Measurement of ^{15}N relaxation rates in perdeuterated proteins by TROSY-based methods

Supporting Information

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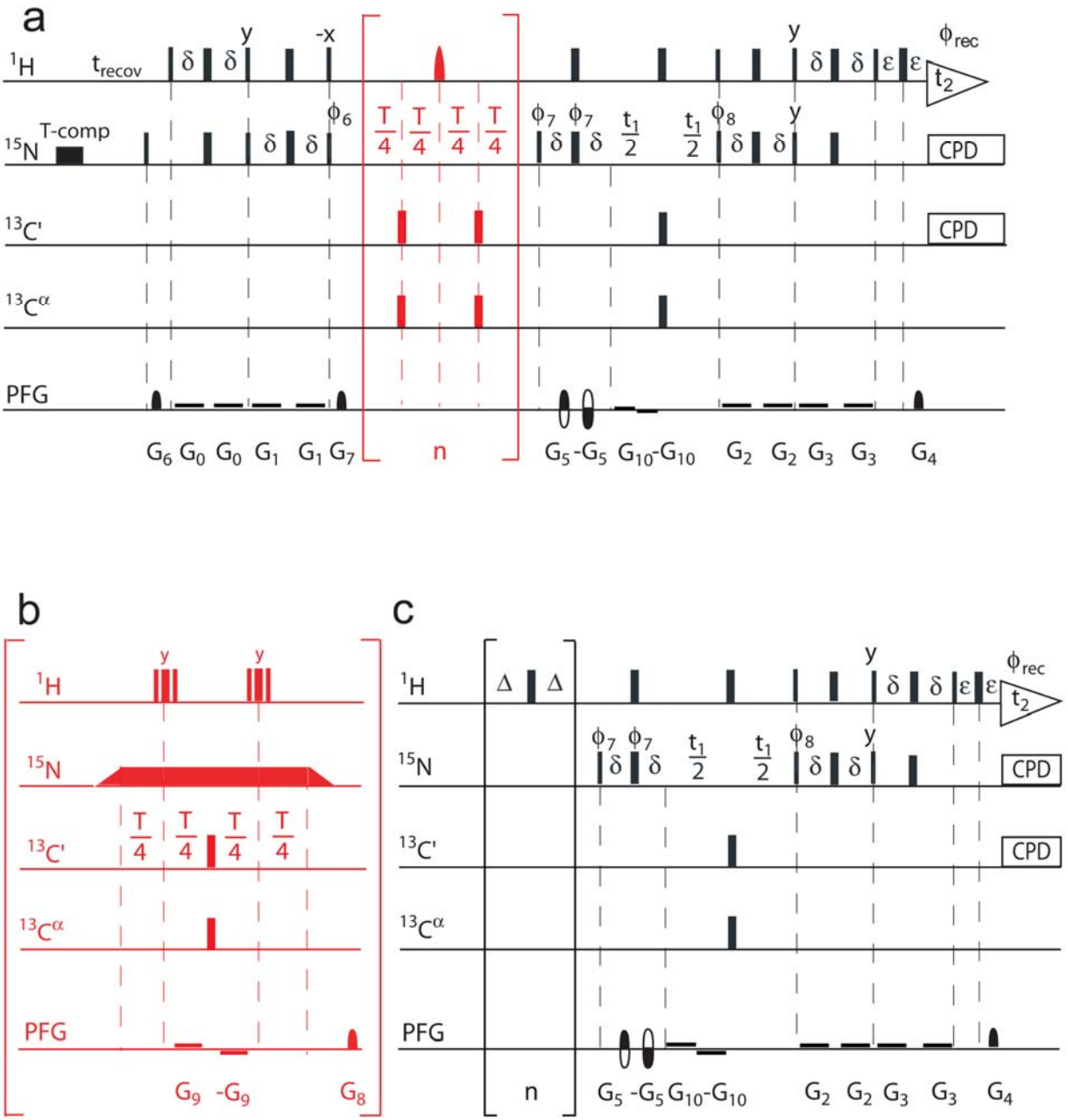

Fig. S1 Pulse schemes for measurement of ^{15}N relaxation rates through sensitivity-enhanced HSQC ^{1}H detection in perdeuterated, amide-protonated proteins. (**a**) Scheme for measurement of ^{15}N R_1. Substitution of the element shown in (**b**) for the red-bracketed element in (**a**) converts the experiment to a ^{15}N $R_{1\rho}$ measurement. (**c**) Pulse scheme for measurement of ^{15}N-{^{1}H} NOE. Narrow and wide pulses correspond to 90° and 180° flip angle pulses, respectively, with phase x unless otherwise indicated. For application to ^{13}C labeled samples: durations of ^{13}C pulses (all

180°) are equal to $\frac{\sqrt{8}}{\Omega}$ (47.4 μs at 600 MHz), where Ω is the frequency difference between $^{13}C^{\alpha}$ and $^{13}C'$. Delay durations: δ = 2.65 ms (≈$1/4^{1}J_{NH}$); ε corresponds to the duration of the decoding gradient G_4 (201 μs) plus a short (250 μs) recovery delay. Gradients G_0 (2.65 ms; 2.1 G/cm), G_1 (2.65 ms; 1.4 G/cm), G_2 (2.65 ms; -3.5 G/cm), G_3 (2.65 ms; -0.35 G/cm), G_9 (T/4; 0.35 G/cm), G_{10} (t_1/4; 1.05 G/cm) are rectangular shaped. Gradients G_4 (201 μs; 28 G/cm), G_5 (1 ms; 28 G/cm) G_6 (1 ms; 21 G/cm), G_7 (200 μs; -35 G/cm) and G_8 (300 μs, 28 G/cm) are sine-bell shaped. Phase cycling: ϕ_6 = 2(y),2(-y); ϕ_7 = y,-y; ϕ_8 = x; ϕ_{rec} = y,-y,-y,y. Quadrature detection is implemented using the gradient-enhanced echo/anti-echo scheme (Kay et al. 1992) with the polarity of gradients G_5 and $-G_5$ inverted, and phase ϕ_8 = -x for the second FID, generated for each quadrature pair. (**a**) The 180° ^{1}H pulse applied at the midpoint of T (40 ms) is of the IBURP2 type and has a 2 ms duration (at 600 MHz), and the loop is repeated an even number of times (n=0, 2, …). ^{13}C 180º pulses serve to eliminate cross correlation effects resulting from ^{15}N-^{13}C dipolar interaction in samples that include ^{13}C labeling. To ensure that the same RF heating applies in the R_1 and $R_{1\rho}$ experiments, immediately following data acquisition, a ^{15}N temperature compensation pulse (Wang and Bax 1993) is applied that corresponds to the longest spin-lock time of the $R_{1\rho}$ experiment (see below). (**b**) The triangle shaped RF fields preceding and following the spin-lock period are adiabatic half passage pulses (see Fig. 1, main text). To eliminate the effect of cross-correlated relaxation, RF-inhomogeneity and offset-compensated 90_x-210_y-90_x ^{1}H pulses are applied at T/4 and 3T/4. H_2O radiation damping between the two composite pulses is prevented by the very weak (0.35 G/cm) gradient G_9. For ^{13}C-enriched samples, 180º $^{13}C^{\alpha}$ and $^{13}C'$ pulses are applied to cancel possible cross-correlated relaxation effects related to the ^{15}N-^{13}C dipolar coupling. Temperature compensation (50 kHz off-resonance) is applied at the RF field strength used during T, for a duration that equals the difference of the current and the maximum spin-lock durations. (**c**) ^{1}H saturation is achieved by *n* repetitions of the symmetric (Δ-180°- Δ) unit (Ferrage et al. 2010), with the ^{1}H carrier switched to 8.6 ppm and Δ equal to≈11 ms which corresponds to $1/J_{NH}$.

