxation delay is not necessary for obtaining accurate diffusion data with this experiment, and shortening the delay is acceptable if a fast DOSY is required. The reduction in AQ (entry 4) also has little or no effect on the observed diffusion coefficients. Improvement in signal to noise at 80° (entry 5) compared with 90° (entry 4) was very small, and there was no significant change in measured diffusion coefficient for the short DOSY experiment compared with the original longer acquisition. Smaller flip angles yielded lower signal:noise (Table S6).

Table S5: Diffusion coefficients and associated errors for entries 1-7 in Table S4.

Entry		1		2		3		4		5		6		7	
	δ / ppm	D / m^2 s^{-1}	error	D / m^2 s^{-1}	error	D / m^2 s^{-1}	error	D / m^2 s^{-1}	error	D / m^2 s^{-1}	error	D / m^2 s^{-1}	error	D / m^2 s^{-1}	error
Mesitylene1	6.83	1.74E-09	6.47E-11	1.75E-09	7.37E-11	1.72E-09	6.97E-11	1.74E-09	1.35E-10	1.74E-09	1.73E-10	1.71E-09	1.6E-10	1.76E-09	1.72E-10
18-crown-6	3.71	1.03E-09	4.96E-12	1.03E-09	4.56E-12	1.03E-09	5.27E-12	1.03E-09	4.84E-12	1.02E-09	3.53E-12	1.03E-09	7.21E-12	1E-09	9.08E-12
Mesitylene2	2.30	1.73E-09	1.03E-11	1.72E-09	1.01E-11	1.72E-09	1.01E-11	1.7E-09	9.74E-12	1.69E-09	8.76E-12	1.69E-09	8.2E-12	1.73E-09	1.89E-11
Adamantane1	2.06	1.12E-09	1.46E-11	1.1E-09	1.63E-11	1.11E-09	1.7E-11	1.07E-09	1.27E-11	1.08E-09	1.85E-11	1.06E-09	1.26E-11	1.11E-09	2.29E-11
Adamantane2	1.94	1.1E-09	1.21E-11	1.1E-09	1.26E-11	1.1E-09	1.25E-11	1.07E-09	1.5E-11	1.07E-09	2.04E-11	1.07E-09	1.26E-11	1.08E-09	1.68E-11
Adamantane3	1.75	1.1E-09	1.42E-11	1.1E-09	1.51E-11	1.1E-09	1.46E-11	1.06E-09	1.22E-11	1.06E-09	1.61E-11	1.06E-09	1.19E-11	1.06E-09	8.68E-12
Cyclohexane	1.45	1.99E-09	3.79E-11	1.98E-09	4.12E-11	1.95E-09	4.73E-11	1.88E-09	6.14E-11	1.89E-09	4.83E-11	1.86E-09	6.47E-11	1.85E-09	4.81E-11
Hexamethyldisiloxane	0.09	1.43E-09	3.33E-11	1.42E-09	3.37E-11	1.41E-09	3.44E-11	1.43E-09	3.63E-11	1.45E-09	2.51E-11	1.42E-09	3.62E-11	1.42E-09	3.46E-11

Table S6: Calculated values of signal-to-noise ratio for the first gradient step of each DOSY experiment with flip angles between 90° and 60°.

Signal range	Molecule	90°	79.5°	70°	60°	Signal-to-noise at 80° wrt 90°
4-3.5	18-crown-6	23569.4	25019.1	24412.3	23532.63	1.061508
2.4-2.3	mesitylene	3801	3896.8	3769	3788.613	1.025204
2.1-1.7	Adamantanecarboxylic acid	2158.9	2244.9	2195.2	2167.822	1.039835
1.6-1.4	cyclohexane	2232.2	2265.4	2153.7	2208.858	1.014873
0.5-0	hexamethyldisiloxane	7203.4	7410.3	7138.4	7172.922	1.028723

c. Hardware optimisation

For the following experiments, a solution of mesitylene, 18-crown-6, cyclohexane and hexamethyldisiloxane, each at 0.02 M in chloroform was circulated through the flow system. The peristaltic pump, PEEK flow tube, and active temperature regulation with heat exchangers connected to the flow path were all used unless stated otherwise. One set of acquisition parameters was used for all the following experiments: Δ was set to 0.025 s, and $\delta/2$ was 1000 µs. Once again, the dstebpgp3s pulse sequence was used with a smooth rectangle gradient pulse, 16 gradient steps, 16 scans, and a linear ramp. The NMR probe remained at 298 K. DOSY data were acquire at flow rates from 0.0 to 4.5 mL min^{-1} to assess the effects on data quality of the following factors.

