Supplementary Material

Automated NMR Backbone Assignment of Large Proteins

Barbara Krähenbühl · Julien Boudet · Gerhard Wider

Institute of Molecular Biology and Biophysics, ETH Zurich, 8093 Zurich, Switzerland

NMR spectroscopy

Setup of APSY experiments

The APSY NMR experiments were performed on a 700 MHz Bruker Avance III spectrometer with cryogenic probe, using the software Topspin 2.1 and 3.0 (Bruker, Karlsruhe, Germany). The APSY series are started from a parent data set which is set up like a conventional 4D experiment. The frequency axes of the four dimensions of the 4D TROSY APSY-HNCOCA (APSY one-letter code, as explained e.g. in Hiller *et al* (Hiller and Wider 2011), and tabulated on the manual on www.apsy.ch: OANH) are defined in Topspin as F1 for $^{13}C'$, F2 for $^{13}C^{\alpha}$, F3 for ^{15}N and acquisition dimension F4 for ^{1}H. For the 4D TROSY APSY-HNCACO (APSY one-letter code: aoNH) they are defined as F1 for $^{13}C^{\alpha}$, F2 for $^{13}C'$, F3 for ^{15}N and acquisition dimension F4 for ^{1}H. For the 4D TROSY APSY-HNCACB (APSY one-letter code: baNH) and the 4D TROSY APSY-HN(CO)CACB (APSY one-letter code: BANH) the frequency channels are defined as F1 for $^{13}C^{\beta}$, F2 for $^{13}C'$, F3 for ^{15}N and acquisition dimension F4 for ^{1}H. The automated setup of the projections is carried out in Topspin with an AU program (provided on www.apsy.ch), which first proposes an angle file, and subsequently creates the corresponding 2-dimensional (2D) data sets for the projection experiments. Based on the number of scans used in the direct projections (with one type of nucleus in the indirect dimension), the number of scans is usually doubled for each additional projected frequency axis, in order to compensate for the sensitivity losses of $\sqrt{2}$. Further criteria for adjustments of the number of scans are the maximal evolution times in the indirect dimension, and the relaxation in the included evolution periods.

In the 4D TROSY APSY-HNCACO experiment the carrier offset is set to the $^{13}C^{\alpha}$ frequency during the evolution time of $^{13}C'$. Thus, for the projection which include $^{13}C'$ evolution the

sweep width (SW) needs to be adjusted so that $SW_{CO} = |(\Omega_{CO}-\Omega_{CA})| / N$, with N being an integer and Ω_{CO}/Ω_{CA} the carrier offsets of $^{13}C'/^{13}C^{\alpha}$ (see manuscript). For the 4D TROSY APSY-HNCOCA experiment the same condition for the sweep widths is applicable. The projections which are set up and measured first are usually those which include the frequency of only one nucleus type in the indirect dimension ("direct" projections), and therefore correspond to conventional two-dimensional spectra (Fig. S5). They can serve to estimate the protein-specific sensitivity (determining the number of scans and recovery delay) and chemical shift distribution (determining sweep widths and frequency offsets) for the individual experiments. The orthogonal 2D $^{1}H–^{15}N$ projections (angles α/β = 0°/0°) are particularly useful, since they correspond to the 2D [^{1}H, ^{15}N]-TROSY spectrum and are therefore ideal to estimate the sensitivity and completeness of the experiment, and to conclude the required measurement time. Further setup instructions and acquisition parameters are provided in the figure captions S1 to S3 as well as figure caption 2, and in the Tables S1 to S8. Additional details about the setup of APSY experiments and their processing have been described previously (Hiller et al. 2005; Hiller et al. 2008).

Using the APSY specific AU program, angle sets for the APSY projections are proposed upon setup of the experiments, and can be created using evolution-time optimization or dispersion optimization.(Krähenbühl and Wider 2012) Evolution-time optimized angles allow the maximal possible - or desired - evolution time for all included indirect dimensions; this option is recommended for experiments with constant time-evolution periods. Dispersion-optimized angles balance out differences in signal dispersion and thus different sweep widths. For the current experiments, the angle sets were mostly evolution-time optimized.