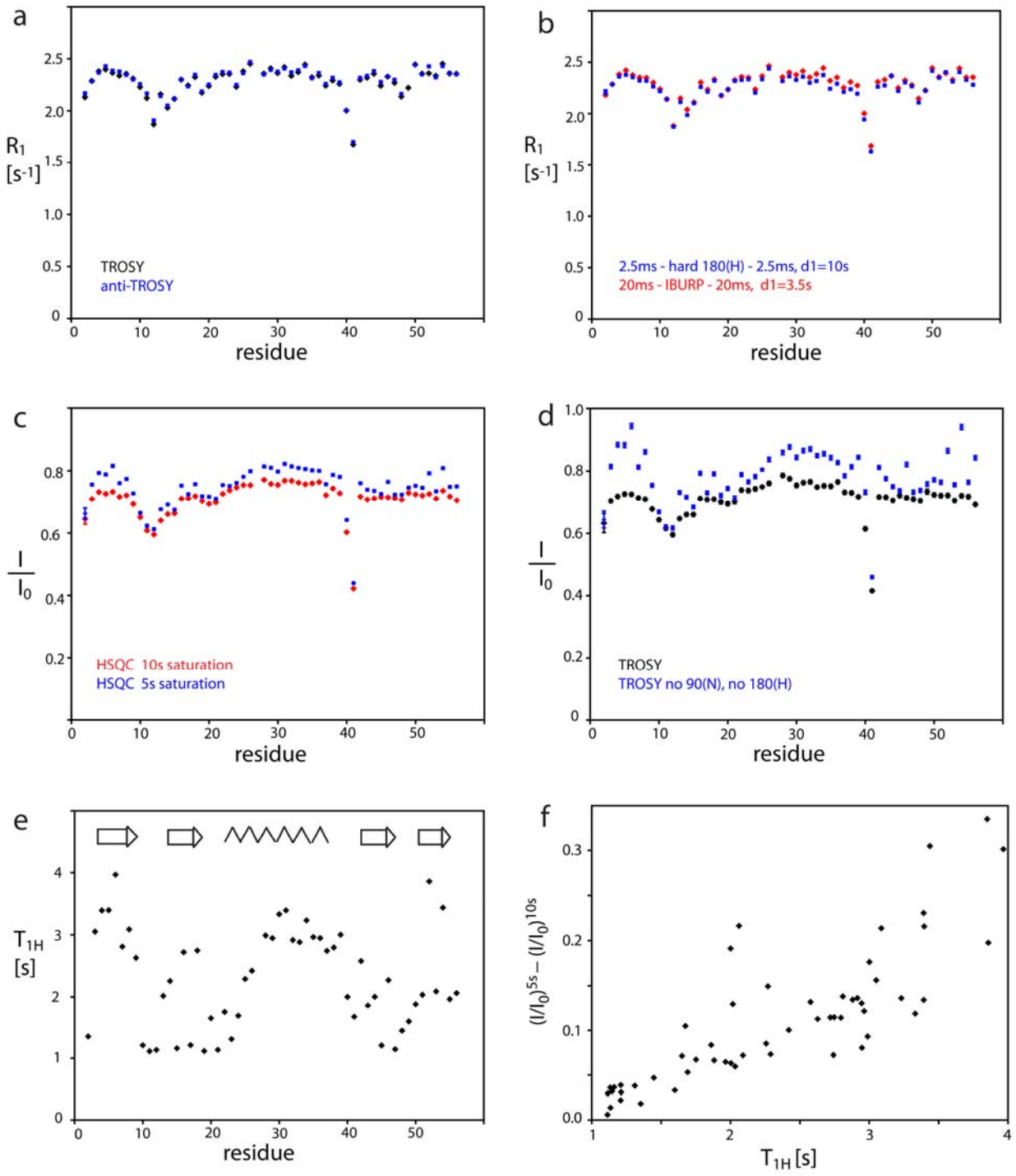

Fig. S2 Extension to the discussion of findings and systematic errors in ^{15}N relaxation experiments. (**a**) R_1 relaxation rates measured as described in Fig. 1 (main text) when reading out the ^{15}N TROSY component (black) and the anti-TROSY component (blue). The effect of cross-correlated relaxation during the R_1 relaxation delay T is sufficiently cancelled by using an even number of 180° inversion IBURP pulses that are separated by 40-ms delays, as evidenced by the

close agreement between relaxation rates extracted from the upfield and downfield doublet components. (**b**) R_1 relaxation rates measured using non-selective 180° (^{1}H) proton pulses to remove cross-correlation, combined with a 10s recovery delay (blue), show less scatter and closely match the R_1 rates (HSQC, red) of Fig. 3**c**, main text (**c**) ^{15}N-^{1}H NOE values measured with gradient-enhanced HSQC read out are systematically too high after 5+1 s of recovery/saturation delay (blue) when comparing to data recorded with 10+1 s recovery/saturation delay (red). (**d**) ^{15}N-^{1}H NOE values are significantly overestimated after a (5+1)-s of recovery/saturation delay when no 90° pulse on ^{15}N and no 180° (H) pulse during the echo/antiecho encoding is applied (blue), compared to the TROSY-detection scheme of Fig. 1c, measured with a 10+1 s recovery/saturation delay (black). (**e**) Apparent ^{1}H T_1 times derived from two sensitivity-enhanced HSQC experiments, one with a 20s recovery delay and one with a 2s recovery delay, assuming the ^{1}H magnetization to be fully relaxed after 20s. (**f**) The difference between NOE values measured using a (5+1)-s delay as described in (d) and a (10+1)-s delay using the pulse sequence described in Fig. 1c (main text) versus the apparent ^{1}H relaxation time, T_{1H}.

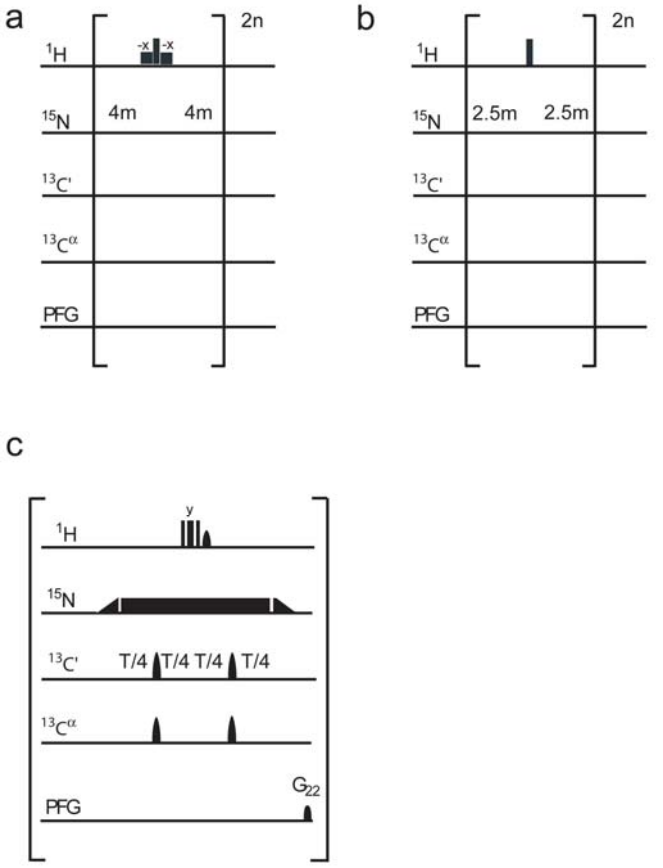

Fig. S3 Different schemes for the suppression of cross-correlated relaxation in R_1 and $R_{1\rho}$ experiments. (**a**) Recently proposed R_1 element that considers the adverse effect of water saturation (Chen and Tjandra 2011). A hard 180°$_x$ (^{1}H) pulse is surrounded by water-selective 90°$_{-x}$ pulses, with the non-selective 180° pulses separated by 8 ms delays. (**b**) Widely used element for the suppression of cross-correlated relaxation during the variable delay period in R_1 relaxation experiment using hard 180° (^{1}H) pulses, separated by 5 ms delays. (**c**) Previously used R_2 element (Chill et al. 2006) which uses only one 180° (^{1}H) pulse followed by a water-flip back pulse, for the cancellation of cross-correlated relaxation during the spin-lock period, but which incompletely suppresses the effect of amide proton spin-spin flips (see Fig. S4).

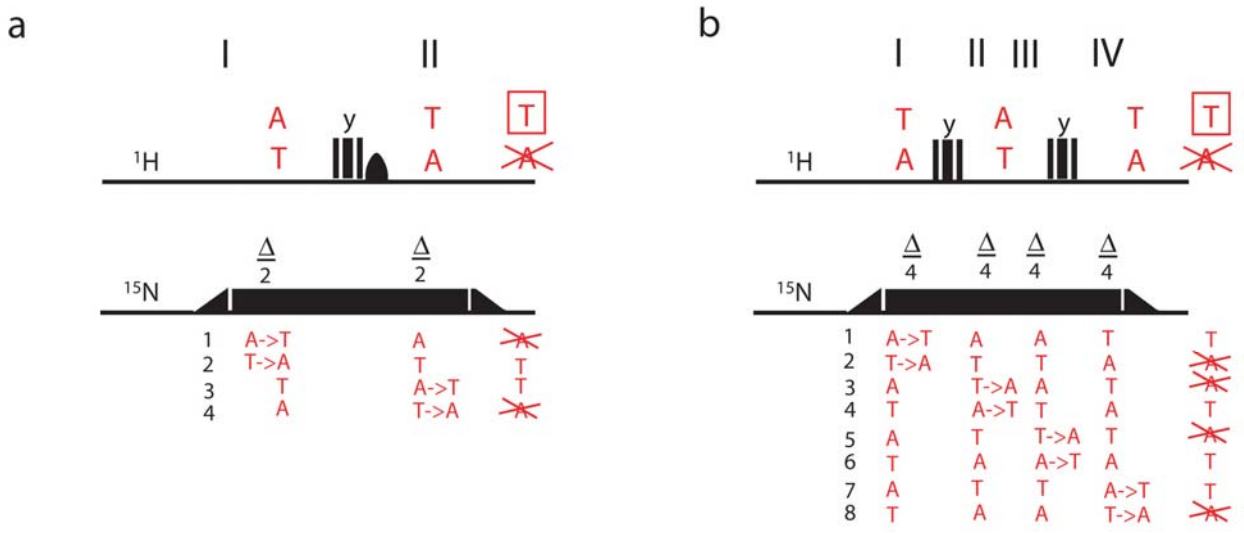

Fig. S4 Schematic diagrams of the magnetization pathway during the $R_{1\rho}$ spin-lock period for TROSY-detected schemes that use (**a**) only a single composite 180° (^{1}H) pulse (plus water-flip back pulse) and (**b**) two composite 180° (^{1}H) pulses, with detection of the TROSY component in both cases. As discussed below, only for (**b**) is the net effect of ^{1}H spin-spin flips during the spin lock duration on the measured $R_{1\rho}$ rate effectively suppressed. A and T refer to the anti-TROSY and TROSY components of the ^{15}N magnetization.

Effect of amide proton spin-spin flips on the measured R_2 rates

R_2 relaxation rates measured with only one 180° (^{1}H) proton pulse (plus one water flip back pulse) significantly underestimate the R_2 rates compared to using two 180° pulses (Fig. 4b, main text). As explained below, the reason for this discrepancy lies in the incomplete cancellation of the effect of amide proton spin-spin flips on cross correlated relaxation. A 180° (^{1}H) applied at the midpoint of the spin-lock period interchanges the $N_{tr}H^{\beta}$ and $N_{tr}H^{\alpha}$ doublet components, below referred to as the TROSY and anti-TROSY components. To first order, the detected magnetization will have existed for an equal amount of time as the TROSY and as the anti-TROSY component, thereby eliminating the effect of cross-correlated relaxation between the NH dipole-dipole interaction and ^{15}N CSA: $R_2(N_{tr})= R_2(N_{tr}E)= R_2(N_{tr}H^{\beta}) + R_2(N_{tr}H^{\alpha})$, where E is the identity matrix and N_{tr} represents the transverse ^{15}N magnetization. However, as explained below, amide ^{1}H spin-spin flips will lead to a bias towards magnetization that exists for a longer period of time as the TROSY component. Only when two ^{1}H pulses are applied at T/4 and 3T/4 will the net effect of such ^{1}H spin-spin flips be cancelled to second order.