i. NMR flow tip geometry

DOSY spectra were acquired using an InsightMR flow tube with a fused silica capillary in the flow tip (Figure S7) and compared with the results from the PEEK flow tube.

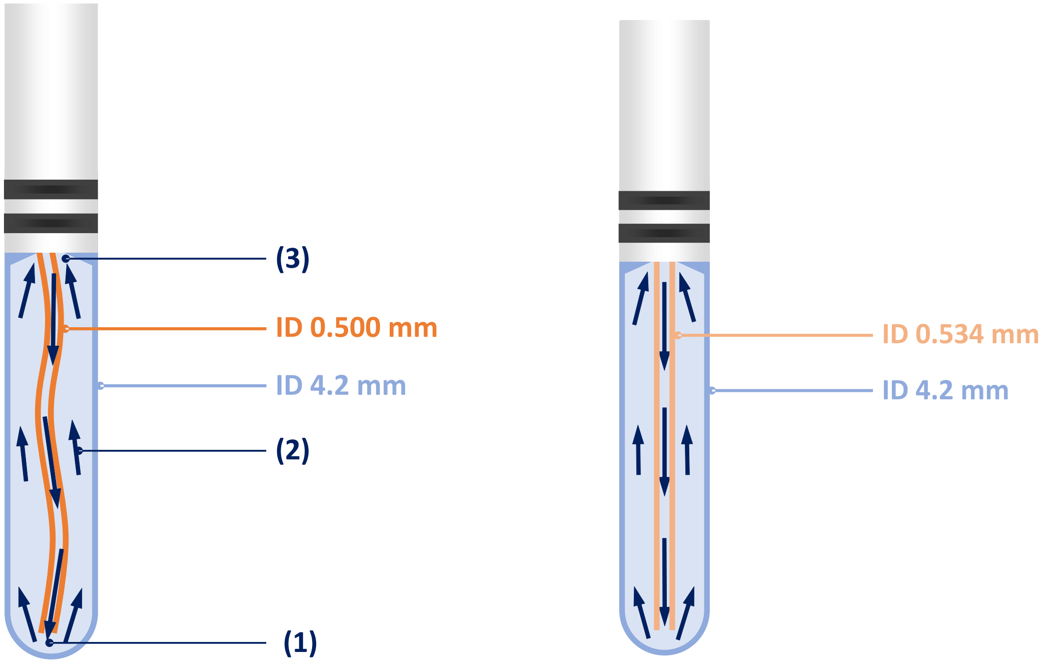


Figure S7: Cartoon representation of the flow of solution through the flow tube tip, for the PEEK (left) and silica flow tubes.