Data analysis

Automated serial processing of the spectra was performed with Prosa 6.4 (Güntert et al. 1992), for which the input files were automatically created by the Topspin AU program during generation of APSY projections. For all spectra, 1k complex data points were measured in the acquisition dimension, multiplied with a 75°-shifted sine-bell function, and zero-filled to 4k complex data points. The same window function was applied in the indirect dimensions which contained between 22 and 212 complex data points; the data was zero-filled to 512 complex data points. More acquisition and processing parameters can be found in tables S1 and S2 for the 4D TROSY APSY-HNCACO experiment, in tables S3-S4 for the 4D TROSY APSY-HNCOCA experiment, in tables S5 and S6 for the 4D TROSY APSY-

HNCACB experiment, and in tables S7 and S8 for the 4D TROSY APSY-HN(CO)CACB experiment. The subsequent peak picking and calculation of the multidimensional peak list was accomplished with the GAPRO software, which integrates the eponymous algorithm (available on www.apsy.ch); parameters specified below for the GAPRO processing can vary depending on the input spectra. The resulting peak list is, however, robust with respect to a range of values. The results were evaluated by overlaying the spectra with the back-projected multidimensional peak list in XEASY format (Bartels et al. 1995; Eccles et al. 1991) with the subroutine NEASY as implemented in CARA 1.5.5 (cara.nmr.ch), and by comparing and analyzing the results with different MATLAB (R2010b, The MathWorks, Natick, MA, USA) scripts.

Further considerations

- Successful APSY experiments with deuterated or perdeuterated proteins require complete amide proton back-exchange which can be challenging for large proteins; back-exchange and potentially accelerating procedures have been extensively studied before (e.g. (Suzuki et al. 2012)).

  A comparison of the ^{1}H-^{15}N spectra of the (per)deuterated and the protonated protein allows an estimation of the degree of protonation.
- Criteria for the selection of the pair of experiments (with or without $^{13}C^{\beta}$):

4D TROSY APSY-HNCOCA/HNCACO	**4D TROSY APSY-HNCACB/HN(CO)CACB**
Only backbone deuteration required, i.e. expression in D_2O with protonated glucose	Perdeuteration required, i.e. expression in D_2O with deuterated glucose
Shorter transfer periods, higher sensitivity	Longer transfer periods, lower sensitivity
Long constant time $^{13}C^{\alpha}$ evolution of 16.5-26.5 ms (MQ parallel) during two transfer periods	Constant time $^{13}C^{\alpha}$ evolution of 11.2 ms during one transfer period
For high magnetic fields CSA dominates relaxation during $^{13}C'$ evolution	No $^{13}C'$ in the HNCACB pathway, hence no CSA relaxation
No $^{13}C^{\beta}$ information included	$^{13}C^{\beta}$ resonances included ▪ well-dispersed ▪ amino-acid specific chemical shifts ▪ connection for NOESY or side-chain experiments
Individual offset and sweep with (SW) for each nucleus. SW of $^{13}C^{\alpha}$ (HNCOCA) or $^{13}C'$ (HNCACO) adjusted for correct off-resonance aliasing.	Same offset and SW for $^{13}C^{\alpha}/^{13}C^{\beta}$, hence SW too large for $^{13}C^{\alpha}$; high number of data points required.

- Connection of the 4D HNCACO/HNCOCA peak lists to one 6D COCAHNCOCA peak list with MATLAB (the same considerations are valid for the connection of the 4D HNCACB/HN(CO)CACB peak lists to one 6D CACBHNCACB peak list): The script first filtered sequential peaks from the HNCACO peak list (which contains a mixture between intra-residue and sequential correlations) by matching corresponding peaks in the HNCOCA peak list (which contains only sequential correlations) via all four dimensions. The remaining HNCACO peak list with exclusively intra-residue correlations and the sequential HNCOCA peak list were joined by matching them via the ^{1}H and ^{15}N chemical shifts of the amide group. Elliptical match criteria were used, i.e. two peaks were considered to match when their chemical shift values lie within an ellipse spanned by the threshold values as axes, e.g., when in four dimensions the condition