Assuming ^{1}H spin flip rates smaller than ~1/T for all durations of T, as will apply in practice for perdeuterated proteins, we can neglect the effect of cases where a given amide ^{1}H undergoes two spin-flips during the spin lock period. Only magnetization that exists as the TROSY component (T) at the end of the spin lock duration will give rise to detectable ^{1}H signal, whereas the anti-TROSY component (A) does not contribute. In the absence of ^{1}H spin flips, only pathway A→T is observed for Fig. S4a, and T→A→T for Fig. S4b, with the impact of cross-correlated relaxation effectively eliminated in both cases. For proteins where a single ^{1}H spin-flip takes place during the spin lock duration, two pathways contribute to observable magnetization in Fig. S4a (marked as pathways 2 and 3). Magnetization that starts out as T at the start of the spin lock period switches to A (pathway 2) if a random ^{1}H spin flip takes place during the first half of the spin lock duration, and is subsequently converted back to T by the pulse applied at the midpoint of the spin lock duration, followed by detection. This component therefore has existed for a longer period of time as the T component than as the A component, resulting in incomplete cancellation of cross correlated relaxation. Similarly, pathway 3 in Fig. S4a corresponds to ^{15}N-^{1}H spin pairs where the ^{1}H flips its spin state during the second half of the spin lock period, again resulting in observed signal that has existed for a longer period as T than as A magnetization.

By contrast, when considering the pathways contributing in the scheme of Fig. S4b for spin pairs where the amide ^{1}H undergoes a spin-flip during the spin lock period, four possible pathways give rise to detectible magnetization, T, at the end of the spin lock period: pathway 1 when the ^{1}H spin flip occurs during the first $\Delta/4$ period, pathway 4 when it occurs during the second $\Delta/4$ period, pathways 6 and 7 when the spin flips occurs during the third and fourth $\Delta/4$ period, respectively, with the population of these pathways being equal. Pathways 1 and 6 correspond to ^{15}N magnetization that has, on average, existed for $^{5}/_{8}\Delta$ as A, and for $^{3}/_{8}\Delta$ as T. Similarly, pathways 4 and 7 correspond to detected magnetization that existed for $^{5}/_{8}\,\Delta$ as T, and for $^{3}/_{8}\,\Delta$ as A. The total detected magnetization corresponds to the sum of pathways 1, 6, 4 and 7, and therefore corresponds to magnetization that existed for equal amounts of time as A and as T components, thereby effectively eliminating the effect of cross-correlated relaxation.

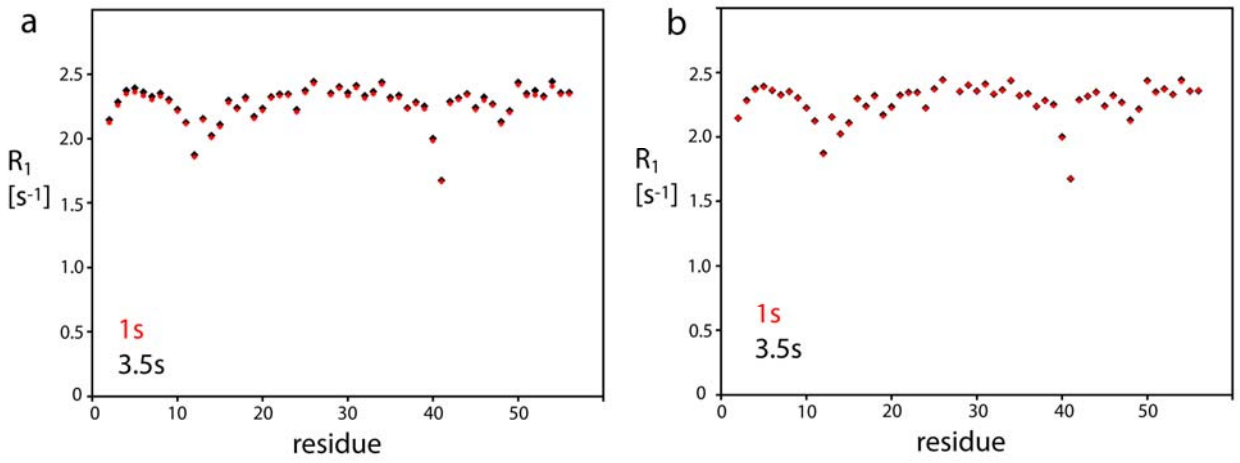

Fig. S5 R_1 relaxation rates measured with TROSY detection and a recycle delay of 3.5s (black) are compared to those measured with a recycle delay of 1s between scans (red). (a) When a short recycle delay of only 1s is used, the ^{15}N R_1 relaxation rates are slightly underestimated (linear regression slope m=0.9921+/-0.0004). This very small systematic difference, seen when using very short recycle delays, appears related to the transfer of ^{1}H magnetization to ^{15}N by the TROSY readout element, as discussed in detail for the ^{15}N-{^{1}H} NOE experiment. (b) The small systematic difference can be eliminated when applying a 90° ^{15}N purge pulse directly after acquisition, which impacts data collected for recycle delays that are not much longer than T_{1N}.

References

Chen, K and Tjandra, N (2011) Water proton spin saturation affects measured protein backbone (15)N spin relaxation rates. J. Magn. Reson. 213: 151-157.

Chill, JH, Louis, JM, Baber, JL and Bax, A (2006) Measurement of N-15 relaxation in the detergent-solubilized tetrameric KcsA potassium channel. J. Biomol. NMR 36: 123-136.

Ferrage, F, Reichel, A, Battacharya, S, Cowburn, D and Ghose, R (2010) On the measurement of (15)N-{(1)H} nuclear Overhauser effects. 2. Effects of the saturation scheme and water signal suppression. J. Magn. Reson. 207: 294-303.

Kay, LE, Keifer, P and Saarinen, T (1992) Pure Absorption Gradient Enhanced Heteronuclear Single Quantum Correlation Spectroscopy with Improved Sensitivity. J. Am. Chem. Soc. 114: 10663-10665.

Wang, AC and Bax, A (1993) Minimizing the Effects of Radiofrequency Heating in Multidimensional NMR Experiments. J. Biomol. NMR 3: 715-720.

Table S1. ^{15}N R_1, ^{15}N R_2 relaxation rates and ^{15}N –{^{1}H} NOE values at 600 MHz, measured with the optimized TROSY-based ^{1}H detection scheme of Fig.1, main text.[a]

residue	^{15}N R_2	Δ ^{15}N R_2	^{15}N R_1	Δ ^{15}N R_1	$^{15}N-\{^1H\}$ NOE	Δ $^{15}N-\{^1H\}$ NOE
Q2	4.292	0.01	2.13	0.007	0.633	0.031
Y3	4.648	0.002	2.288	0.001	0.704	0.006
K4	4.833	0.002	2.38	0.002	0.719	0.005
L5	4.857	0.003	2.398	0.002	0.726	0.006
V6	4.852	0.003	2.366	0.002	0.725	0.006
I7	4.709	0.002	2.338	0.001	0.714	0.005
N8	4.825	0.002	2.356	0.001	0.710	0.005
G9	4.652	0.002	2.311	0.001	0.678	0.005
K10	4.491	0.002	2.229	0.001	0.644	0.005
T11	4.333	0.002	2.123	0.001	0.617	0.007
L12	3.727	0.001	1.87	0.001	0.595	0.006
K13	4.352	0.001	2.16	0.001	0.647	0.005
G14	4.06	0.002	2.029	0.001	0.660	0.005
E15	4.231	0.001	2.115	0.001	0.660	0.005
T16	4.576	0.002	2.3	0.001	0.712	0.005
T17	4.532	0.002	2.244	0.001	0.708	0.006
T18	4.69	0.002	2.324	0.001	0.710	0.005
19K	4.426	0.002	2.174	0.001	0.701	0.007
A20	4.507	0.002	2.238	0.001	0.695	0.006
V21	4.622	0.002	2.327	0.001	0.703	0.008
D22	4.985	0.002	2.353	0.001	0.739	0.006
A23	5.082	0.002	2.355	0.001	0.738	0.006
E24	5.026	0.002	2.231	0.001	0.743	0.006
T25	5.257	0.001	2.38	0.001	0.750	0.004
A26	5.276	0.003	2.452	0.001	0.761	0.006
K28	5.345	0.003	2.358	0.001	0.786	0.006
A29	5.257	0.003	2.408	0.002	0.776	0.006
F30	5.238	0.003	2.363	0.002	0.754	0.006
K31	5.565	0.003	2.419	0.002	0.763	0.006
Q32	5.396	0.003	2.337	0.002	0.766	0.006
Y33	5.288	0.002	2.372	0.001	0.749	0.005
A34	5.453	0.003	2.445	0.002	0.753	0.006
N35	5.276	0.003	2.322	0.002	0.751	0.006
D36	5.312	0.003	2.34	0.001	0.765	0.005
N37	4.668	0.002	2.242	0.001	0.732	0.006
G38	4.837	0.002	2.292	0.001	0.730	0.006
V39	5.053	0.002	2.259	0.001	0.718	0.005
D40	4.248	0.002	2.002	0.001	0.615	0.005
G41	3.33	0.001	1.676	0.001	0.416	0.005

V42	4.783	0.002	2.299	0.001	0.717	0.005
W43	4.792	0.002	2.319	0.001	0.716	0.006
T44	4.738	0.002	2.356	0.001	0.706	0.006
Y45	4.588	0.002	2.241	0.001	0.722	0.006
D46	4.74	0.002	2.329	0.001	0.714	0.006
D47	4.585	0.002	2.27	0.001	0.710	0.007
A48	4.575	0.002	2.136	0.001	0.705	0.006
T49	4.637	0.003	2.221	0.001	0.734	0.008
K50	4.979	0.002	2.446	0.001	0.724	0.006
T51	4.773	0.002	2.357	0.001	0.721	0.006
F52	4.919	0.003	2.36	0.002	0.722	0.006
T53	4.727	0.002	2.337	0.001	0.705	0.006
V54	4.918	0.003	2.452	0.002	0.721	0.006
T55	4.859	0.002	2.359	0.001	0.718	0.006
E56	4.735	0.002	2.356	0.001	0.693	0.005

[a] Measured at 600 MHz ^{1}H frequency, at 25 ºC, for a 1.3 mM sample of uniformly $^{2}H/^{15}N$-enriched GB3, at pH 6.4, in 25 mM NaH_2PO_4 buffer containing 0.01% NaN_3 and 5% D_2O.

