SUPPLEMENTARY INFORMATION

TROSY-selected ZZ-exchange experiment for characterizing slow chemical exchange in large proteins

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Derivation of eq. 1 in the main text

In the absence of S^3CT element, evolution of magnetization during the mixing period is given by

$$\frac{dM(t)}{dt} = -RM(t) \tag{S1}$$

$$M(t) = \begin{bmatrix} I_z(t) \\ 2I_zS_z(t) \\ S_z(t) \end{bmatrix} \tag{S2}$$

$$R = \begin{bmatrix} R_{1H} & \eta_H & \sigma \\ \eta_H & R_{1HN} & \eta \\ \sigma & \eta & R_{1N} \end{bmatrix} \tag{S3}$$

in which R_{1H} is the ^{1}H longitudinal relaxation rate, σ is the ^{1}H-^{15}N heteronuclear cross relaxation rate, R_{1N} is the relaxation rate of ^{15}N longitudinal magnetization, R_{1N} is the relaxation rate of ^{15}N longitudinal two-spin order, η_z is the longitudinal ^{15}N-^{1}H dipole/^{15}N CSA cross-correlated relaxation rate, η_H is the longitudinal ^{15}N-^{1}H dipole/^{1}H CSA cross-correlated relaxation rate. Equilibrium magnetizations are cancelled by the phase cycle and are not included in the equations. The solution to eqs. (S1-S3) is $M(t) = \exp(-Rt)M(0)$.

With the S^3CT element present in the middle of the mixing period, the evolution of magnetization is given by

$$\begin{aligned} M(t) &= \exp(-Rt/2)U\exp(-Rt/2)M(0) \\ &= \exp(-Rt/2)\exp(-URUt/2)UM(0) \\ &= [E-(R+URU)t/2+...]UM(0) \\ &\approx \exp[-(R+URU)t/2]UM(0) \end{aligned} \tag{S4}$$

in which the transformation matrix $U = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{bmatrix}$ represents the net effect of S^3CT element.

Taking the derivatives of both left-hand side and right-hand side yields

$$\frac{dM(t)}{dt} \approx -\frac{R+URU}{2}\exp[-(R+URU)t/2]UM(0) \tag{S5}$$

Substituting eq. (S4) into eq. (S5) yields

$$\frac{dM(t)}{dt} \approx -\frac{R+URU}{2}M(t) = -R_{eff}M(t) \tag{S6}$$

To first order approximation, the effective rate matrix is

$$R_{eff} = \frac{R+URU}{2} = \begin{bmatrix} R_{1H} & \frac{\eta_H+\sigma}{2} & \frac{\eta_H+\sigma}{2} \\ \frac{\eta_H+\sigma}{2} & \overline{R}_1 & \eta_z \\ \frac{\eta_H+\sigma}{2} & \eta_z & \overline{R}_1 \end{bmatrix} \tag{S7}$$

in which $\overline{R}_1 = \left(R_{1N} + R_{1HN}\right)/2$.

Transforming eq. (S6) from Cartesian to single-element basis set yields

$$\frac{dM'(t)}{dt} = -R'_{eff}M'(t) \tag{S8}$$

$$M'(t) = \begin{bmatrix} I_z(t) \\ S_zI^{\alpha}(t) \\ S_zI^{\beta}(t) \end{bmatrix} = U'M(t) \tag{S9}$$

$$R'_{eff} = U'R_{eff}U'^{-1} = \begin{bmatrix} R_{1H} & \eta_H+\sigma & 0 \\ \frac{\eta_H+\sigma}{2} & \overline{R}_1+\eta_z & 0 \\ 0 & 0 & \overline{R}_1-\eta_z \end{bmatrix} \tag{S10}$$

in which the transformation matrix $U' = \frac{1}{2}\begin{bmatrix} 2 & 0 & 0 \\ 0 & 1 & 1 \\ 0 & -1 & 1 \end{bmatrix}$. Combining eqs. (S8-S10) yields

$$\frac{d}{dt}\begin{bmatrix} I_z \\ S_zI^{\alpha} \\ S_zI^{\beta} \end{bmatrix} = -\begin{bmatrix} R_{1H} & \eta_H+\sigma & 0 \\ \frac{\eta_H+\sigma}{2} & \overline{R}_1+\eta_z & 0 \\ 0 & 0 & \overline{R}_1-\eta_z \end{bmatrix}\begin{bmatrix} I_z \\ S_zI^{\alpha} \\ S_zI^{\beta} \end{bmatrix} \tag{S11}$$

