Electronic Supplementary Material (ESI) for Analyst.

Supporting information for
Online reaction monitoring by single-scan 2D NMR under flow conditions

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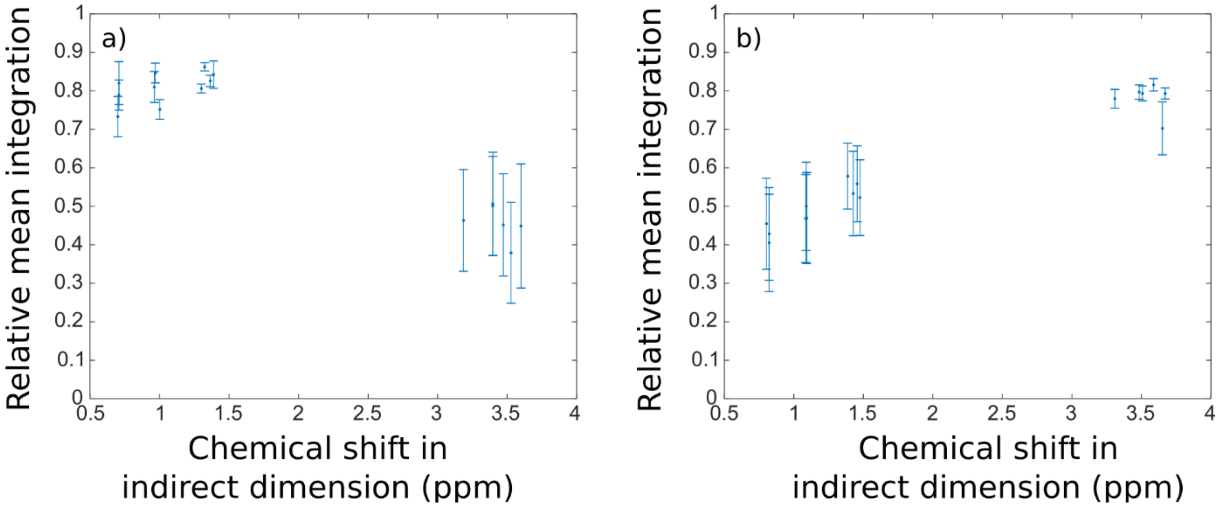
I. Effect of the direction of the frequency sweep



Figure_SI 1: Mean integration of several peaks in ufCOSY with flow of 2.5 mL/min relative to maximum integration without flow as a function of chemical shift of the peaks in indirect dimension using chirp sweeping high to low field (a) and low to high field (b).

II. Kinetics at several temperatures

A. Experimental section

Saponification reaction. The saponification reaction was prepared according the following procedure. First, temperature regulation of the probe was set to the desired temperature (25, 35 or 45°C) and the probe was tuned on a tube containing ethanol and sodium acetate at 25 mM in purified undeuterated water. At room temperature, in a 100 mL round-bottom flask with magnetic agitation, 50 mL of purified and degassed water were used to dissolve 119 µL of ethyl acetate (1 eq, 25 mM). The flask was heated to the selected temperature (25, 35 or 45°C) in a bain-marie. The solution was fed to the flow tube with a flow rate of 3 mL/min until the solution returned to the flask while the transfer line was heated to the desired temperature using a thermostatic water bath. With this setup, the dead time before the system equilibrates is about 1 minute and 40 seconds. A series of acquisition were repeated every 15 s interlacing 1D ^{1}H and ufCOSY experiments with spatial encoding on X-axis. This 15 s delay is chosen to avoid any damage to the probe, and provides a repetition time that is still much shorter than with conventional (non-UF) experiments). After the end of the fourth UF experiment, 125 µL of 10M NaOH (1 eq, 25 mM) was added in the reaction mixture. The acquisitions continued for about 40 min.

NMR experiments. For UFCOSY experiments on the saponification reaction at 25 °C, bipolar encoding gradients of ±0.0075 (on X-axis) T/m were used in combination with 15 ms chirp pulses with 12 kHz bandwidth. The acquisition consisted of a train bipolar gradient pulses of or ±0.374 (X-axis) T/m acquisition gradient, with 150 loops and a duration of 190 µs for each gradient pulse. A relaxation delay of 14.79 s was used including 5 s of presaturation. The excitation sculpting block was composed of a pair a 2 ms trapezoidal pulses and hard π pulse surrounded by a pair of 1 ms gradient pulses (Y axis).
In order to avoid water peak to fold back on peaks of interests, UFCOSY for saponification at 35 and 45°C were slightly changed to use these parameters. For spatial encoding, bipolar encoding gradients of ±0.0064 (on X-axis) T/m were used in combination with 15 ms chirp pulses with 12 kHz bandwidth. The acquisition consisted of a train bipolar gradient pulses of or ±0.374 (X-axis) T/m acquisition gradient, with 150 loops and a duration of 225 µs for each gradient pulse. A relaxation delay of 14.80 s was used including 5 s of presaturation. The excitation sculpting block was composed of a pair a 2 ms trapezoidal pulses and hard π pulse surrounded by a pair of 1 ms gradient pulses (Y axis).