Table S2. ^{15}N R_1, ^{15}N R_2 relaxation rates and ^{15}N–{^1H} NOE values at 600 MHz, measured with the optimized HSQC-based ^1H detection scheme of Fig. S1.[a]

residue	^{15}N R_2	Δ ^{15}N R_2	^{15}N R_1	Δ ^{15}N R_1	^{15}N –{^1H} NOE	Δ ^{15}N –{^1H} NOE
Q2	4.42	0.017	2.182	0.012	0.646	0.017
Y3	4.588	0.003	2.286	0.002	0.709	0.003
K4	4.8	0.004	2.383	0.002	0.732	0.003
L5	4.9	0.004	2.421	0.002	0.726	0.003
V6	4.815	0.004	2.375	0.003	0.732	0.003
7I	4.702	0.003	2.351	0.002	0.717	0.003
N8	4.811	0.004	2.349	0.002	0.721	0.003
G9	4.608	0.003	2.302	0.002	0.693	0.003
K10	4.465	0.002	2.239	0.002	0.651	0.003
T11	4.35	0.003	2.14	0.002	0.608	0.004
L12	3.74	0.002	1.881	0.001	0.595	0.003
K13	4.299	0.002	2.149	0.001	0.641	0.003
G14	4.083	0.002	2.04	0.001	0.661	0.003
E15	4.207	0.002	2.111	0.001	0.663	0.003
T16	4.568	0.003	2.304	0.002	0.710	0.003
T17	4.495	0.002	2.233	0.001	0.712	0.003
T18	4.677	0.003	2.333	0.002	0.718	0.003
K19	4.411	0.003	2.175	0.002	0.703	0.003
A20	4.471	0.003	2.234	0.002	0.694	0.003
V21	4.626	0.003	2.328	0.002	0.699	0.004
D22	4.93	0.003	2.358	0.002	0.726	0.003
A23	5.042	0.003	2.35	0.002	0.737	0.003
E24	5	0.003	2.236	0.002	0.746	0.003
T25	5.277	0.002	2.365	0.001	0.754	0.002
A26	5.33	0.004	2.463	0.002	0.754	0.003
K28	5.349	0.004	2.355	0.002	0.771	0.003
A29	5.249	0.004	2.4	0.002	0.758	0.003
F30	5.25	0.004	2.377	0.002	0.755	0.003
K31	5.579	0.005	2.414	0.003	0.768	0.004
Q32	5.411	0.004	2.353	0.002	0.768	0.003
Y33	5.267	0.003	2.386	0.002	0.762	0.003
A34	5.411	0.004	2.443	0.003	0.757	0.003
N35	5.315	0.004	2.321	0.002	0.760	0.003
D36	5.333	0.004	2.352	0.002	0.764	0.003
N37	4.698	0.003	2.253	0.002	0.722	0.003
G38	4.804	0.003	2.308	0.002	0.743	0.003

V39	5.071	0.003	2.273	0.002	0.728	0.003
D40	4.188	0.003	2.002	0.001	0.603	0.003
G41	3.291	0.002	1.686	0.001	0.422	0.002
V42	4.785	0.003	2.309	0.002	0.717	0.003
W43	4.812	0.003	2.331	0.002	0.707	0.003
T44	4.755	0.003	2.366	0.002	0.711	0.003
Y45	4.514	0.002	2.251	0.001	0.716	0.003
D46	4.671	0.003	2.324	0.002	0.715	0.003
D47	4.623	0.003	2.278	0.002	0.712	0.003
A48	4.508	0.002	2.147	0.002	0.707	0.003
T49	4.662	0.004	2.227	0.002	0.729	0.004
K50	4.925	0.003	2.443	0.002	0.724	0.003
T51	4.734	0.003	2.357	0.002	0.720	0.003
F52	4.897	0.005	2.395	0.003	0.726	0.003
T53	4.704	0.003	2.329	0.002	0.711	0.003
V54	4.899	0.004	2.441	0.002	0.736	0.003
T55	4.828	0.003	2.353	0.002	0.718	0.003
E56	4.732	0.003	2.354	0.002	0.705	0.003

[a] Measured at 600 MHz ^{1}H frequency, at 25 ºC, for a 1.3 mM sample of uniformly $^{2}H/^{15}N$-enriched GB3, at pH 6.4, in 25 mM NaH_2PO_4 buffer containing 0.01% NaN_3 and 5% D_2O. Reported uncertainties, Δ, correspond to the statistical error in the fit (R_1, R_2) or to the propagated error in the intensity ratio, based on the signal to noise ration observed for intensities in the reference and attenuated spectra (NOE).

Pulse sequence code for the measurement of ^{15}N R_1, ^{15}N $R_{1\rho}$ relaxation rates and ^{15}N –{^1H} NOE values at 600 MHz with a TROSY based ^1H detection scheme on Bruker Avance spectrometers

15N R1 relaxation experiment (TROSY)

```
; 15N-T1 relaxation experiment with TROSY read-out
; for 15N, 15N13C, 2H15N and 2H15N13C labelled proteins
; written by NL 10/25/11
; see footnotes

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

;#define LABEL_CN  ; switch on for 13C labeled samples
#define TEMP_COMPENSATION


"in0=inf1*0.5"

# ifdef LABEL_CN
"d0=97u-p4*2+p7*0.66-p1*0.5"
"d25=20m-p15*0.5-p4*4-8u"
#else
"d0=100u+p7*0.66-p1*0.5"
"d25=20m-p15*0.5"
#endif /*LABEL_CN*/

"d11=30m"
"DELTA=2.65m"
"DELTA1=2.65m"
"DELTA2=2.65m-p22-p11-300u"
"DELTA3=2.65m-p23-p10-300u"
"DELTA4=260u-p24-p1*0.66"

"d27=p24+35u"

"l1=1"
"l2=1"


"cnst21=176"
"cnst22=56"
"cnst18=-800"

"spoff4=bf2*((cnst22-cnst21)/1000000)"
```

```
1       ze
        1m
2       d11 do:f2
        1m LOCKH_OFF
        3m
3       1m
        1m
4       3m
5       2m BLKGRAD
        10u pl1:f1
        10u pl4:f2
        10u pl7:f3

       (p7 ph0):f3
       10u

;---------temperature compensation and d1 recovery delay---------
# ifdef TEMP_COMPENSATION

"d17=d1-p18"

        10u  fq=cnst18(bf ppm):f3
        10u pl8:f3
        (p18 ph0):f3    ; 15N pulse is applied far off-resonance
        10u
        10u  fq=0:f3
        d17
# else
        d1
# endif
        1m UNBLKGRAD
        10u pl7:f3

;------- kill steady state 15N ------------
        (p7 ph0):f3
        5u
        p20:gp6
        200u

;------- first INEPT Hz-> 2HxNz -----------
        (p1 ph0):f1
        5u
        DELTA gron0 ; soft gradient to prevent radiation damping
        5u  groff
        (center(p1*2 ph0):f1 (p7*2 ph0):f3)
        5u
        DELTA gron0
        5u groff

;------- rephase  2HxNz to Nz------ --------
        (p1 ph5):f1  (p7 ph0):f3
        5u
        DELTA1 gron1 ; soft gradient to prevent radiation damping
        5u groff
        (center (p1*2 ph0):f1 (p7*2 ph0):f3)
```

```
        5u
        DELTA1 gron1
        5u groff
        (p7 ph6):f3 ; phase-cycle Nz, -Nz for Freeman-Hill decay
        5u
;-----------------------------------------------
        (p1 ph2):f1 ; purge pulse to kill any residual HzNz
        5u
        p21:gp7  ; cleaning gradient
        100u
        100u pl0:f1
;------15N T1 relaxation period--------------

if "l2==1" goto 77  ; jump to 77 for first relaxation data point, needs to be 0 in vclist

70      d25*0.5
#  ifdef LABEL_CN
        3u pl4:f2
        (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
#  endif
        d25*0.5
        (p15:sp5 ph0):f1
        d25*0.5
#  ifdef LABEL_CN
        3u pl4:f2
        (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
# endif
        d25*0.5
        lo to 70 times c   ; delay=2*c*d25 (20ms)

;------Echo/ Anti-echo encoding for TROSY read-out------------
 77   3u
        3u pl4:f2
        3u pl1:f1
        if "l1==1"
        {
        (p7 ph7):f3
        10u
        p25:gp5
        200u
        (p7*2 ph7):f3
        10u
        p25:gp5*-1
        }
        else
        {
        (p7 ph17):f3
       10u
        p25:gp5*-1
        200u
        (p7*2 ph17):f3
        10u
        p25:gp5
        }
;------ t1 (15N) evolution period -------------------------------
```

```
        d0
# ifdef LABEL_CN
        (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
# endif
        d0
;------ start TROSY read-out-----------------------------------
        if "l1==1"
        {
        (p1 ph1):f1     ; Echo
        3u
        3u pl0:f1
        (p11:sp11 ph11:r):f1
        6u
        }
        else
        {
        (p1 ph3):f1    ; Anti-Echo
        3u
        3u pl0:f1
        (p11:sp11 ph13:r):f1
        6u
        }
        5u pl1:f1
;goto 999 ; optimization of  water suppression
        DELTA2
        p22:gp2
        300u
        (center (p1*2 ph0):f1 (p7*2 ph0):f3)
        7u
        p22:gp2
        DELTA2
        300u pl0:f1
;-------------------------------------------------
        (p11:sp12 ph12:r):f1
        5u
        3u pl1:f1
        if "l1==1"
        {
        (p1 ph0):f1 (p7 ph1):f3  ; Echo
        }
        else
        {
        (p1 ph0):f1 (p7 ph3):f3     ; Anti-Echo
        }
        ;goto 999 ; for optimization of  water suppression
        DELTA3
        p23:gp3
        200u
        100u pl10:f1
        (center(p10 ph10:r 5u pl1 p1*2 ph0 5u pl10 p10 ph10:r):f1 (p7*2 ph0 d27):f3)
        5u
;goto 999 ; for optimization of  water suppression
        p23:gp3
        DELTA3
        DELTA4
```

```
      (p7 ph0):f3
      5u
      p24:gp4 ; Echo/Anti-echo decoding gradient
999   5u
      5u pl31:f2
      20u BLKGRAMP
      go=2 ph31 cpds2:f2
      1m do:f2
      1m LOCKH_OFF
      d11 wr #0 if #0 zd
      1m ivc
      1m iu2
      lo to 3 times l6
      1m iu1
      1m igrad EA
      1m ru2
      lo to 4 times 2
      1m id0
      1m ru1
      lo to 5 times l3
1m
1m BLKGRAD
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph5=1
ph6=1 1 1 1  3 3 3 3
ph10=2
ph11=3
ph12=0
ph13=1
ph7=1 0 3 2
ph17=1 2 3 0
ph31=1 2 3 0  3 0 1 2


;-------------NOTES----------------------

;o1p = 4.7 ppm
;o2p=176 ppm (CO)
;o3p=119 ppm

;NS=8*n
;in0=inf/2
;SW=1/(2*in0)
;echo-antiecho in N15 (process as Complex in NmrDraw before splitting the spectra)


; 1H pulses

;p1:  90 deg hard 1H pulse @pl1
;pl1: 1H 90 deg
```