$$\left(\frac{\delta_{CA1}-\delta_{CA2}}{\Delta CA}\right)^2+\left(\frac{\delta_{CA1}-\delta_{CA2}}{\Delta CO}\right)^2+\left(\frac{\delta_{N1}-\delta_{N2}}{\Delta N}\right)^2+\left(\frac{\delta_{H1}-\delta_{H2}}{\Delta H}\right)^2\leq 1$$

  is fulfilled, with chemical shifts δ and ellipse axes Δ. If there was more than one matching HN peak, the one with the smallest ellipse value was selected.
- For an evaluation of the peak lists we recommend to visualize the resulting peak lists by plotting them with MATLAB or any other program
- MATLAB scripts for matching and evaluations are available upon request
- The highest percentage of assigned peaks was obtained with MARS(Jung and Zweckstetter 2004) for the HNCACO/HNCOCA approach, and reached only when the result of one run was fed as fixed assignment (all assignments with high and medium reliability) for a next run. This procedure led after 4-7 runs to the mentioned assignment. The quality of the resulting assignment was in our hands still quite high, and the unreliable parts were easily recognizable since they were correctly ranked “L” (low reliability) by MARS. The details in this statistic will, however, be strongly dependent on the quality of the peak lists.

4D TROSY APSY-HNCOCA experiment

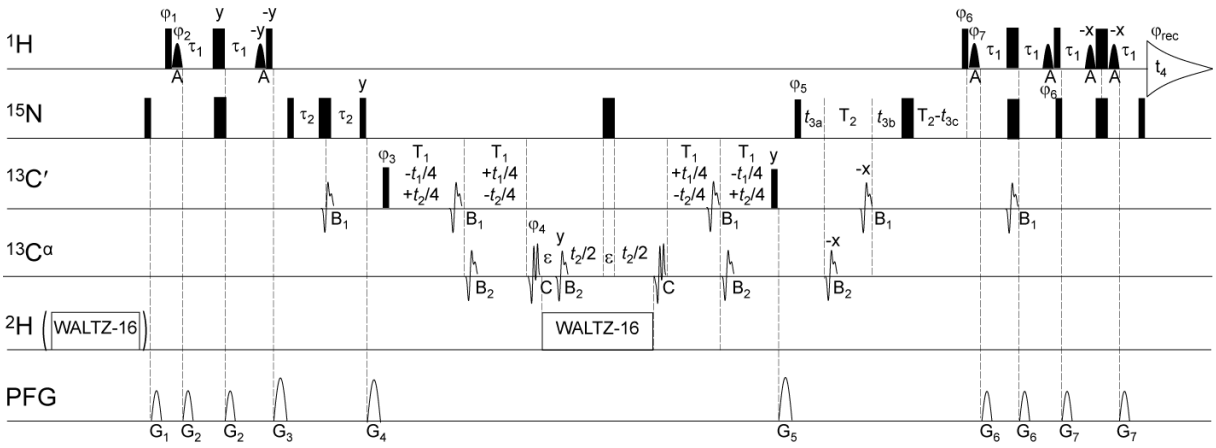

Fig. S1 Pulse sequence of the 4D TROSY APSY-HNCOCA experiment (APSY one-letter code: OANH). Black thin and wide rectangular bars represent 90° and 180° high-power pulses, respectively. The carrier frequencies were set to 4.7 ppm, 118 ppm, 177 ppm, and 3.8 ppm on the ^{1}H, ^{15}N, ^{13}C, and ^{2}H channel, respectively. During the $^{13}C^{\alpha}$ evolution period (t_2), ^{2}H was decoupled with WALTZ-16 (Shaka et al. 1983); for constant average heating with increasing t_2, a corresponding decoupling period at the beginning of the recycle delay is decreased accordingly .