Bruker pulse sequence for TROSY selected ZZ-exchange experiment

```
#include <Avance.incl>
#include <Delay.incl>
#include <UNBLKGRAD.incl>

define delay GRDELAY

;***** POWER LEVELS *****
; pl1 = hard 1H pulse
; pl3 = 15N hard pulse

;***** PULSES *****
; p1=1H 90
"p2=p1*2"
; p3=15N hard 90
"p4=p3*2"

; ***** RELAXATION DELAYS *****
; d1 = relaxation delay
; cnst20 =  zz exchange delay in units of ms

"d17=(cnst20*1m)/2.0-GRDELAY-p26-3u"
"d18=(cnst20*1m)/2.0-GRDELAY-p27-3u"

; ***** PRESET DELAYS *****
"GRDELAY=200u"
"d20=2.68m"
"d21=2.5m"
"DELTA1=d21-p22-GRDELAY-3u"
"DELTA2=(d20/2.0)-p22-GRDELAY-3u"
"DELTA3=d20-p22-GRDELAY-3u"
"DELTA4=DELTA1-p9"
"DELTA5=d21-p25-GRDELAY-3u-3.0*p1-d19*2.5+p4/2"
"d4=(p3-p1)/2"
"d3=p4-p1"

;d19: delay for binomial water suppression
"d30=p1*2.385+2.5*d19-p4/2"

"d0=in0/2-p3*0.6369"
;in0=1/2SW1
;l0=number of complex points
;td1=2*l0
;MC2=States-TPPI
;ND0=2
```

```
"l0=td1/2"

  ze
  20u pl3:f3
1 30m
  30m*3
2 30m
  30m
3 30m
  20u BLKGRAD
  d1

;***** INEPT *****
  3u UNBLKGRAD
  p21:gp21
 GRDELAY pl8:f1
 (p8 ph3):f1
 10u pl1:f1
  (p1 ph4):f1
  3u
  p22:gp22
  GRDELAY
  DELTA1
  (d4 d4 p2 ph4):f1 (p4 ph5):f3
  3u
  DELTA1
  p22:gp22
  GRDELAY
  (p1 ph3):f1

;
;***** S3E element *****
  (p3 ph5):f3
  3u
  p22:gp22
  GRDELAY
  DELTA2
  (d3 p2 ph1):f1 (p3 ph5 p4 ph25 p3 ph5):f3
  3u
  DELTA2
  p22:gp22
  GRDELAY
  (p3 ph6):f3
  3u
  p23:gp23
  GRDELAY BLKGRAD
```

```
  (p3 ph26):f3
;

;***** t1 LABELING *****
  d0
  d0
;

;****** T/2 zz exchange *****
 (p3  ph14):f3    ;Quadrature detection
 3u UNBLKGRAD
 p26:gp26
 GRDELAY BLKGRAD
 d17
;

;***** S3CT inversion  *****
  (p3 ph4):f3
  3u UNBLKGRAD
  p22:gp22
  GRDELAY
  DELTA3
  (d4 d4 p2 ph4):f1 (p4 ph4):f3
  3u
  DELTA3
  p22:gp22
  GRDELAY BLKGRAD
  (p2 ph4):f1 (p3 ph13):f3


;***** T/2 zz exchange  *****
 3u
 p27:gp27
 GRDELAY
 d18
;***** TROSY period  *****

  (p1 ph7):f1 (p3 ph15):f3
  10u pl9:f1
  (p9 ph17):f1
  13u UNBLKGRAD
  p22:gp22
  GRDELAY pl1:f1
  DELTA4
  (d4 d4 p2 ph4):f1 (p4 ph4):f3
  3u
```

```
  DELTA4
  p22:gp22
  GRDELAY pl9:f1
  (p9 ph4):f1
  10u pl1:f1
  (p1 ph4):f1
  (p3 ph1):f3

; WATERGATE with 3-9-19 pulse
  3u
  p25:gp25
  GRDELAY
  DELTA5
  (p1*0.231 ph10 d19 p1*0.692 ph10 d19 p1*1.462 ph10 d19):f1 (d30 p4 ph4):f3
  (p1*1.462 ph11 d19 p1*0.692 ph11 d19 p1*0.231 ph11):f1
  DELTA5
  p25:gp25
  GRDELAY
  (p3 ph8):f3
  3u  BLKGRAD

; ***** ACQUISITON *****
  go=1 ph0
  30m wr #0 if #0 dp14 zd
  lo to 2 times 2
  30m ip0*2
  30m id0
  lo to 3 times l0
exit

ph0=0 2 1 3 0 2 3 1
       2 0 3 1 2 0 1 3

ph1=1
ph2=2
ph3=3
ph4=0

ph5=0 2 3 1
ph25=1 3 0 2
ph6=(8) 3 7 1 5 3 7 1 5
           7 3 5 1 7 3 5 1
ph26=0 2 3 1
ph7=1 1 1 1 3 3 3 3
ph17=3 3 3 3 1 1 1 1
ph8=0 0 0 0 2 2 2 2
```