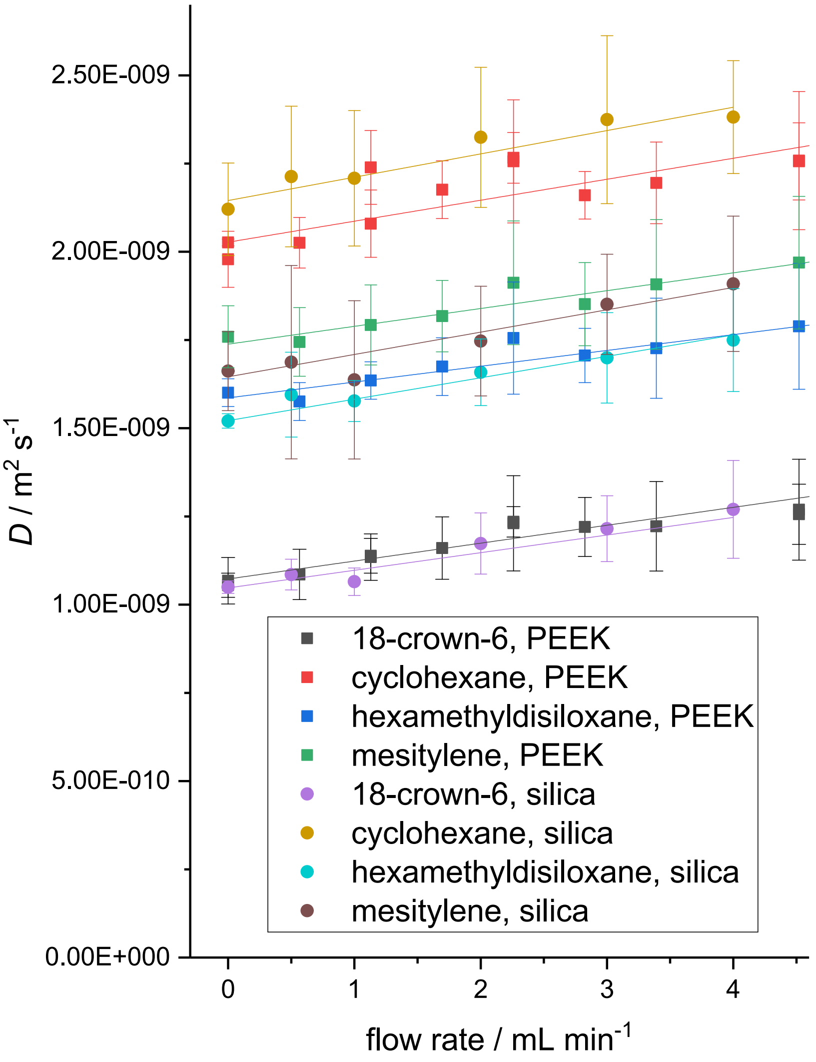


Figure S8: Measured diffusion coefficients as a function of flow rate show no significant difference when acquired with silica of PEEK capillary in flow tip ($\delta/2$ = 1000 µs; Δ = 0.025 s). Diffusion coefficient for mesitylene is the average of both ^{1}H signals.

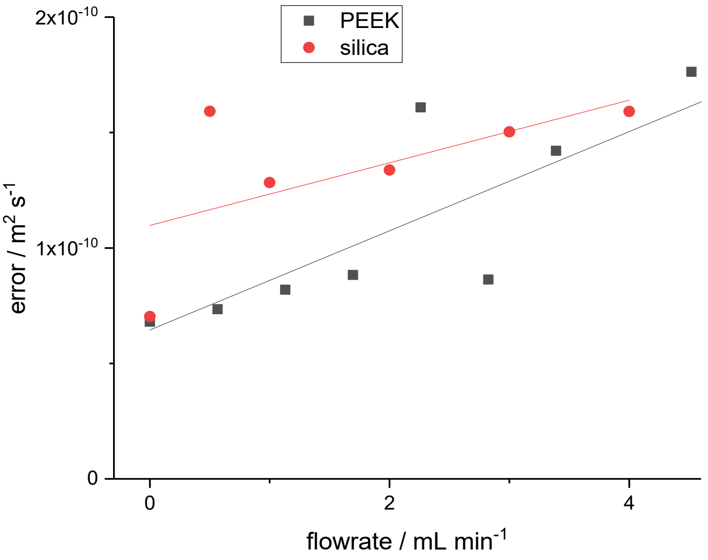


Figure S9: Errors associated with diffusion measurements using silica-tipped flow tube are no smaller than with PEEK.

ii. Temperature regulation

Equivalent DOSY experiments were carried out with and without active temperature regulation from the flow tube heat exchanger. These experiments were performed with both the PEEK flow tube and the silica tipped flow tube.