;pl0: 120 dB
;p10: 1200u (@ 600 MHz) 180 deg soft rectangular water flip-back pulse
;p11: 1900u (@ 600 MHz) 90 deg Sinc1.1000 water flip-back pulse (sp11,sp12)
;p15: 2000u (@ 600 MHz) 180 deg IBurp2 pulse on 1H (sp15)
;sp5: 180 deg IBurp2 pulse on 1H (sp15)
;sp11: 90 deg Sinc1.1000 water flip-back pulse
;sp12: 90 deg Sinc1.1000 water flip-back pulse
;spnam5: IBurp2
;spnam11: Sinc1.1000
;spnam12: Sinc1.1000
;spoffs5: 2340Hz @ 600 MHz (8.6 ppm) , should be centered in amide region but not touch the water

; 13C pulses

;p4: 13CO selective 180 deg (23.7*2us @ 600 MHz) @pl4
;pl2: 120 dB
;pl4: 13C 90 deg

;sp4: 13CA selective 180 deg (23.7*2us @ 600 MHz)
;CPDPRG2: garp (aq C' decoupling)
;pcpd5: C' decoupling (140u or 280u @pl31)
;pl31: C' decoupling power

;15N pulses
;p7 : 90 deg hard 15N pulse @pl7
;p18 maximum duration of spinlock; temperature compensation
;pl7 :15N 90 deg
;pl8: 15N spin-lock power

; gradients
;p20: 1000u
;p21: 200u
;p22: 300u
;p23: 1000u
;p24: 60.8u Echo/Anti-echo decoding gradient
;p25: 300u Echo/Anti-echo half-encoding gradient

;for z-only gradients
;gpz0: 3%
;gpz1: 2%
;gpz2: 10%
;gpz3: 50%
;gpz4: 33%
;gpz5: -33%
;gpz6: 30%
;gpz7: -50%

;gpnam2 SINE.10
;gpnam3 SINE.50
;gpnam4 SINE.10
;gpnam5 SINE.10
;gpnam6 SINE.10
;gpnam7 SINE.10

^{15}N $R_{1\rho}$ relaxation experiment (TROSY)

```
; 15N-T1rho relaxation experiment with TROSY read-out
; for 15N, 2H15N, 15N13C and 2H15N13C labelled proteins
; written by NL 10/25/11
; see footnotes

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

;#define LABEL_CN  ; switch on for 13C labelled samples
#define TEMP_COMPENSATION

"in0=inf1*0.5"

# ifdef LABEL_CN
"d0=97u-p4*2+p7*0.66-p1*0.5"
#else
"d0=100u+p7*0.66-p1*0.5"
#endif /*LABEL_CN*/

"d11=30m"
"DELTA=2.65m"
"DELTA1=2.65m"
"DELTA2=2.65m-p22-p11-300u"
"DELTA3=2.65m-p23-p10-300u"
"DELTA4=260u-p24-p1*0.66"

"d27=p24+35u"

"l1=1"
"l2=1"

"cnst21=176"
"cnst22=56"
"cnst18=-800"


"spoff4=bf2*((cnst22-cnst21)/1000000)"

1       ze
        1m
2       d11 do:f2
        1m LOCKH_OFF
        3m
3       1m
        1m
4       3m
5       2m BLKGRAD
        10u pl1:f1
        10u pl4:f2
```

```
        10u pl7:f3

       (p7 ph0):f3
       10u


;---------temperature compensation---------
# ifdef TEMP_COMPENSATION
"p17=p18+1m-vp"
"d17=d1-p17"
# endif

"d8=p8+vp*0.25-p1*2-2u"
"d9=vp*0.5-p1*4-24u"
 "d29=p8"

;-------- Temperature compensation and d1 recovery delay----------------
#ifdef TEMP_COMPENSATION

       10u  fq=cnst18(bf ppm):f3
        10u pl8:f3
       (p17 ph0):f3    ; 15N pulse is applied far off-resonance
       10u
       10u  fq=0:f3
        d17
#else
       d1
#endif
       1m UNBLKGRAD
       10u pl7:f3

;------- kill steady state 15N ------------
       (p7 ph0):f3
       5u
       p20:gp6
       200u

;------- first INEPT Hz-> 2HxNz -----------
       (p1 ph0):f1
       5u
       DELTA gron0 ; soft gradient to prevent radiation damping
       5u  groff
       (center(p1*2 ph0):f1 (p7*2 ph0):f3)
       5u
       DELTA gron0
       5u groff

;------- rephase  2HxNz to Nz------ ---------
       (p1 ph5):f1  (p7 ph0):f3
       5u
       DELTA1 gron1 ; soft gradient to prevent radiation damping
       5u groff
       (center (p1*2 ph0):f1 (p7*2 ph0):f3)
       5u
       DELTA1 gron1
```

```
	5u groff
	(p7 ph6):f3
	5u
;-----------------------------------------------
	(p1 ph2):f1 ; purge pulse to kill any residual HzNz
;goto 999
	5u
	p21:gp7  ; cleaning gradient
	100u
	100u pl8:f3

;------ N15 T1rho relaxation period-----------
# ifdef LABEL_CN
	(d8 p1 ph0 3u p1*2.3 ph1 3u p1 ph0 3u d9*0.5 gron9 3u groff 6u d9*0.5 gron9*-1 3u groff 6u p1 ph0 3u
p1*2.3 ph1 3u p1 ph0):f1 (p8:sp8 ph0 3u pl8 vp ph0 p8:sp9 ph0):f3 (d29 d9 p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
#else
	(d8 p1 ph0 3u p1*2.3 ph1 3u p1 ph0 3u d9*0.5 gron9 3u groff 6u d9*0.5 gron9*-1 3u groff 6u p1 ph0 3u
p1*2.3 ph1 3u p1 ph0):f1 (p8:sp8 ph0 3u pl8 vp ph0 p8:sp9 ph0):f3
#endif
	5u
	p21:gp8
	200u

;------Echo/ Anti-echo encoding for TROSY read-out------------
	3u pl7:f3
	3u pl4:f2
	3u pl1:f1
	if "l1==1"
	{
	(p7 ph7):f3
	10u
	p25:gp5
	200u
	(p7*2 ph7):f3
	10u
	p25:gp5*-1
	}
	else
	{
	(p7 ph17):f3
	10u
	p25:gp5*-1
	200u
	(p7*2 ph17):f3
	10u
	p25:gp5
	}
;------ t1 (15N) evolution period -----------------------------
	d0
# ifdef LABEL_CN
	(p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
# endif
	d0
;------ start TROSY read-out-------------------------------------
	if "l1==1"
```

```
	{
	(p1 ph1):f1	; Echo
	3u
	3u pl0:f1
	(p11:sp11 ph11:r):f1
	6u
	}
	else
	{
	(p1 ph3):f1	; Anti-Echo
	3u
	3u pl0:f1
	(p11:sp11 ph13:r):f1
	6u
	}
	5u pl1:f1
;goto 999 ; optimization of  water suppression
	DELTA2
	p22:gp2
	300u
	(center (p1*2 ph0):f1 (p7*2 ph0):f3)
	7u
	p22:gp2
	DELTA2
	300u pl0:f1
;-------------------------------------------------
	(p11:sp12 ph12:r):f1
	5u
	3u pl1:f1
	if "l1==1"
	{
	(p1 ph0):f1 (p7 ph1):f3  ; Echo
	}
	else
	{
	(p1 ph0):f1 (p7 ph3):f3	; Anti-Echo
	}
;goto 999 ; for optimization of  water suppression
	DELTA3
	p23:gp3
	200u
	100u pl10:f1
	(center(p10 ph10:r 5u pl1 p1*2 ph0 5u pl10 p10 ph10:r):f1 (p7*2 ph0 d27):f3)
	5u
;goto 999 ; for optimization of  water suppression
	p23:gp3
	DELTA3
	DELTA4
	(p7 ph0):f3
	5u
	p24:gp4 ; Echo/Anti-echo decoding gradient
999  5u
	5u pl31:f2
	20u BLKGRAMP
	go=2 ph31 cpds2:f2
```

```
      1m do:f2
      d11 wr #0 if #0 zd
      1m LOCKH_OFF
      2m ivp
      lo to 3 times l6
      3m iu1
      lo to 4 times 2
      1m id0
      1m ru1
      lo to 5 times l3
1m
1m BLKGRAD
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph5= 1
ph6= 1 1 1 1 3 3 3 3
ph9=2
ph10=2
ph11=3
ph12=0
ph13=1
ph7=1 0 3 2
ph17=1 2 3 0
ph31=1 2 3 0 3 0 1 2

;----------NOTES----------

;o1p = 4.7 ppm
;o2p=176 ppm (CO)
;o3p=119 ppm

;NS=8*n
;in0=inf/2
;SW=1/(2*in0)
;echo-antiecho in N15 (process as Complex in NmrDraw before splitting the spectra)


; 1H pulses
;p1:  90 deg hard 1H pulse @pl1
;pl1: 1H 90 deg
;pl0: 120 dB
;p10: 1200u (@ 600 MHz) 180 deg soft rectangular water flip-back pulse
;p11: 1900u (@ 600 MHz) 90 deg Sinc1.1000 water flip-back pulse (sp11,sp12)
;sp11: 90 deg Sinc1.1000 water flip-back pulse
;sp12: 90 deg Sinc1.1000 water flip-back pulse
;spnam11 Sinc1.1000
;spnam12 Sinc1.1000


; 13C pulses
```