ph10=1
ph11=3
ph13=3
ph14=0 2 1 3
ph15=2 0 3 1

Example Bruker acquisition file (acqus) for TROSY-selected ZZ-exchange experiment

```
##TITLE= Parameter file, XWIN-NMR              Version 3.5
##JCAMPDX= 5.0
##DATATYPE= Parameter Values
##ORIGIN= UXNMR, Bruker Analytische Messtechnik GmbH
##OWNER=
$$
$$
##$AMP= (0..31)
100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100
100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100
##$AQSEQ= 0
##$AQ_mod= 3
##$AUNM= <au_zg>
##$AUTOPOS= <>
##$BF1= 600.13
##$BF2= 600.13
##$BF3= 60.810645
##$BF4= 600.13
##$BF5= 600.13
##$BF6= 600.12
##$BF7= 600.12
##$BF8= 600.12
##$BYTORDA= 1
##$CFDGTYP= 2
##$CFRGTYP= 5
##$CHEMSTR= <none>
##$CNST= (0..31)
1 1 145 1 1 1 1 1 1 1 1 1 1 1 10 8 12 1 -40 400 20 1 1 1 1 10 30 50
25.33 1 1
##$CPDPRG= <>
##$CPDPRG1= <>
##$CPDPRG2= <garp>
##$CPDPRG3= <garp>
##$CPDPRG4= <mlev>
##$CPDPRG5= <mlev>
##$CPDPRG6= <mlev>
##$CPDPRG7= <mlev>
##$CPDPRG8= <mlev>
##$CPDPRGB= <>
##$CPDPRGT= <>
##$D= (0..31)
7.709842e-05 1.3 0.0027 7.55e-05 1.65e-05 0 0 0.001166 3e-06 0 5.75e-05
0.0002 0.0019115 4e-06 0 0 0 0.198997 0.199497 0.000231 0.00268 0.0025
0 0 0 0 0.0027 0 0 1.5 0.0005576575 0
```

##$DATE= 1248041096
##$DBL= (0..7)
120 120 120 120 120 120 120 120
##$DBP= (0..7)
150 150 150 150 150 150 150 150
##$DBP07= 0
##$DBPNAM0= <>
##$DBPNAM1= <>
##$DBPNAM2= <>
##$DBPNAM3= <>
##$DBPNAM4= <>
##$DBPNAM5= <>
##$DBPNAM6= <>
##$DBPNAM7= <>
##$DBPOAL= (0..7)
0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5
##$DBPOFFS= (0..7)
0 0 0 0 0 0 0 0
##$DE= 6
##$DECBNUC= <off>
##$DECIM= 16
##$DECNUC= <off>
##$DECSTAT= 4
##$DIGMOD= 1
##$DIGTYP= 8
##$DL= (0..7)
0 120 120 120 120 120 120 120
##$DP= (0..7)
150 150 150 150 150 150 150 150
##$DP07= 0
##$DPNAME0= <>
##$DPNAME1= <>
##$DPNAME2= <>
##$DPNAME3= <>
##$DPNAME4= <>
##$DPNAME5= <>
##$DPNAME6= <>
##$DPNAME7= <>
##$DPOAL= (0..7)
0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5
##$DPOFFS= (0..7)
0 0 0 0 0 0 0 0
##$DQDMODE= 0
##$DR= 18
##$DS= 128
##$DSLIST= <SSSSSSSSSSSSSSS>