Data processing. 1D ^{1}H were processed with Topspin 3.6.1 and integration was done using Dynamics Center 2.5.6. The integration values were then imported for processing in MATLAB. ufCOSY processing and integration were done in MATLAB. For fitting, only point from 150 to 700 s after NaOH introduction were taken into account in order to eliminate first points, which correspond to the homogenization in the flow tube, and the last points, which suffer from a too low signal to noise ratio.

Kinetic measurement. The saponification of ethyl acetate is known to be a reaction of order 2. In order to evaluate the kinetics of the reaction, the saponifications were done with equal quantities of ethyl acetate and NaOH, so that [AcOEt] = [NaOH]. Then the decay of the concentration of ethyl acetate can be fitted to the equation:

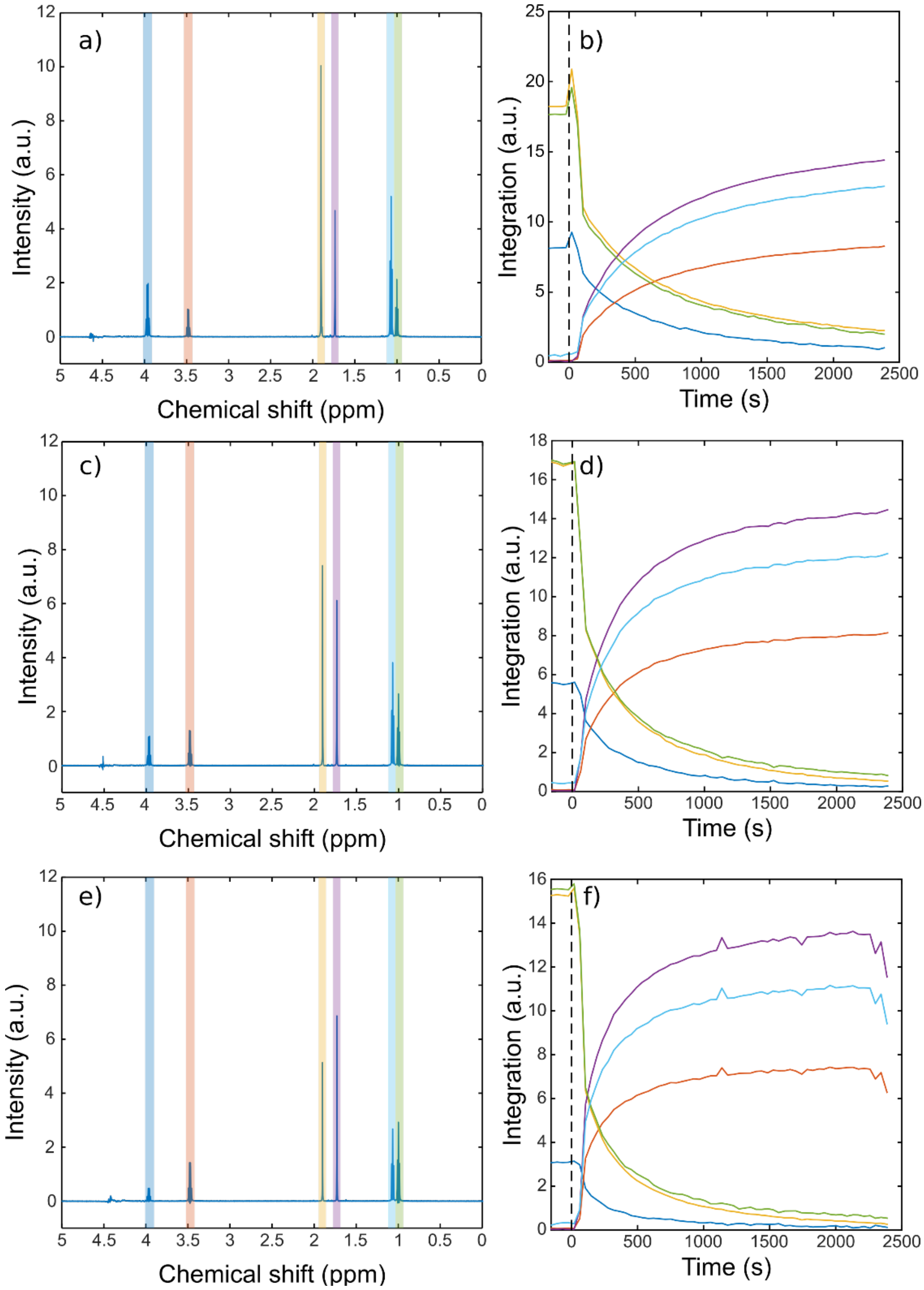
$$\frac{1}{[\mathrm{AcOEt}](t)} = at + b$$