;p4: 13CO selective 180 deg (23.7*2us @ 600 MHz) @pl4
;pl4: 13C 90 deg
;sp4: 13CA selective 180 deg (23.7*2us @ 600 MHz)
;CPDPRG2: garp (aq C' decoupling)
;pcpd5: C' decoupling (140u or 280u @pl31)
;pl31: C' decoupling power

;15N pulses
;p7 : 90 deg hard 15N pulse @pl7
;pl7 :15N 90 deg
;p8: adiabatic half-passage pulse (sp8,sp9) to rotate magnetization along spin-lock axis (sp8) and on to -z (sp9)
;p18 maximum duration of spinlock; temperature compensation
;pl8: 15N spin-lock power
;sp8 @pl8 spin-lock power
;sp9 @pl8 spin-lock power
;spnam8 AHP TanhTan (1st half)
;spnam9 AHP TanhTan (2nd half)
;spoal8 1
;spoal9 0

; gradients
;p20: 1000u
;p21: 200u
;p22: 300u
;p23: 1000u
;p24: 60.8u Echo/Anti-echo decoding gradient
;p25: 300u Echo/Anti-echo half-encoding gradient

;for z-only gradients
;gpz0: 3%
;gpz1: 2%
;gpz2: 10%
;gpz3: 50%
;gpz4: 33%
;gpz5: -33%
;gpz6: 30%
;gpz7: -50%
;gpz8: 40%
;gpz9: 0.5%

;gpnam2 SINE.10
;gpnam3 SINE.50
;gpnam4 SINE.10
;gpnam5 SINE.10
;gpnam6 SINE.50
;gpnam7 SINE.10
;gpman8 SINE.10

^{15}N –{^{1}H} NOE experiment (TROSY)

```
; 15N NOE experiment with TROSY read-out
; for 15N, 2H15N, 15N13C and 2H15N13C labeled proteins
; written by NL 10/25/11
; see footnotes

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

;#define LABEL_CN  ; switch on for 13C labeled samples


"in0=inf1*0.5"

# ifdef LABEL_CN
"d0=97u-p4*2+p7*0.66-p1*0.5"
"d25=20m-p15*0.5-p4*4-5u"
#else
"d0=100u+p7*0.66-p1*0.5"
"d25=20m-p15*0.5"
#endif /*LABEL_CN*/

"d11=30m"
"DELTA=2.65m-p25-200u-p10"
"DELTA1=2.65m"
"DELTA2=2.65m-p22-p11-300u"
"DELTA3=2.65m-p23-p2-310u"
"DELTA4=260u-p24-p1*0.37"

"d27=p24+35u"

"l1=1"
"l2=1"


"cnst21=176"
"cnst22=56"
"cnst23=8.5"
"spoff4=bf2*((cnst22-cnst21)/1000000)"
"spoff18=bf3*((cnst18)/1000000)"
"d8=p1"


1       ze
        1m
2       d11 do:f2
        1m LOCKH_OFF
        3m
3       1m
        1m
4       3m
5       2m BLKGRAD
```

```
        10u pl1:f1
        10u pl4:f2
        10u pl7:f3

        (p7 ph0):f3     ; 90N pulse before d1
        d1

;---------------- 1H saturation period-----------------------
     10u fq=cnst23(bf ppm):f1

if "l2 == 1" goto 9
8    11m
     (p1*2 ph0):f1
     11m
lo to 8 times l8

goto 10

9   11m
     d8*2
    11m
lo to 9 times l8


10    10u fq=0:f1
      10u UNBLKGRAD

;------Echo/ Anti-echo encoding for TROSY read-out------------
        if "l1==1"
        {
        (p7 ph7):f3
        10u
        DELTA pl14:f1
        p25:gp5
        200u
        (center(p10 ph14:r 3u pl1 p1 ph0 3u p1*2.3 ph1 3u p1 ph0 3u pl14 p10 ph15:r):f1 (p7*2 ph7):f3)
;goto 999
        10u
        p25:gp5*-1
        DELTA
        }
        else
        {
        (p7 ph17):f3
       10u
        DELTA pl14:f1
        p25:gp5*-1
        200u
        (center(p10 ph14:r 3u pl1 p1 ph0 3u p1*2.3 ph1 3u p1 ph0 3u pl14 p10 ph15:r):f1 (p7*2 ph17):f3)
        10u
        p25:gp5
        DELTA
        }

;------ t1 (15N) evolution period ------------------------------
```

```
        d0
# ifdef LABEL_CN
        (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
# endif
        d0
;------ start TROSY read-out-----------------------------------
        3u pl1:f1
        if "l1==1"
        {
        (p1 ph1):f1     ; Echo
        3u
        3u pl0:f1
        (p11:sp11 ph11:r):f1
        6u
        }
        else
        {
        (p1 ph3):f1    ; Anti-Echo
        3u
        3u pl0:f1
        (p11:sp11 ph13:r):f1
        6u
        }
        5u pl1:f1
;goto 999 ; optimization of  water suppression
        DELTA2
        p22:gp2
        300u
        (center (p1*2 ph0):f1 (p7*2 ph0):f3)
        7u
        p22:gp2
        DELTA2
        300u pl0:f1
;-------------------------------------------------
        (p11:sp12 ph12:r):f1
        5u
        3u pl1:f1
        if "l1==1"
        {
        (p1 ph0):f1 (p7 ph1):f3  ; Echo
        }
        else
        {
        (p1 ph0):f1 (p7 ph3):f3     ; Anti-Echo
        }
;goto 999 ; for optimization of  water suppression
        DELTA3
        p23:gp3
        200u
        100u pl10:f1
       (center(p10 ph10:r 5u pl1 p1*2 ph0 5u pl10 p10 ph10:r):f1 (p7*2 ph0 d27):f3)
        5u
        5u pl1:f1
;goto 999 ; for optimization of  water suppression
        p23:gp3
```

```
      DELTA3
      DELTA4
      (p7 ph0):f3
      5u
      p24:gp4 ; Echo/Anti-echo decoding gradient
999   5u
      5u pl31:f2
      20u BLKGRAMP
      go=2 ph31 cpds2:f2
      1m do:f2
      d11 wr #0 if #0 zd
      1m LOCKH_OFF
      2m iu2
      lo to 3 times 2
      1m iu1
      1m igrad EA
      1m ru2
      lo to 4 times 2
      1m id0
      1m ru1
      lo to 5 times l3
1m
1m BLKGRAD
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph5=1
ph6=1
ph10=2
ph11=3
ph12=0
ph13=1
ph14=2
ph15=0
ph7=1 0 3 2
ph17=1 2 3 0
ph31=1 2 3 0


;----------NOTES----------

;o1p = 4.7 ppm
;o2p=176 ppm (CO)
;o3p=119 ppm

;NS=8*n
;in0=inf/2
;SW=1/(2*in0)
;echo-antiecho in N15 (process as Complex in NmrDraw before splitting the spectra)


; 1H pulses
```

;p1: 90 deg hard 1H pulse @pl1
;pl1: 1H 90 deg
;pl0: 120 dB
;p10: 1200u (@ 600 MHz) 180 deg soft rectangular water flip-back pulse
;p11: 1900u (@ 600 MHz) 90 deg Sinc1.1000 water flip-back pulse (sp11,sp12)
;sp11: 90 deg Sinc1.1000 water flip-back pulse
;sp12: 90 deg Sinc1.1000 water flip-back pulse
;spnam11: Sinc1.1000
;spnam12: Sinc1.1000

; 13C pulses

;p4: 13CO selective 180 deg (23.7*2us @ 600 MHz) @pl4
;pl4: 13C 90 deg
;sp4: 13CA selective 180 deg (23.7*2us @ 600 MHz)
;CPDPRG2: garp (aq C' decoupling)
;pcpd5: C' decoupling (140u or 280u @pl31)
;pl31: C' decoupling power

;15N pulses
;p7 : 90 deg hard 15N pulse @pl7
;pl7 :15N 90 deg

; gradients
;p20: 1000u
;p21: 200u
;p22: 300u
;p23: 1000u
;p24: 60.8u Echo/Anti-echo decoding gradient
;p25: 300u Echo/Anti-echo half-encoding gradient

;for z-only gradients
;gpz0: 3%
;gpz1: 2%
;gpz2: 10%
;gpz3: 50%
;gpz4: 33%
;gpz5: -33%
;gpz20: 28%
;gpz21: -50%

;gpnam2 SINE.10
;gpnam3 SINE.50
;gpnam4 SINE.10
;gpnam5 SINE.10
;gpnam20 SINE.50
;gpnam21 SINE.10

Pulse sequences for the measurement of ^{15}N R_1, ^{15}N $R_{1\rho}$ relaxation rates and ^{15}N –{^{1}H} NOE values at 600 MHz with a sensitivity-enhanced HSQC based ^{1}H detection scheme