(Wang and Bax 1993) Time periods: τ_1 = 2.4 ms, τ_2 = 14.0 ms, T_1 = 4.0 ms, and $T_2 = \tau_2-\tau_1$ = 11.6 ms and ε = 180° ^{15}N (initial value). The pulses labeled (A) are selective 180° Sinc (central lobe) pulses on water with a length of 1 ms; (B_1) and (B_2) are 180° Gaussian cascades (Q3) (Emsley and Bodenhausen 1990) with a duration of 309 µs, on-resonance on 13C′ at 177 ppm and off-resonance at 56 ppm on $^{13}C^{\alpha}$, respectively; pulses (C) are off-resonance 90° Gaussian cascades (Q5) (Emsley and Bodenhausen 1990) at 56 ppm ($^{13}C^{\alpha}$) with a duration of 300 µs on a 700 MHz spectrometer. All pulses were applied along the *x*-axis unless indicated otherwise above the pulse symbol. The following phase cycles were used: φ_1= 4(*x*), 4(-*x*); $\varphi_2 = -\varphi_1$; φ_3 = *x*; φ_4 = 2(*x*), 2(-*x*); φ_5 = -*x*, -*y* or -*x*, *y* (even or odd number of increments); φ_6 = -*y*; $\varphi_7 = -\varphi_6$; φ_{rec} = -*y*, *x*, *y*, -*x*, *y*, -*x*, -*y*, *x* (receiver phase). Quadrature detection for the indirect dimensions was achieved for t_1(^{13}C′) and t_2($^{13}C^{\alpha}$) by *States-TPPI* (Marion et al. 1989) incrementing phases φ_3 and φ_4, respectively, and for t_3(^{15}N) by the *echo-antiecho* method (Kay et al. 1992) incrementing φ_6 and φ_7. In order to preserve the ^{13}C′ evolution with incremented t_2($^{13}C^{\alpha}$), the 180° pulses on ^{13}C between a and d are shifted appropriately. For ^{13}C′ and ^{15}N, constant time evolution and semi-constant time evolution were used, respectively. The trigonometric addition theorem was used to obtain pure cosine and sine terms for a subsequent hypercomplex Fourier transformation (Brutscher et al. 1995; Kupce and Freeman 2004). The sine-bell shaped pulsed field gradient (PFG) pulses were applied with a length of 800 µs and the following strengths: G_1: 27.5 G/cm; G_2: 10.5 G/cm; G_3: 27.5 G/cm; G_4: 33 G/cm; G_5: 40.2 G/cm; G_6: 8.3 G/cm; G_7: 17.6 G/cm.