where t is the reaction time. The reaction rate constant is then equal to a. Peak volumes can be converted to concentrations by using the known initial concentration of ethyl acetate:

$$[\mathrm{AcOEt}](t) = [\mathrm{AcOEt}]_0 \frac{I(t)}{I_0}$$

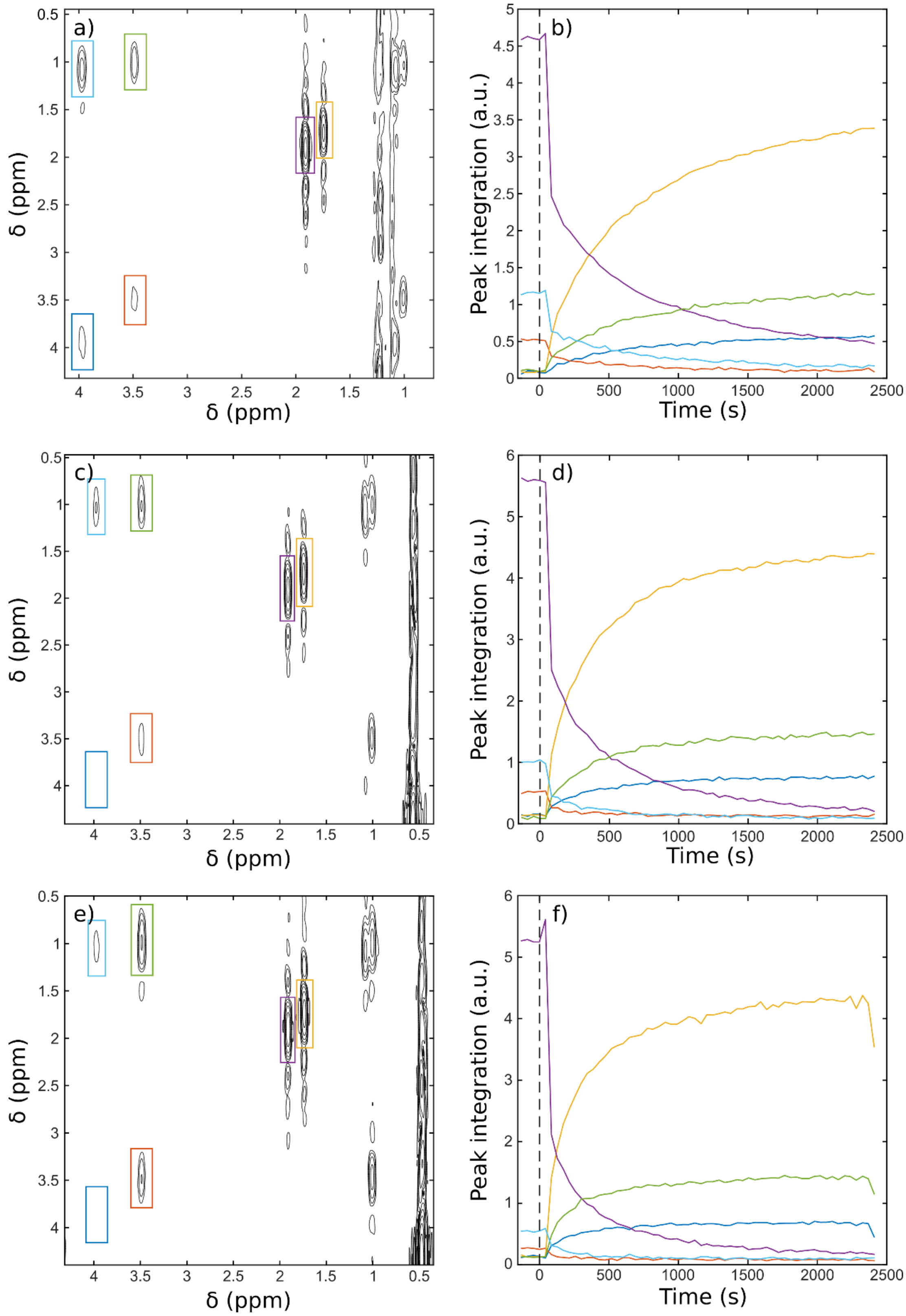
Where I is the peak volume at time t, I_0 is the peak volume before the reaction starts, and $[\mathrm{AcOEt}]_0$ is the initial concentration in ethyl acetate.