^{15}N R_1 relaxation experiment (HSQC)

```
; 15N-T1 relaxation experiment with sensitivity enhanced (Rance-Kay) read-out
; for 15N, 2H15N, 15N13C and 2H15N13C labelled proteins
; written by NL 10/27/11
; see footnotes

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


;#define LABEL_CN  ; switch on for 13C labeled samples
#define TEMP_COMPENSATION

"in0=inf1*0.5"

"d0=10u"
"d11=30m"
"d26=p7-p1"
"DELTA=2.65m"
"DELTA1=2.65m"
"DELTA2=2.65m-200u"
"DELTA3=2.65m-200u"
"DELTA4=p24-p19*0.63"
"DELTA5=2.65m-p25-200u"
"DELTA7=2.65m"
"l1=1"
"l2=1"

#  ifdef LABEL_CN
"DELTA6=35u+p4*4"
"d25=20m-p15*0.5-p4*4-5u"
# else
"DELTA6=30u"
"d25=20m-p15*0.5"
# endif

"cnst21=176"
"cnst22=56"
"cnst18=-800"


"spoff4=bf2*((cnst22-cnst21)/1000000)"


1      ze
       1m
```

```
2       2m do:f3
        1m do:f2
        1m LOCKH_OFF
        d11
3       3m
4       1m
5       1m do:f3
        1m do:f2
        1m BLKGRAD
        10u pl1:f1
        10u pl4:f2



;**********  echo/anti-echo encoding *******

;;---------temperature compensation and d1 recovery delay---------
# ifdef TEMP_COMPENSATION
"d17=d1-p18"

        10u  fq=cnst18(bf ppm):f3
        10u pl8:f3
        (p18 ph0):f3    ; 15N pulse is applied far off-resonance
        10u
        10u  fq=0:f3
         d17
# else
        d1
# endif
        1m UNBLKGRAD
        10u pl7:f3

;------- kill steady state 15N ------------
        (p7 ph0):f3
        5u
        p20:gp6
        200u

;------- first INEPT Hz-> 2HxNz -------------------------
        (p1 ph0):f1
        5u
        DELTA gron0 ; soft gradient to prevent radiation damping
        5u  groff
        (center(p1*2 ph0):f1 (p7*2 ph0):f3)
        5u
        DELTA gron0
        5u groff

;------- rephase  2HxNz to Nz-----------------------------
        (center(p1 ph5):f1  (p7 ph0):f3)
        5u
        DELTA1 gron1 ; soft gradient to prevent radiation damping
        5u groff
        (center (p1*2 ph0):f1 (p7*2 ph0):f3)
        5u
```

```
        DELTA1 gron1
        5u groff
        (p7 ph6):f3 ; phase-cycle Nz, -Nz for Freeman-Hill decay
        5u
;-----------------------------------------------------------
        (p1 ph2):f1 ; purge pulse to kill any residual HzNz
;goto 999
        5u
        p21:gp7  ; cleaning gradient
        100u
        100u pl0:f1
;------15N T1 relaxation period --------------------------

if "l2==1" goto 77

70    d25*0.5
#       ifdef LABEL_CN
         3u pl4:f2
         (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
#      endif
         d25*0.5
         (p15:sp5 ph0):f1
         d25*0.5
#      ifdef LABEL_CN
          3u pl4:f2
         (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
#      endif
         d25*0.5
         lo to 70 times c   ; delay=2*c*d25 (20ms)

;---------- Echo- Antiecho encoding------------------------

77    10u pl1:f1
        5u pl4:f2
        5u pl7:f3
        (p7 ph7):f3
;goto 999
        DELTA6 ;compensation for d0 15N evolution
        DELTA5
        190u
        p25:gp5*EA
        10u
        (center (p1*2 ph0):f1 (p7*2 ph7):f3)
        10u
        p25:gp5*EA*-1
        190u
        DELTA5

; t1 evolution ----------------------------------------------
89    d0*0.5 gron1
        5u groff
        d0*0.5 gron1*-1
        5u groff
#      ifdef LABEL_CN
        (center (p1*2 ph0):f1 (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2)
```

```
#    else
      (p1*2 ph0):f1
#    endif
     d0
;goto 999
;--------Rance-Kay transfer back ----------------------------

98    (center (p1 ph0):f1 (p7 ph8):f3)
      DELTA2 gron2
      200u groff
     (center(p1*2 ph0):f1 (p7*2 ph0):f3)
      DELTA2 gron2
      200u groff

; ---second INEPT ---------------------------------------
     (center (p1 ph1):f1 (p7 ph1):f3)    ;DOUBLE 90
      DELTA3 gron3
      200u groff
      (center(p1*2 ph0):f1 (p7*2 ph0):f3)
      DELTA3 gron3
      200u groff
      (d26*0.5 p19 ph0:r):f1
998  245u
      DELTA4
      (p1*2 ph0):f1
      5u
      p24:gp4
      200u
999  5u
      5u pl30:f2
      10u pl31:f3
      20u BLKGRAMP
      go=2 ph31 cpds3:f3 ;cpd2:f2
      500u do:f3
      500u do:f2
      1m LOCKH_OFF
      d11 wr #0 if #0 zd
      1m ivc
      1m iu2
      lo to 3 times l6
      1m ip8*2
      1m igrad EA
      1m ru2
      lo to 4 times 2
      1m id0
      lo to 5 times l3
1m do:f3
1m BLKGRAD
exit

ph0=0
ph1=1
ph2=2
ph5=1 1 1 1
ph6=1 1 3 3
```

```
ph7= 1 3
ph8= 0
ph31=1 3 3 1


;-------------NOTES----------------------

;o1p = 4.7 ppm
;o2p=176 ppm (CO)
;o3p=119 ppm

;NS=4*n
;in0=inf/2
;SW=1/(2*in0)
;echo-antiecho in N15 (process as Complex in NmrPipe before splitting the spectra)

; 1H pulses
;p1:  90 deg hard 1H pulse @pl1
;p19 last 90 deg hard 1H pulse @pl1, can be adjusted for improving water-suppression
;pl1: 1H 90 deg
;pl0: 120 dB
;p15: 2000u (@ 600 MHz) 180 deg IBurp2 pulse on 1H (sp5)
;sp5: 180 deg IBurp2 pulse on 1H (sp5)
;spnam5: IBurp2
;spoffs5: 2340Hz @ 600 MHz (8.6 ppm) , should be centered in amide region but not touch the water

; 13C pulses
;p4: 13CO selective 180 deg (23.7*2us @ 600 MHz) @pl4
;pl4: 13C 90 deg
;sp4: 13CA selective 180 deg (23.7*2us @ 600 MHz)


;15N pulses
;p7 : 90 deg hard 15N pulse @pl7
;pl7 :15N 90 deg
;p17  maximum duration of spinlock; temperature compensation
;pl8: 15N spin-lock power;CPDPRG3: garp (aq 15N decoupling)
;pcpd3: 15N decoupling (200u @pl31)
;pl31: 15N decoupling power

; gradients
;p20: 1000u
;p21: 200u
;p24: 201u Echo/Anti-echo decoding gradient
;p25: 1000u Echo/Anti-echo half-encoding gradient

;for z-only gradients
;gpz10: -1.5%
;gpz2: -5%
;gpz3: -0.5%
;gpz4: 40%
;gpz5: 40%
;gpz0: 3%
;gpz1: 2%
;gpz6: 30%
```

;gpz7: -50%

;gpnam4 SINE.10
;gpnam5 SINE.10
;gpnam6 SINE.50
;gpnam7 SINE.10

^{15}N $R_{1\rho}$ relaxation experiment (HSQC)

```
; 15N-T1rho relaxation experiment with sensitivity enhanced (Rance-Kay) read-out
; for 15N, 2H15N, 15N13C and 2H15N13C labelled proteins
; written by NL 10/27/11
; see footnotes

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
;#define LABEL_CN  ; switch on for 13C labeled samples
#define TEMP_COMPENSATION

"in0=inf1*0.5"

"d0=10u"
"d11=30m"
"d26=p7-p1"
"DELTA=2.65m"
"DELTA1=2.65m"
"DELTA2=2.65m-200u"
"DELTA3=2.65m-200u"
"DELTA4=p24-p19*0.63"
"DELTA5=2.65m-p25-200u"
"DELTA7=2.65m"
"l1=1"
"l2=1"

#  ifdef LABEL_CN
"DELTA6=35u+p4*4"
# else
"DELTA6=30u"
# endif

"cnst21=176"
"cnst22=56"
"cnst18=-800"
"spoff4=bf2*((cnst22-cnst21)/1000000)"

1       ze
        1m
2       2m do:f3
        1m do:f2
        1m
        d11 LOCKH_OFF
```

```
3       3m
4       1m
5       1m do:f3
        1m do:f2
        1m BLKGRAD
        10u pl1:f1
        10u pl4:f2

;---------temperature compensation---------
# ifdef TEMP_COMPENSATION
"p17=p18+1m-vp"
"d17=d1-p17"
# endif


"d8=p8+vp*0.25-p1*2-2u"
"d9=vp*0.5-p1*4-24u"
"d29=p8"

;-------- Temperature compensation and d1 recovery delay----------------
#ifdef TEMP_COMPENSATION
        10u fq=cnst18(bf ppm):f3
        10u pl8:f3
        (p17 ph0):f3    ; 15N pulse is applied far off-resonance
        10u
        10u fq=0:f3
        d17
#else
        d1
#endif
        1m UNBLKGRAD
        10u pl7:f3
;------- kill steady state 15N ------------
        (p7 ph0):f3
        5u
        p20:gp6
        200u

;------- first INEPT Hz-> 2HxNz -------------------------
        (p1 ph0):f1
        5u
        DELTA gron0 ; soft gradient to prevent radiation damping
        5u  groff
        (center(p1*2 ph0):f1 (p7*2 ph0):f3)
        5u
        DELTA gron0
        5u groff

;------- rephase  2HxNz to Nz------------------------------
        (p1 ph5):f1  (p7 ph0):f3
        5u
        DELTA1 gron1 ; soft gradient to prevent radiation damping
        5u groff
        (center (p1*2 ph0):f1 (p7*2 ph0):f3)
        5u
```

```
      DELTA1 gron1
      5u groff
      (p7 ph6):f3 ; phase-cycle Nz, -Nz for Freeman-Hill decay
      5u
;-------------------------------------------------------
      (p1 ph2):f1 ; purge pulse to kill any residual HzNz
;goto 999
      5u
      p21:gp7  ; cleaning gradient
      100u
      100u pl8:f3

;------ N15 T1rho relaxation period-----------
# ifdef LABEL_CN
       (d8 p1 ph0 3u p1*2.3 ph1 3u p1 ph0 3u d9*0.5 gron9 3u groff 6u d9*0.5 gron9*-1 3u groff 6u p1 ph0 3u
p1*2.3 ph1 3u p1 ph0):f1 (p8:sp8 ph0 3u pl8 vp ph0 p8:sp9 ph0):f3 (d29 d9 p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2
#else
       (d8 p1 ph0 3u p1*2.3 ph1 3u p1 ph0 3u d9*0.5 gron9 3u groff 6u d9*0.5 gron9*-1 3u groff 6u p1 ph0 3u
p1*2.3 ph1 3u p1 ph0):f1 (p8:sp8 ph0 3u pl8 vp ph0 p8:sp9 ph0):f3
#endif
      5u
      p22:gp8
      200u
;---------- Echo- Antiecho encoding------------------------

77     3u pl7:f3
      3u pl4:f2
      3u pl1:f1
                                          (p7 ph7):f3
;goto 999
      DELTA6;compensation for d0 15N evolution
      DELTA5
      190u
      p25:gp5*EA
      10u
      (center (p1*2 ph0):f1 (p7*2 ph7):f3)
      5u
      p25:gp5*EA*-1
      195u
      DELTA5

; t1 evolution ------------------------------------------------
89     d0*0.5 gron1
                                          5u groff
      d0*0.5 gron1*-1
      5u groff
#     ifdef LABEL_CN
      (center (p1*2 ph0):f1 (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2)
#     else
      (p1*2 ph0):f1
#     endif
      d0
;goto 999
;--------Rance-Kay back transfer  ----------------------------
```

```
98    (center (p1 ph0):f1 (p7 ph8):f3)
      DELTA2 gron2
      200u groff
     (center(p1*2 ph0):f1 (p7*2 ph0):f3)
      DELTA2 gron2
      200u groff
      (center (p1 ph1):f1 (p7 ph1):f3)
      DELTA3 gron3
      200u groff
      (center(p1*2 ph0):f1 (p7*2 ph0):f3)
      DELTA3 gron3
      200u groff
      (d26*0.5 p19 ph0:r):f1
998   245u
      DELTA4
      (p1*2 ph0):f1
      5u
      p24:gp4
      200u
999   5u
      5u pl30:f2
      10u pl31:f3
      20u BLKGRAMP
      go=2 ph31  cpds3:f3 ;cpd2:f2
      500u do:f3
      500u do:f2
      d11 wr #0 if #0 zd
      1m LOCKH_OFF
      2m ivp
      lo to 3 times l6
      1m ip8*2
      1m igrad EA
      1m ru2
       lo to 4 times 2
      1m id0
      lo to 5 times l3
1m do:f3
1m BLKGRAD
exit

ph0=0
ph1=1
ph2=2
ph5=1
ph6=1 1 3 3
ph7= 1 3
ph8= 0
ph31=1 3 3 1


;-------------NOTES----------------------

;o1p = 4.7 ppm
;o2p=176 ppm (CO)
;o3p=119 ppm
```