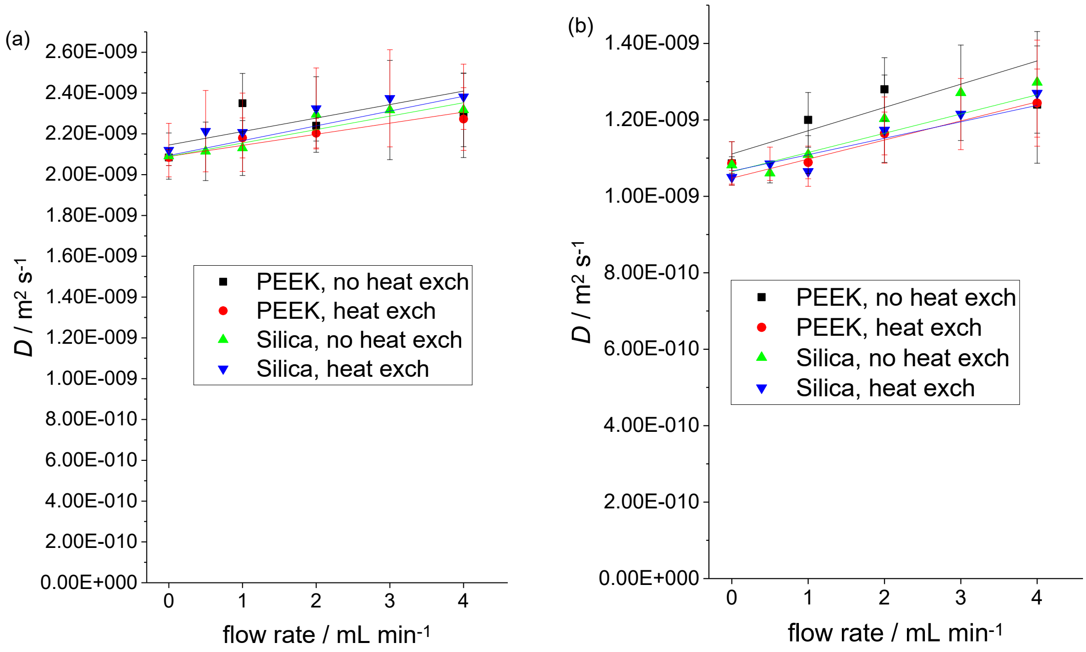


Figure S10: Observed diffusion coefficients acquiredusing silica and PEEK tipped flow tubes with and without active temperature regulation. (*δ*/2 = 1000 µs; *Δ* = 0.025 s)

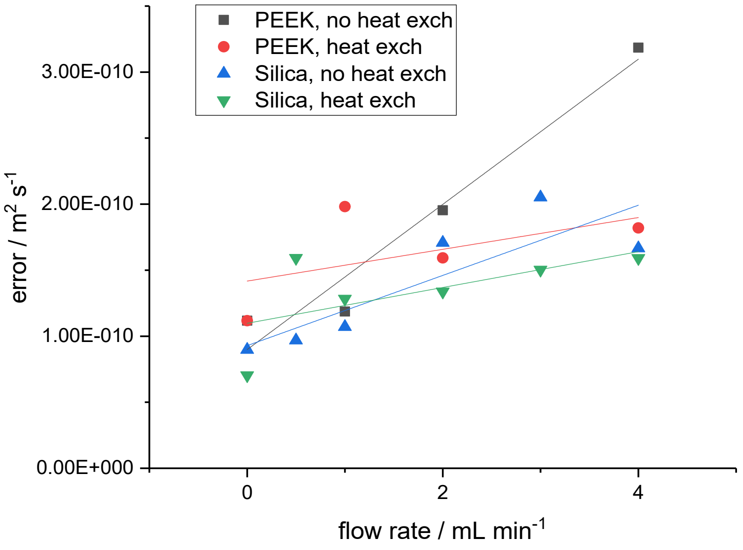


Figure S11 (*δ*/2 = 1000 µs; *Δ* = 0.025 s)

iii. Pump pulsation

DOSY spectra were acquired while circulating the test solution using a Vici M6 HP rotating tetra-piston pump, and the results were compared with those from a Vapourtec SF-10 peristaltic pump.