B. Kinetic monitoring using 1D ^{1}H NMR



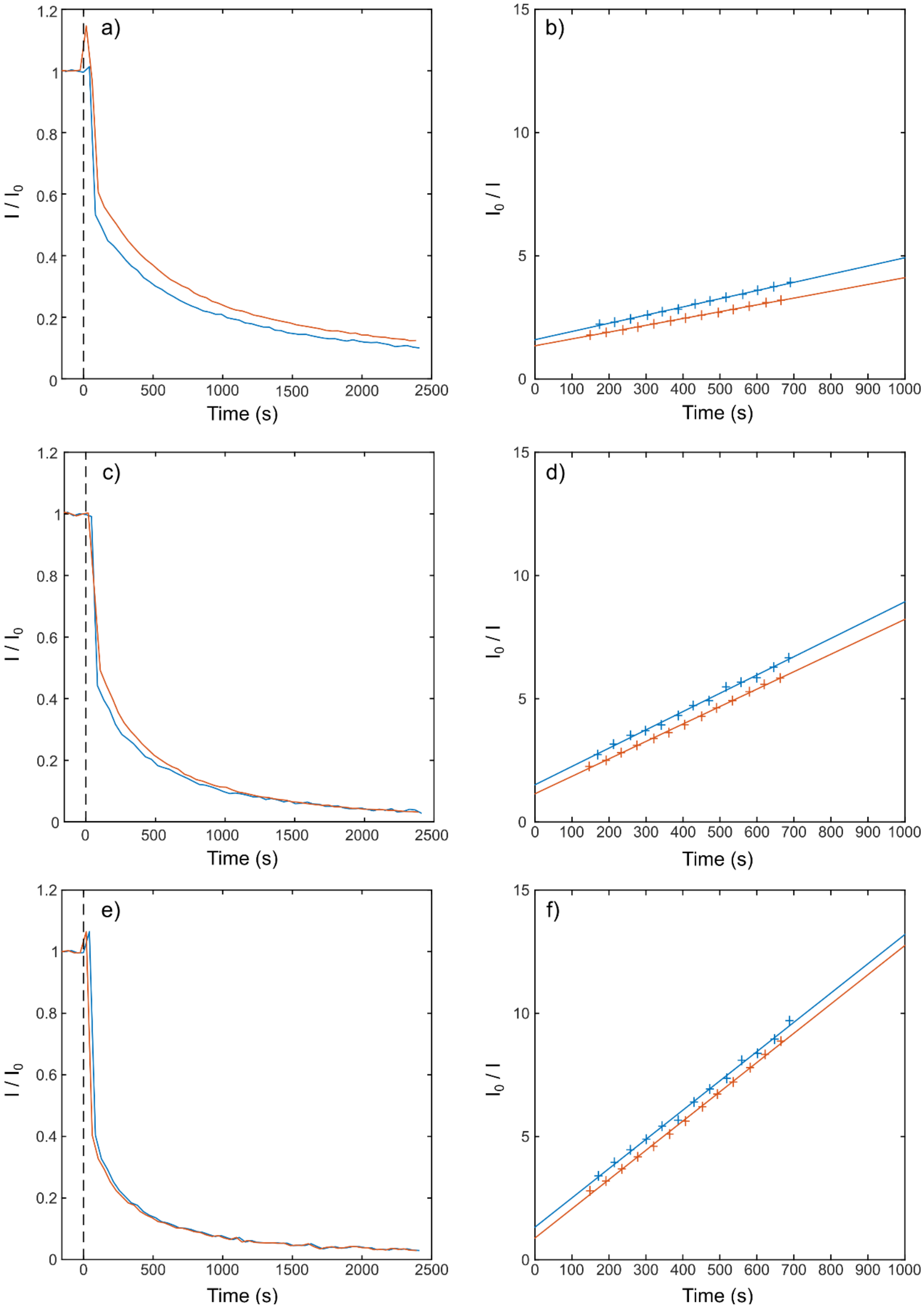
Figure_SI 2: 1D ^{1}H spectra at t= 150 s after the addition of NaOH and evolution of peak integrations for saponifications at T=25°C (a and b), 35°C (c and d) and 45°C (e and f). Vertical axes of the three graphs (b, d, f) have the same scale. Dashed lines correspond to the moment the NaOH was introduced in the reaction mixture.