4D TROSY APSY-HNCACB experiment

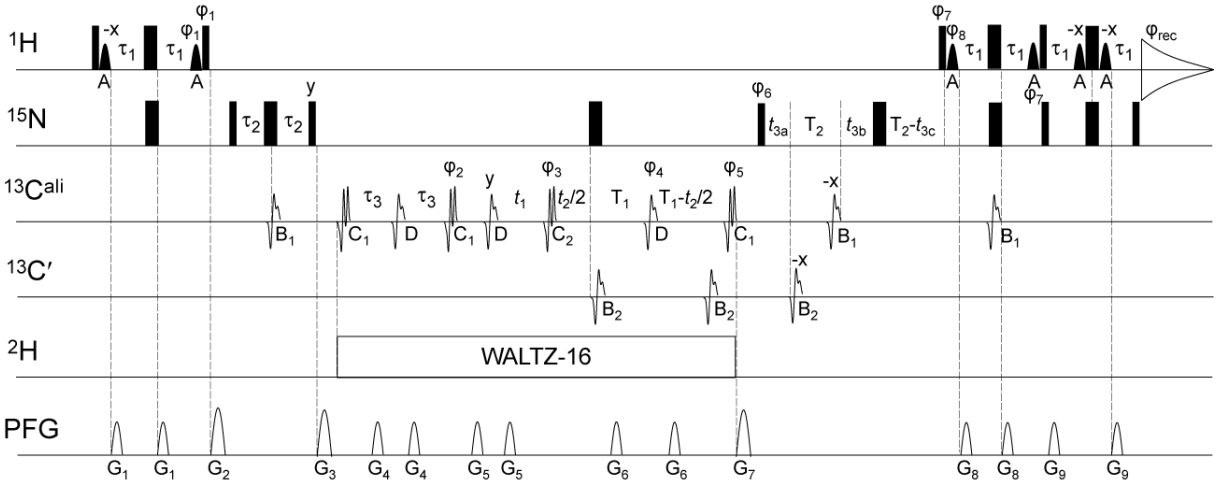

Fig. S2 Pulse sequence of the 4D TROSY APSY-HNCACB experiment (APSY one-letter code: baNH). Black thin and wide rectangular bars represent 90° and 180° high-power pulses, respectively. The carrier frequencies were set to 4.7 ppm, 118 ppm, 42 ppm, and 3.8 ppm on the ^{1}H, ^{15}N, $^{13}C^{ali}$ (aliphatic carbons), and ^{2}H channel, respectively. $^{13}C^{ali}$ is decoupled from ^{2}H with WALTZ-16 (Shaka et al. 1983). Time periods: τ_1 = 2.4 ms, τ_2 = 12.4 ms, τ_3 = 6.8 ms, $T_1 = \tau_3$ and $T_2 = \tau_2$-τ_1 = 10.0 ms. The shaped pulses (A) are selective 90° pulses on water protons with Sinc shape (central lobe), and are applied during 1 ms; pulses (B_1) on $^{13}C^{\alpha}$ and (B_2) on $^{13}C'$ are off-resonance 180° IBurp1 pulses (Geen and Freeman 1991) with a duration of 275 µs; (C) are on-resonance 90° Gaussian cascades (Q5) (Emsley and Bodenhausen 1990) on $^{13}C^{ali}$ with a duration of 350 µs, with (C_2) having a time-reversed profile with respect to (C_1); pulses (D) are on-resonance 180° Gaussian cascades (Q3) (Emsley and Bodenhausen 1990) on $^{13}C^{ali}$ with a duration of 210 µs; the pulse durations were adjusted for a 700 MHz spectrometer. All pulses were applied along the *x*-axis unless indicated otherwise above the pulse symbol. The following phase cycle was used: φ_1= *y*; φ_2 = 2(*y*), 2(*-y*); φ_3 = *y*; φ_4 = *x*; φ_5 = 2(*x*), 2*(-x)*; φ_6 = *-x*, *-y* or *-x*, *y* (even or odd number of increments); φ_7 = *-y*; φ_8 = - φ_7; φ_{rec} = *-y*, *x*, *y*, *-x* (receiver phase). Quadrature detection for the indirect dimensions was achieved for t_1(^{13}C′) and t_2($^{13}C^{\alpha}$) by *States-TPPI* (Marion et al. 1989) incrementing phases $\varphi_3 - \varphi_5$ and φ_5, and for t_3(^{15}N) by the *echo-antiecho* method (Kay et al. 1992) incrementing $\varphi_6 - \varphi_8$. For $^{13}C^{\alpha}$ and ^{15}N constant time and semi-constant time evolution periods were used, respectively. The trigonometric addition theorem was used to obtain pure cosine and sine terms for a subsequent hypercomplex Fourier transformation (Brutscher et al. 1995; Kupce and Freeman 2004). The sine-bell shaped pulsed field gradient (PFG) pulses were applied with the following lengths and strengths: G_1: 600 µs, 13.8 G/cm; G_2: 1000 µs, 38.5 G/cm; G_3: 1000 µs, 33.0 G/cm; G_4: 800 µs, 13.8 G/cm; G_5: 1000 µs, 33.0 G/cm; G_6: 600 µs, 27.5 G/cm; G_7: 800 µs, 30.3 G/cm; G_8: 800 µs, 19.3 G/cm; G_9: 800 µs, 30.3 G/cm.