;NS=4*n
;in0=inf/2
;SW=1/(2*in0)
;echo-antiecho in N15 (process as Complex in NmrPipe before splitting the spectra)

; 1H pulses

;p1: 90 deg hard 1H pulse @pl1
;p19 last 90 deg hard 1H pulse @pl1, can be adjusted for improving water-suppression
;pl1: 1H 90 deg

; 13C pulses

;p4: 13CO selective 180 deg (23.7*2us @ 600 MHz) @pl4
;pl4: 13C 90 deg
;sp4: 13CA selective 180 deg (23.7*2us @ 600 MHz)

;15N pulses
;p7 : 90 deg hard 15N pulse @pl7
;p17 maximum duration of spinlock; temperature compensation
;pl7: 15N 90 deg
;pl8: 15N spin-lock power
;sp8 @pl8 spin-lock power
;sp9 @pl8 spin-lock power
;spnam8 AHP TanhTan (1st half)
;spnam9 AHP TanhTan (2nd half)
;spnam18 rect.1000
;spoal8 1
;spoal9 0
;CPDPRG3: garp (aq 15N decoupling)
;pcpd3: 15N decoupling (200u @pl31)
;pl31: 15N decoupling power

; gradients
;p20: 1000u
;p21: 200u
;p22: 300u
;p24: 201u Echo/Anti-echo decoding gradient
;p25: 1000u Echo/Anti-echo half-encoding gradient

;for z-only gradients
;gpz0: 3%
;gpz1: 2%
;gpz2: -5%
;gpz3: -0.5%
;gpz4: 40%
;gpz5: 40%
;gpz6: 30%
;gpz7: -50%
;gpz8: 40%
;gpz9: 0.5%
;gpz10:-1.5%

;gpnam4 SINE.10
;gpnam5 SINE.10
;gpnam6 SINE.50
;gpnam7 SINE.10
;gpnam8 SINE.10

^{15}N –{^{1}H} NOE experiment (HSQC)

; 15N1H HetNoe relaxation experiment with sensitivity enhanced (Rance-Kay) read-out
; for 15N, 2H15N, 15N13C and 2H15N13C labelled proteins
; written by NL 10/27/11
; see footnotes

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
#define LABEL_CN ; switch on for 13C labelled samples

"in0=inf1*0.5"

"d0=10u"
"d11=30m"
"d26=p7-p1"
"DELTA2=2.65m"
"DELTA3=2.65m"
"DELTA4=p24-p19*0.63"
"DELTA5=2.65m-p25-200u"
"DELTA7=2.65m"
"cnst22=176"
"cnst21=56"
"l1=1"
"l2=1"

ifdef LABEL_CN
"DELTA6=35u+p4*4"
"d25=20m-p15*0.5-p4*4-5u"
else
"DELTA6=30u"
"d25=20m-p15*0.5-p4*4-5u"
endif

"cnst21=176"
"cnst22=56"
"cnst23=8.5"
"cnst18=-800"
"spoff4=bf2*((cnst22-cnst21)/1000000)"
"spoff18=bf3*((cnst18)/1000000)"
"d8=p1"

```
1       ze
        1m
2       3m do:f3
        d11
        1m LOCKH_OFF
3       3m
4       1m
5       1m do:f3
        1m BLKGRAD
        10u pl1:f1
        10u pl4:f2
        10u pl7:f3

        (p7 ph0):f3     ; 90N pulse before d1
        d1

;---------- 1H saturation period----------
       10u fq=cnst23(bf ppm):f1

     if "l2 == 1" goto 9
8    11m
     (p1*2 ph0):f1
     11m
lo to 8 times l8
goto 10
9    11m
     d8*2
    11m
lo to 9 times l8

10     10u fq=0:f1
      10u UNBLKGRAD
;---------- Echo- Antiecho encoding----------

       (p7 ph7):f3
;goto 999
       DELTA6;compensation for d0 15N evolution
       DELTA5
       190u
       p25:gp5*EA
       10u
       (center (p1*2 ph0):f1 (p7*2 ph7):f3)
       10u
       p25:gp5*EA*-1
       190u
       DELTA5
; t1 evolution ----------------------------------------------
89     d0*0.5 gron1
       5u groff
       d0*0.5 gron0
       5u groff
#      ifdef LABEL_CN
       (center (p1*2 ph0):f1 (p4*2 ph0 3u 3u pl2 p4*2:sp4 ph0):f2)
```

```
#    else
      (p1*2 ph0):f1
#    endif
      d0
;goto 999
;--------Rance-Kay transfer back ----------------------------

98    (center (p1 ph0):f1 (p7 ph8):f3)
      DELTA2 gron2
      10u groff
     (center(p1*2 ph0):f1 (p7*2 ph0):f3)
      DELTA2 gron2
      10u groff

; ---second INEPT ----------------------------------------
      (center (p1 ph1):f1 (p7 ph1):f3)    ;DOUBLE 90
      DELTA3 gron3
      10u groff
      (center(p1*2 ph0):f1 (p7*2 ph0):f3)
      DELTA3 gron3
      10u groff
      (d26*0.5 p19 ph0:r):f1
998  245u
      DELTA4
      (p1*2 ph0):f1
      5u
      p24:gp4
      200u
999   5u
      10u pl31:f3
      20u BLKGRAMP
      go=2 ph31 cpds3:f3
      1m do:f3
      d11 wr #0 if #0 zd
      1m LOCKH_OFF
      2m iu2
      lo to 3 times 2
      1m ip8*2
      1m igrad EA
      1m ru2
       lo to 4 times 2
      1m id0
      lo to 5 times l3
1m do:f3
1m BLKGRAD
exit

ph0=0
ph1=1          ;check right phase for Boltzmann !!!!!
ph2=2
ph7= 1 3 3 1
ph8= 0
ph31=1 3 3 1

;-------------NOTES----------------------
```

```
;o1p = 4.7 ppm
;o2p=176 ppm (CO)
;o3p=119 ppm

;NS=4*n
;in0=inf/2
;SW=1/(2*in0)
;echo-antiecho in N15 (process as Complex in NmrDraw before splitting the spectra)

; 1H pulses
;p1:  90 deg hard 1H pulse @pl1
;p19 last 90 deg hard 1H pulse @pl1, can be adjusted for improving water-suppression
;pl1: 1H 90 deg
;pl0: 120 dB

; 13C pulses
;p4: 13CO selective 180 deg (23.7*2us @ 600 MHz) @pl4
;pl4: 13C 90 deg
;sp4: 13CA selective 180 deg (23.7*2us @ 600 MHz)


;15N pulses
;p7 : 90 deg hard 15N pulse @pl7
;pl7 :15N 90 deg
;CPDPRG3: garp (aq 15N decoupling)
;pcpd3: 15N decoupling (200u @pl31)
;pl31: 15N decoupling power



; gradients
;p20: 1000u
;p21: 200u
;p24: 201u Echo/Anti-echo decoding gradient
;p25: 1000u Echo/Anti-echo half-encoding gradient

;for z-only gradients
;gpz0: 1.5%
;gpz1: -1.5%
;gpz2: -3%
;gpz3: 0.5%
;gpz4: 40%
;gpz5: 40%
;gpz10: 1%
;gpz11: 2%
;gpz20: 28%
;gpz21: 50%

;gpnam4 SINE.10
;gpnam5 SINE.10
;gpnam20 SINE.50
;gpnam21 SINE.10
```