C. Kinetic monitoring using ufCOSY



Figure_SI 3: ufCOSY spectra at t= 175 s after the addition of NaOH and evolution of peak integrations for saponifications at T=25°C (a and b), 35°C (c and d) and 45°C (e and f). Vertical axes of the three graphs (b, d, f) have the same scale. Dashed lines correspond to the moment the NaOH was introduced in the reaction mixture.

D. Comparison between 1D ^{1}H NMR and ufCOSY



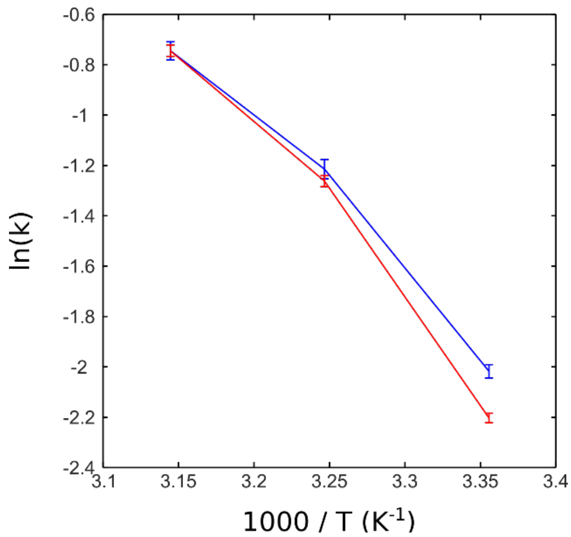
Figure_SI 4: (a, c, e) Evolution of ethyl acetate $CH_3(CO)$ peak integration as a function of time for saponification at 25°C (a), 35°C(c) and 45°C (e). Blue lines correspond to results obtained with UF experiments and red lines correspond to results obtained with 1D ^{1}H experiments. (b, d, f) Fit of the data for times between 150 and 700 s. Blue crosses and lines respectively corresponds to the experimental values and to the fit of results obtained with UF results and red crosses and lines correspond to the experimental values and to the fit of results obtained with 1D ^{1}H experiments.

Table 1: Reaction rates determined by fitting the data shown in Fig.SI 4, obtained with 1D ^{1}H NMR experiments and UFCOSY for saponification at 25°C, 35°C and 45°C.

Experiment	k[(a)] at 25°C ($L.mol^{-1}s^{-1}$)	k at 35°C ($L.mol^{-1}s^{-1}$)	k at 45°C ($L.mol^{-1}s^{-1}$)
1D ^{1}H NMR	0.11	0.28	0.47
ufCOSY	0.13	0.30	0.48

(a) the difference in rate is tentatively assigned to an inaccurate determination of the proportionality constant between peak volume and concentration in the 1H 2D NMR data.

E. Arrhenius Plot



Figure_SI 5: ln(k) as a function of 1000/T for rate constants measured by 1D NMR an UF monitoring at 25, 35 and 45°C. Blue and red lines respectively correspond to the results obtained with UFCOSY and 1D ^{1}H experiments. The error bars correspond to the error of the fit.