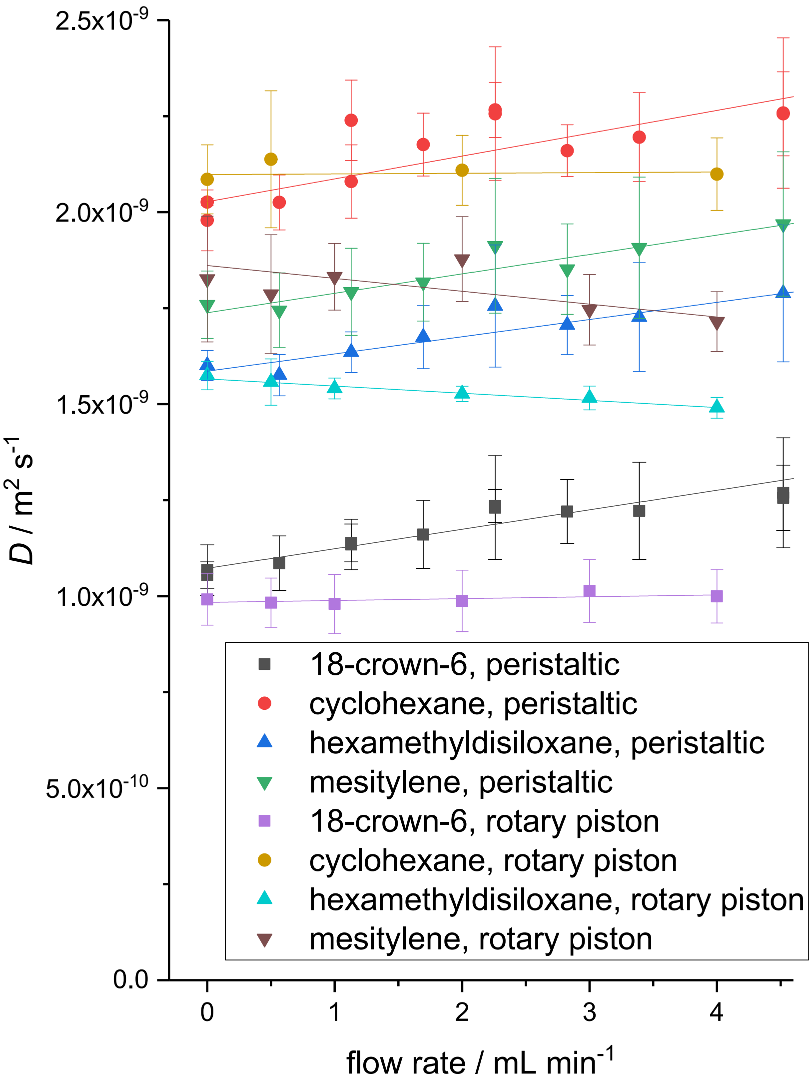


Figure S12: Difference in flow effects on measured diffusion coefficient of four molecules in chloroform using peristaltic and rotary piston pumps ($\delta/2$ = 1000 µs; Δ = 0.025 s).