##$DSPFIRM= 0
##$DSPFVS= 12
##$DTYPA= 0
##$EXP= <>
##$F1LIST= <111111111111111>
##$F2LIST= <222222222222222>
##$F3LIST= <333333333333333>
##$FCUCHAN= (0..9)
0 1 0 4 0 0 0 0 0 0
##$FL1= 90
##$FL2= 90
##$FL3= 90
##$FL4= 90
##$FOV= 20
##$FQ1LIST= <freqlist>
##$FQ2LIST= <freqlist>
##$FQ3LIST= <freqlist>
##$FQ4LIST= <freqlist>
##$FQ5LIST= <freqlist>
##$FQ6LIST= <freqlist>
##$FQ7LIST= <freqlist>
##$FQ8LIST= <freqlist>
##$FS= (0..7)
83 83 83 83 83 83 83 83
##$FTLPGN= 0
##$FW= 125000
##$FnMODE= 0
##$GP031= 0
##$GPNAM0= <sine.100>
##$GPNAM1= <SINE.100>
##$GPNAM10= <sine.100>
##$GPNAM11= <sine.100>
##$GPNAM12= <sine.100>
##$GPNAM13= <sine.100>
##$GPNAM14= <sine.100>
##$GPNAM15= <sine.100>
##$GPNAM16= <sine.100>
##$GPNAM17= <sine.100>
##$GPNAM18= <sine.100>
##$GPNAM19= <sine.100>
##$GPNAM2= <SINE.100>
##$GPNAM20= <sine.100>
##$GPNAM21= <sine.100>
##$GPNAM22= <sine.100>
##$GPNAM23= <sine.100>
##$GPNAM24= <sine.100>

##$GPNAM25= <sine.100>
##$GPNAM26= <sine.100>
##$GPNAM27= <sine.100>
##$GPNAM28= <sine.100>
##$GPNAM29= <sine.100>
##$GPNAM3= <SINE.100>
##$GPNAM30= <sine.100>
##$GPNAM31= <sine.100>
##$GPNAM4= <sine.100>
##$GPNAM5= <sine.100>
##$GPNAM6= <sine.100>
##$GPNAM7= <sine.100>
##$GPNAM8= <sine.100>
##$GPNAM9= <sine.100>
##$GPX= (0..31)
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
##$GPY= (0..31)
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
##$GPZ= (0..31)
0 10 25 50 25.33 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 25 15 20 0 45 20 20 0
0 0 0
##$GRDPROG= <grad_out>
##$HDDUTY= 20
##$HDRATE= 20
##$HGAIN= (0..3)
0 0 0 0
##$HL1= 256
##$HL2= 35
##$HL3= 16
##$HL4= 17
##$HOLDER= 0
##$HPMOD= (0..7)
0 0 0 0 0 0 0 0
##$HPPRGN= 0
##$IN= (0..31)
0.0002083375 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001
0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001
0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001
##$INP= (0..31)
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
##$INSTRUM= <spect>
##$L= (0..31)
170 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
##$LFILTER= 200
##$LGAIN= -5
##$LOCKED= yes

##$LOCKFLD= 1531
##$LOCKGN= 133.100006103516
##$LOCKPOW= -20
##$LOCKPPM= 4.69999980926514
##$LOCNUC= <2H>
##$LOCPHAS= 355
##$LOCSHFT= no
##$LTIME= 0.200000002980232
##$MASR= 0
##$MASRLST= <masrlst>
##$NBL= 1
##$NC= -2
##$NS= 48
##$NUC1= <1H>
##$NUC2= <off>
##$NUC3= <15N>
##$NUC4= <off>
##$NUC5= <off>
##$NUC6= <off>
##$NUC7= <off>
##$NUC8= <off>
##$NUCLEI= 0
##$NUCLEUS= <off>
##$O1= 2809.55
##$O2= 0
##$O3= 7193.65823400153
##$O4= 0
##$O5= 0
##$O6= -99990000
##$O7= -99990000
##$O8= -99990000
##$OBSCHAN= (0..9)
0 0 0 0 0 0 0 0 0 0
##$OVERFLW= 0
##$P= (0..31)
10 9.5 19 42.5 85 1400 116 0 1600 1600 0 0 0 0 0 1500 500 1500 1000 1000
500 1500 500 1500 0 1000 1000 500 2000 400 0 0
##$PAPS= 2
##$PARMODE= 1
##$PCPD= (0..9)
100 250 200 200 100 100 100 100 100 100
##$PHCOR= (0..31)
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
##$PHP= 1
##$PH_ref= 0
##$PL= (0..31)