III. Pulse sequences

A. ufCOSY with excitation sculpting

```
;ufcosyes.3
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Grad.incl>
#include <De.incl>
#include <Delay.incl>

"p2=2*p1"
"d11=30m"
"p15=(td*dw/(2*l3))-d6"
"p24=p15"
"DELTA = d1-d3"
"TAU=de+p1*2/3.1416+50u"

1  ze
   d11 pl9:f2
   20u st0

; -----------------------------relaxation and presaturation
2  30m
   10u ;reset:f1:f2
   DELTA
   d3 cw:f2 ph29
   4u do:f2
   100u UNBLKGRAD
   20u pl1:f1

;------------------------------excitation
   p1 ph1
   d10
;------------------------------spatial encoding step
   p20:gp20
   20u gron0
   p11:sp1:f1 ph1
   20u groff
   p20:gp21
   20u gron1
   p11:sp1:f1 ph2
   20u groff
   p20:gp22

;------------------------------pre-mixing gradient
  20u
   p24:gp24
  10u
```

```
;------------------------------mixing
   p23:gp23
   10u
   5u pl1:f1
   p1 ph1
   10u
   p26:gp26
   10u

;--------------------------------excitation sculpting
   p6:gp7
   d16 pl0:f2
   (p12:sp2 ph2:r):f2
   4u
   d12 pl1:f1
   p2 ph3
   4u
   p6:gp7
   d16
   TAU
   p6:gp8
   d16 pl0:f1
   (p12:sp2 ph4:r):f2
   4u
   d12 pl1:f1
   p2 ph3
   4u
   p6:gp8
   d16

;-------------------------------post-mixing gradient
   10u
   p24:gp25
   10u

;-------------------------------acquisition
   ACQ_START(ph30,ph31)
   1u DWELL_GEN:f1
3   p15:gp15
   d6
   p15:gp16
   d6
   lo to 3 times l3
            100u BLKGRAD
   rcyc=2
   100u UNBLKGRAD
   p27:gp27
   100u BLKGRAD
   30m mc #0 to 2 F1QF()
   d17
  exit

ph1=0
ph2=0 1
ph3=0
ph4=0
ph29=0
```

```
ph30=0
ph31=0 2


;pl1 : f1 channel - power level for pulse (default)
;sp1: shaped pulse power level for spatial encoding
;sp2: shaped pulse power level for excitation sculpting
;p1 : f1 channel - 90 degree high power pulse
;p12 : f2 channel – 180 shaped pulse for excitation sculpting [2 ms]
;p23 and p26: coherence-selection gradients for mixing(p23=p26)
;p20: coherence-selection gradient duration for spatial encoding
;spnam1 : shaped pulse for spatial encoding
;spnam2 : shaped pulse for excitation sculpting
;d1 : relaxation delay; 1-5 * T1
;d3 : presaturation delay, < D1
;d20 + d6 : acquisition gradient duration
;p24 : pre and post-mixing gradient duration
;d17 : delay after experiment (not necessary)
;GPZ0 : strength for encoding gradient [0-100]
;GPZ1 : strength for reversed encoding gradient GPZ1 = -GPZ0
;GPZ2 : strength for acquisition gradient [0-100]
;GPZ3 : strength for reversed acquisition gradient GPZ3 = -GPZ2
;GPZ20 : with GPZ21 and GPZ22 coherence-selection gradients for spatial encoding (21 = 20 + 22)
;GPZ21 : with GPZ20 and GPZ22: coherence-selection gradients for spatial encoding (21 = 20 + 22)
;GPZ22 : with GPZ20 and GPZ21: coherence-selection gradients for spatial encoding (21 = 20 + 22)
;GPZ23 and GPZ26: coherence-selection gradients for mixing (GPZ26=-GPZ23)
;GPZ24 and GPZ25: pre- and post-mixing gradients
;GPNAM20=GPNAM21= GPNAM22=GPNAM23=GPNAM26=SINE.100
;GPNAM24=GPNAM25=RECT.1
;GPNAM15=GPNAM16=SMSQ10.32
;NS: 1 (or more if necessary)
;l3=number of loops for acquisition
;IMPORTANT: set d20 + d6 = DW x TD(F2)/(2xL3)
```