4D TROSY APSY-HN(CO)CACB experiment

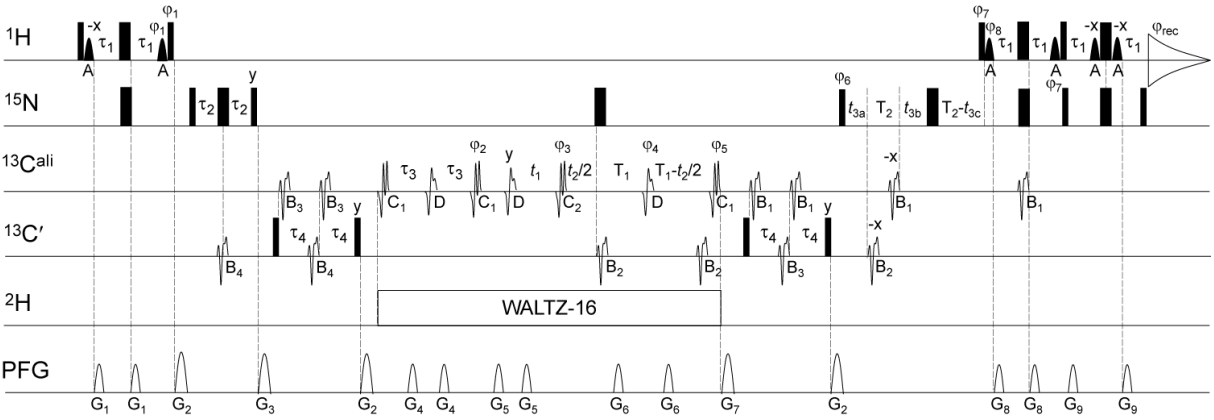

Fig. S3 Pulse sequence of the 4D TROSY APSY-HN(CO)CACB experiment (APSY one-letter code: BANH). Most parameters are identical to those described for the 4D TROSY APSY-HNCACB pulse sequence (Fig. S3). Differences are: Time periods τ_2 = 14.0 ms and τ_4 = 4.7 ms. The carrier frequency of the carbon channel is set to $^{13}C'$ (176 ppm) in the beginning, and switched to $^{13}C^{ali}$ (42 ppm) in the period from the first to the last C_1 pulse. Pulses B_3 and B_4 are off-resonance on $^{13}C^{\alpha}$ and on-resonance on $^{13}C'$, and otherwise identical to the already described pulses B. The experiment can be extended to a 5D TROSY APSY-HNCOCACB experiment (BAONH) by including a constant time $^{13}C'$ evolution by shifting the central 180° pulses in the second τ_4 period accordingly, and by incrementing the phase of the 90° pulse concluding this period in *States-TPPI* (Marion et al. 1989) manner for quadrature detection.

Parameters used for the TROSY APSY experiments

4D TROSY APSY-HNCACO experiment (aoNH)

Spectrometer: Bruker Avance III 700 MHz with cryogenic probe

Temperature: 50°C

Total experiment time: 33 h

Nr. of projections: 33

Interscan delay: 1.5 s

GAPRO parameters: $S_{min,1} = S_{min,2} = 7$, $R_{min} = 22.0$ Hz, $\Delta\upsilon = 5.0$ pt, S/N = 3.4 (Hiller et al. 2005)

(parameters are usually variable by approximately ±20-30% without a significant impact on the result)

Table S1 Acquisition parameters of the 4D TROSY APSY-HNCACO experiment

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]	Maximal evolution time [ms]
ω_1	CA	13.1	5000	56.0	26.5
ω_2	CO	28.4	2300	177.0	10.0
ω_3	N	33.8	2270	118.0	50.2
ω_4	H	14.3	10000	4.7	102.5

Table S2 Angles and parameters for 2D projections of the 4D TROSY APSY-HNCACO experiment.

α [°]	β [°]	Time [min]	# of complex points in indirect dimension	Maximal evolution time [ms]	Nr. of scans	Indirect dimension frequencies
0	0	53	114	50.2	8	N
0	90	38	84	16.4	8	CA
90	0	22	22	9.7	16	CO
90	±69.4	97	212	26.5 / 10.0	8	CA / CO
0	±39.4	44	94	16.5 / 20.0	8	CA / N
±26.3	0	49	52	10.0 / 20.1	16	CO / N
±26.3	±49.7	97	212	26.5 / 10.0 / 20.1	8	CA / CO / N
90	±76.7	50	108	21.6 / 5.1	8	CA / CO
0	±28.6	50	106	16.4 / 30.2	8	CA / N
±14.0	0	49	52	5.0 / 20.0	16	CO / N
±14.6	±44.0	102	110	20.0 / 5.2 / 20.0	16	CA / CO / N
90	±24.7	33	34	4.5 / 9.9	16	CA / CO
0	±24.4	54	116	16.5 / 36.5	8	CA / N
±44.6	0	33	34	9.9 / 10.0	16	CO / N
44.6	±17.9	39	42	4.5 / 9.9 / 10.0	16	CA / CO / N

4D TROSY APSY-HNCOCA experiment (OANH)