120 2 53 -0.5 120 13 120 120 44.5 47 120 120 120 120 120 120 13.55 120
120 120 120 120 120 120 120 120 120 120 120 120 120 120 120 120
##$POWMOD= 0
##$PR= 1
##$PRECHAN= (0..15)
-1 1 -1 -1 0 4 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1
##$PRGAIN= 0
##$PROBHD= <5 mm CPTXI 1H-13C/15N/2H Z-GRD Z44866/0137
>
##$PROSOL= no
##$PULPROG= <yl.ts.zzex_c.f3>
##$PW= 0
##$QNP= 1
##$QS= (0..7)83 83 83 83 83 83 83 22
##$QSB= (0..7)83 83 83 83 83 83 83 83
##$RD= 0
##$RECCHAN= (0..15)
-1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1
##$RECPH= 0
##$RG= 256
##$RO= 0
##$ROUTWD1= (0..23)
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0
##$ROUTWD2= (0..23)
0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 1 1 0 0
##$RPUUSED= (0..8)
0 0 0 0 0 0 0 0 0
##$RSEL= (0..9)
0 2 0 0 5 0 0 0 0 0
##$S= (0..7)
83 4 83 83 83 83 83 83
##$SEOUT= 0
##$SFO1= 600.13280955
##$SFO2= 600.13
##$SFO3= 60.817838658234
##$SFO4= 600.13
##$SFO5= 600.13
##$SFO6= 500.13
##$SFO7= 500.13
##$SFO8= 500.13
##$SOLVENT= <MeOD>
##$SP= (0..31)
1 17.4 0 0 0 0 17.4 0 40.4 40.4 0 0 0 0 0 0 150 150 150 150 150 150 150
150 150 150 150 150 150 150 150 150 150 150
##$SP07= 0
##$SPECTR= 0

##$SPNAM0= <gauss>
##$SPNAM1= <rsnob.512>
##$SPNAM10= <gauss>
##$SPNAM11= <gauss>
##$SPNAM12= <gauss>
##$SPNAM13= <gauss>
##$SPNAM14= <gauss>
##$SPNAM15= <gauss>
##$SPNAM16= <gauss>
##$SPNAM17= <gauss>
##$SPNAM18= <gauss>
##$SPNAM19= <gauss>
##$SPNAM2= <gauss>
##$SPNAM20= <gauss>
##$SPNAM21= <gauss>
##$SPNAM22= <gauss>
##$SPNAM23= <gauss>
##$SPNAM24= <gauss>
##$SPNAM25= <gauss>
##$SPNAM26= <gauss>
##$SPNAM27= <gauss>
##$SPNAM28= <gauss>
##$SPNAM29= <gauss>
##$SPNAM3= <gauss>
##$SPNAM30= <gauss>
##$SPNAM31= <gauss>
##$SPNAM4= <gauss>
##$SPNAM5= <gauss>
##$SPNAM6= <rsnob.512>
##$SPNAM7= <gauss>
##$SPNAM8= <square.1000>
##$SPNAM9= <square.1000>
##$SPOAL= (0..31)
0.5 0 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5
0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5
##$SPOFFS= (0..31)
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
##$SUBNAM0= <"">
##$SUBNAM1= <"">
##$SUBNAM2= <"">
##$SUBNAM3= <"">
##$SUBNAM4= <"">
##$SUBNAM5= <"">
##$SUBNAM6= <"">
##$SUBNAM7= <"">
##$SUBNAM8= <"">

##$SUBNAM9= <"">
##$SW= 16.0220946001578
##$SWIBOX= (0..15)
0 0 4 4 0 5 6 0 0 0 0 0 0 0 0 0
##$SW_h= 9615.38461538462
##$TD= 2048
##$TD0= 1
##$TE= 309.6
##$TE2= 300
##$TE3= 300
##$TEG= 300
##$TL= (0..7)
0 120 120 120 120 120 120 120
##$TP= (0..7)
150 150 150 150 150 150 150 150
##$TP07= 0
##$TPNAME0= <>
##$TPNAME1= <>
##$TPNAME2= <>
##$TPNAME3= <>
##$TPNAME4= <>
##$TPNAME5= <>
##$TPNAME6= <>
##$TPNAME7= <>
##$TPOAL= (0..7)
0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5
##$TPOFFS= (0..7)
0 0 0 0 0 0 0 0
##$TUNHIN= 0
##$TUNHOUT= 0
##$TUNXOUT= 0
##$USERA1= <user>
##$USERA2= <user>
##$USERA3= <user>
##$USERA4= <user>
##$USERA5= <user>
##$V9= 5
##$VALIST= <valist>
##$VCLIST= <CCCCCCCCCCCCCCC>
##$VD= 0
##$VDLIST= <DDDDDDDDDDDDDDD>
##$VPLIST= <PPPPPPPPPPPPPPP>
##$VTLIST= <TTTTTTTTTTTTTTT>
##$WBST= 1024
##$WBSW= 2
##$WS= (0..7)83 83 83 83 83 83 83 83

##$XGAIN= (0..3)
0 0 0 0
##$XL= 0
##$YL= 0
##$YMAX_a= 23313
##$YMIN_a= -20015
##$ZGOPTNS= <>
##$ZL1= 120
##$ZL2= 120
##$ZL3= 120
##$ZL4= 120
##END=