B. ufCOSY with WET

```
;ufcosywetdc.gron
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Grad.incl>
#include <De.incl>
#include <Delay.incl>

"d11=30m"
"d15=(td*dw/(2*l3))-d6"
"p24=d15"
"DELTA = d1-d3"
"TAU=de+p1*2/3.1416+50u"

1 ze
  d11 pl9:f2
  20u st0

; ----------------------------relaxation and presaturation
2 30m
   10u pl9:f2 ;reset:f1:f2
    DELTA
    d3 cw:f2 ph29
    4u do:f2

;----------------------------WET block with 13C decoupling
    50u UNBLKGRAD
    d12 pl0:f2
    20u cpd2:f3
    (p12:sp7 ph5):f2
    4u
    p16:gp11
    d16 pl0:f2
   (p12:sp8 ph6):f2
    4u
    p16:gp12
    d16 pl0:f2
    (p12:sp9 ph6):f2
    4u
    p16:gp13
    d16 pl0:f2
    (p12:sp10 ph6):f2
    4u do:f3
    p16:gp14
    d16
   10m pl1:f1

;-------------------------------excitation

  p1 ph1
```

```
 d10
;-----------------------------spatial encoding step
 p20:gp20
 20u gron0
 p11:sp1:f1 ph1
 20u groff
 p20:gp21
 20u gron1
 p11:sp1:f1 ph2
 20u groff
 p20:gp22

;------------------------------pre-mixing gradient
 20u
 p24:gp24
 10u

;-------------------------------mixing
 p23:gp23
 10u
 5u pl1:f1
 p1 ph1
 10u
 p26:gp26
 10u

;--------------------------------post-mixing gradient
 10u
 p24:gp25
 10u

;--------------------------------acquisition
  ACQ_START(ph30,ph31)
  5u cpd2:f3
  1u DWELL_GEN:f1
3 d15 gron15
  d6 groff
  d15 gron16
  d6 groff
  lo to 3 times l3
  100u BLKGRAD
  rcyc=2
  100u UNBLKGRAD
  p27:gp27
  100u BLKGRAD
  20u do:f3
  30m mc #0 to 2 F1QF()
  d17
exit

ph1=0
ph2=0 1
ph3=0
ph4=0
ph5=0
ph6=1
ph29=0
```

```
ph30=0
ph31=0 2


;pl1 : f1 channel - power level for pulse (default)
;sp1: shaped pulse power level for spatial encoding
;sp7: f2 channel - shaped pulse (to be optimized)
;sp8: f2 channel - shaped pulse
;sp9: f2 channel - shaped pulse
;sp10: f2 channel - shaped pulse
;p1 : f1 channel - 90 degree high power pulse
;p12 : f2 channel –90 degree shaped pulse for WET [20 ms]
;p23 and p26: coherence-selection gradients for mixing (p23=p26)
;p20: coherence-selection gradient duration for spatial encoding
;spnam1 : adiabatic chirp pulse for spatial encoding
;spnam7: Sinc1.1000
;spnam8: Sinc1.1000
;spnam9: Sinc1.1000
;spnam10: Sinc1.1000
;spnam7 : first selective pulse for WET block
;spnam8 : second selective pulse for WET block
;spnam9 : third selective pulse for WET block
;spnam10 : fourth selective pulse for WET block
;d1 : relaxation delay; 1-5 * T1
;d3 : presaturation delay, < D1
;d20 + d6 : acquisition gradient duration
;p16: gradient duration for WET solvent suppression
;p24 : pre and post-mixing gradient duration
;d17 : delay after experiment (not necessary)
;GPZ0 : strength for excitation gradient [0-100]
;GPZ1 : strength for reversed excitation gradient GPZ1 = -GPZ0
;GPZ2 : strength for acquisition gradient [0-100]
;GPZ3 : strength for reversed acquisition gradient GPZ3 = -GPZ2
;GPZ11: 80%
;GPZ12: 40%
;GPZ13: 20%
;GPZ14: 10%
;GPZ20 : with GPZ21 and GPZ22 coherence-selection gradients for spatial encoding (21 = 20 + 22)
;GPZ21 : with GPZ20 and GPZ22: coherence-selection gradients for spatial encoding (21 = 20 + 22)
;GPZ22 : with GPZ20 and GPZ21: coherence-selection gradients for spatial encoding (21 = 20 + 22)
;GPZ23 and GPZ26: coherence-selection gradients for mixing (GPZ26=-GPZ23)
;GPZ24 and GPZ25: pre- and post-mixing gradients
;GPNAM20=GPNAM21= GPNAM22=GPNAM23=GPNAM26=SINE.100
;GPNAM11=GPNAM12= GPNAM13=GPNAM14=SMSQ10.100
;GPNAM24=GPNAM25=RECT.1
;NS: 1 (or more if necessary)
;l3=number of loops for acquisition
;IMPORTANT: set d20 + d6 = DW x TD(F2)/(2xL3)

;use power levels                                  sp7     sp8     sp9     sp10
; shaped pulse powerlevel for 90 degree pulse     +0.87   -1.04   +2.27   -5.05
```