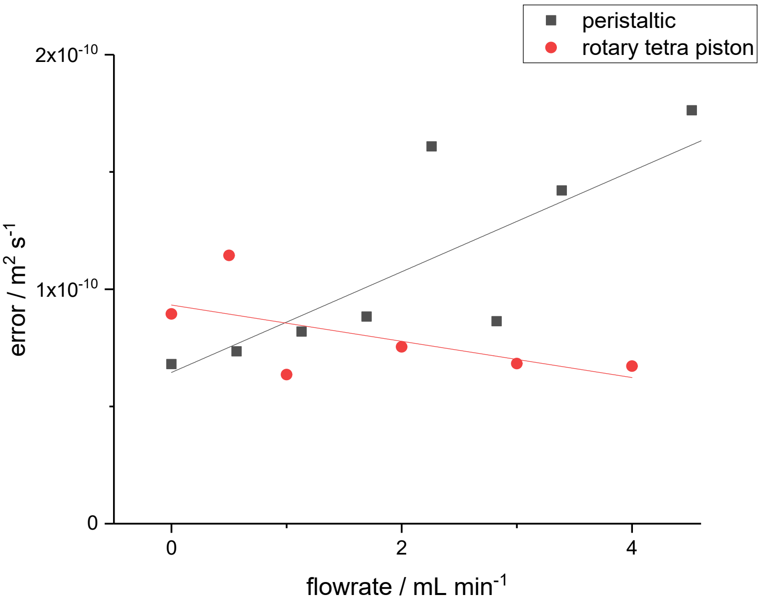


Figure S13: Mean error in *D* for mesitylene, 18-crown-6, cyclohexane and hexamethyldisiloxane plotted against flow rate ($\delta/2$ = 1000 µs; Δ = 0.025 s)

d. Correcting for flow effects: calculating static D

Plotting the observed diffusion coefficient measured on a flowing sample, D_{flow}, against the value obtained with no flow, D_{static}, gives a straight line with a gradient around 1 and a vertical intercept slightly above zero. The faster the flow, the greater the flow effect, and the higher the vertical intercept. After making a calibration curve using two or more signals whose diffusion coefficient has been measured at zero flow rate and the desired flow rate, the static diffusion coefficient of unknowns may be calculated from their flow values using the equation of the curve.

Table 7: Linear fits for plots of D_{flow} vs D_{static} for flow rates up to 6.78 mL min^{-1} (Figure 5 in paper).

Equation	y = a + b*x									
Flow rate / mL/min	0.00	0.57	1.13	1.7	2.26	2.83	3.39	4.52	5.65	6.78
Intercept	0 ± 0	1.03375E-10 ± 3.88405E-11	1.51215E-10 ± 4.97257E-11	1.07079E-10 ± 1.0983E-10	1.64536E-10 ± 8.87536E-11	2.43403E-10 ± 7.39186E-11	2.02992E-10 ± 3.48563E-11	2.35108E-10 ± 3.41097E-11	3.05191E-10 ± 8.46769E-12	2.3971E-10 ± 4.6434E-11
Slope	1 ± 0	0.94765 ± 0.02386	0.95076 ± 0.03055	1.00789 ± 0.06747	1.02306 ± 0.05452	0.93925 ± 0.04541	0.98255 ± 0.02141	0.99917 ± 0.02095	0.98171 ± 0.0052	1.08061 ± 0.02852
Residual Sum of Squares	0	5.91E-22	9.69E-22	4.73E-21	3.09E-21	2.14E-21	4.76E-22	4.56E-22	2.81E-23	8.45E-22
Pearson's r	1	0.99937	0.99897	0.99555	0.99717	0.99767	0.99953	0.99956	0.99997	0.9993
R-Square(COD)	1	0.99873	0.99794	0.99112	0.99435	0.99535	0.99905	0.99912	0.99994	0.99861
Adj. R-Square	1	0.9981	0.99691	0.98668	0.99153	0.99302	0.99858	0.99868	0.99992	0.99791

3. Recommendations for FlowDOSY acquisition

Table S8: Best practice recommendations for obtaining reliable FlowDOSY data. Using these as a starting point, optimal conditions for new reaction systems may be found.

Variable	**Recommendation**
Pulse sequence	Convection compensation (e.g. dstebpgp3s) is essential.
Δ	Smaller values lead to smaller flow effects. 0.025–0.050 s works well.
$\delta/2$	Larger values are preferable for larger molecules. Must not exceed the limits of the probe: in this case 1250 µs for dstebpgp3s pulse sequence.
Flow rate	Lower flow rates give better results. Recommend ≤ 4.0 mL min^{-1}.
d1	Can be reduced to nearly zero for fast acquisition.
AQ	Can be reduced to 1 s.
Internal references	Use at least two molecules to create a calibration curve relating D_{flow} and D_{static}, so diffusion coefficients of unknowns can be calculated.
Flow tip geometry	Any. No significant difference between polymer capillary and fused silica capillary.
Temperature regulation	Use if available to minimise complications from thermal gradients.
Pump choice	A pump with minimal pulsation is preferable. In cases where pulsation leads to significant flow effects, D_{static} can be calculated from a flow effect calibration curve.