C. ufCOSY with WET solvent suppression and chirp sweeping opposite ways

```
;ufcosywetdc.gron-ChInv
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Grad.incl>
#include <De.incl>
#include <Delay.incl>

"p2=2*p1"
"d11=30m"
"d15=(td*dw/(2*l3))-d6"
"p24=d15"
"DELTA = d1-d3"

1 ze
  d11 pl9:f2
  20u st0
; -----------------------------relaxation and presaturation
2 30m
  10u pl9:f2 ;reset:f1:f2
  DELTA
  d3 cw:f2 ph29
  4u do:f2

; ----------------------------WET block with 13C decoupling
 50u UNBLKGRAD
 d12 pl0:f2
 20u cpd2:f3
 (p12:sp7 ph5):f2
 4u
 p16:gp11
 d16 pl0:f2
 (p12:sp8 ph6):f2
 4u
 p16:gp12
 d16 pl0:f2
 (p12:sp9 ph6):f2
 4u
 p16:gp13
 d16 pl0:f2
 (p12:sp10 ph6):f2
 4u do:f3
 p16:gp14
 d16
 10m pl1:f1

;--------------------------------excitation
 p1 ph1
 d10 pl0:f1

;-----------------------------spatial encoding step
```

```
  p20:gp19
  20u gron0
  p11:sp1:f1 ph1
  20u groff
  p20:gp20
  20u pl1:f1
  p2 ph1
  p20:gp21
  20u pl0:f1
  20u gron1
  p11:sp2:f1 ph2
  20u groff
  p20:gp22

;-------------------------------pre-mixing gradient
  20u
  p24:gp24
  10u

;--------------------------------mixing
  p23:gp23
  10u
  5u pl1:f1
  p1 ph1
  10u
  p26:gp26
  10u

;-------------------------------post-mixing gradient
  10u
  p24:gp25
  10u

;--------------------------------acquisition
   ACQ_START(ph30,ph31)
   5u cpd2:f3
   1u DWELL_GEN:f1
3 d15 gron15
    d6 groff
   d15 gron16
   d6 groff
   lo to 3 times l3
   100u BLKGRAD
   rcyc=2
   100u UNBLKGRAD
   p27:gp27
   100u BLKGRAD
   20u do:f3
   30m mc #0 to 2 F1QF()
   d17
exit

ph1=0
ph2=0 1
ph3=0
ph4=0
ph5=0
```

```
ph6=1
ph29=0
ph30=0
ph31=0 2


;pl1 : f1 channel - power level for pulse (default)
;sp1: shaped pulse power level for spatial encoding
;sp2: shaped pulse power level for spatial encoding
;sp7: f2 channel - shaped pulse (to be optimized)
;sp8: f2 channel - shaped pulse
;sp9: f2 channel - shaped pulse
;sp10: f2 channel - shaped pulse
;p1 : f1 channel - 90 degree high power pulse
;p12 : f2 channel –90 degree shaped pulse for WET [20 ms]
;p23 and p26: coherence-selection gradients for mixing(p23=p26)
;p20: coherence-selection gradient duration for spatial encoding
;spnam1 : adiabatic chirp pulse for spatial encoding
;spnam2 : adiabatic chirp pulse for spatial encoding with sweeping direction opposite to spnam1
;spnam7: Sinc1.1000
;spnam8: Sinc1.1000
;spnam9: Sinc1.1000
;spnam10: Sinc1.1000
;d1 : relaxation delay; 1-5 * T1
;d3 : presaturation delay, < D1
;d20 + d6 : acquisition gradient duration
;p24 : pre and post-mixing gradient duration
;d17 : delay after experiment (not necessary)
;GPZ0 : strength for excitation gradient [0-100]
;GPZ1 : strength for reversed excitation gradient GPZ1 = -GPZ0
;GPZ2 : strength for acquisition gradient [0-100]
;GPZ3 : strength for reversed acquisition gradient GPZ3 = -GPZ2
;GPZ11: 80%
;GPZ12: 40%
;GPZ13: 20%
;GPZ14: 10%
;GPZ19 and GPZ20 coherence-selection gradients for spatial encoding (19 = 20)
;GPZ20 and GPZ19 coherence-selection gradients for spatial encoding (19 = 20)
;GPZ21 and GPZ22: coherence-selection gradients for spatial encoding (21 = 22)
;GPZ22 and GPZ21: coherence-selection gradients for spatial encoding (21 = 22)
;GPZ23 and GPZ26: coherence-selection gradients for mixing (GPZ26=-GPZ23)
;GPZ24 and GPZ25: pre- and post-mixing gradients
;GPNAM19= GPNAM20=GPNAM21=GPNAM23=GPNAM26=SINE.100
;GPNAM11=GPNAM12= GPNAM13=GPNAM14=SMSQ10.100
;GPNAM24=GPNAM25=RECT.1
;NS: 1 (or more if necessary)
;l3=number of loops for acquisition
;IMPORTANT: set d20 + d6 = DW x TD(F2)/(2xL3)


;use power levels                                  sp7     sp8     sp9     sp10
; shaped pulse powerlevel for 90 degree pulse    +0.87   -1.04   +2.27   -5.05
```