Spectrometer: Bruker Avance III 700 MHz with cryogenic probe

Temperature: 50°C

Total experiment time: 20.5 h

Nr. of projections: 29

Interscan delay: 1.5 s

GAPRO parameters: $S_{min,1} = S_{min,2} = 7$, $R_{min} = 22.0$ Hz, $\Delta\upsilon = 4.0$ pt, S/N = 4.2 (Hiller et al. 2005)

(parameters are usually variable by approximately ±20-30% without a significant impact on the result)

Table S3 Acquisition parameters of the 4D TROSY APSY-HNCOCA experiment

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]	Maximal evolution time [ms]
ω_1	CO	13.1	2300	177.0	25.0
ω_2	CA	28.4	5000	56.0	8.0
ω_3	N	33.8	2270	118.0	50.2
ω_4	H	14.3	10000	4.7	102.5

Table S4 Angles and parameters for 2D projections of the 4D TROSY APSY-HNCOCA experiment.

α [°]	β [°]	Time [min]	# of complex points in indirect dimension	Maximal acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	0	52	114	50.2	8	N
0	90	18	38	17.0	8	CO
90	0	19	42	7.9	8	CA
90	±72.3	39	84	25.0 / 8.0	8	CO / CA
0	±36.3	30	64	16.9 / 23.0	8	CO / N
±19.1	0	39	84	8.0 / 23.0	8	CA / N
±19.1	±45.7	77	170	25.0 / 8.0 / 23.0	8	CO / CA / N
90	±65.3	25	56	17.1 / 7.9	8	CO / CA
0	±44.6	25	54	16.9 / 17.1	8	CO / N
±24.4	0	25	56	7.9 / 17.4	8	CA / N
±24.4	±42.0	38	84	17.4 / 7.9 / 17.6	8	CO / CA / N
90	±79.0	39	86	21.0 / 4.1	8	CO / CA
0	±26.0	40	88	16.9 / 34.7	8	CO / N
±13.0	0	77	170	8.0 / 34.6	8	CA / N

4D TROSY APSY-HNCACB experiment (baNH)

Spectrometer: Bruker Avance III 700 MHz with cryogenic probe

Temperature: 50°C

Total experiment time: 30 h

Nr. of projections: 35

Interscan delay: 1.5 s

GAPRO parameters: $S_{min,1} = S_{min,2} = 7$, $R_{min} = 22.0$ Hz, $\Delta\upsilon = 5.0$ pt, S/N = 3.8 (Hiller et al. 2005)

(parameters are usually variable by approximately ±20-30% without a significant impact on the result)

Table S5 Acquisition parameters of the 4D TROSY APSY-HNCACB experiment

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]	Maximal evolution time [ms]
ω_1	CB	80.0	14086	42.0	8.0
ω_2	CA	60.0	10565	42.0	11.2
ω_3	N	35.0	2484	118.0	19.7
ω_4	H	14.3	10000	4.7	102.5

Table S6 Angles and parameters for 2D projections of the 4D TROSY APSY-HNCACB experiment

α [°]	β [°]	Time [min]	# of complex points in indirect dimension	Maximal acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	0	23	48	19.3	8	N
0	90	52	112	8.0	8	CB
90	0	55	118	11.2	8	CA
90	±35.7	75	162	8.0 / 11.1	8	CB / CA
0	±22.1	57	122	7.9 / 19.6	8	CB / N
±29.5	0	59	126	11.0 / 19.5	8	CA / N
±29.5	±19.5	79	170	8.0 / 11.1 / 19.7	8	CB / CA / N
90	±55.2	59	126	7.9 / 5.5	8	CB / CA
±15.8	0	36	76	5.5 / 19.6	8	CA / N
0	±11.5	35	74	4.0 / 19.5	8	CB / N
±90	±19.8	60	130	4.0 / 11.2	8	CB / CA
±48.5	0	56	120	11.2 / 9.9	8	CA / N
0	±39.1	53	114	7.9 / 9.7	8	CB / N
±13.2	±9.7	39	84	3.4 / 14.6 / 19.6	8	CB / CA / N
90	±9.7	56	120	1.9 / 11.1	8	CB / CA
±6.7	0	26	54	2.3 / 19.4	8	CA / N
0	±5.0	26	54	1.7 / 19.5	8	CB / N

4D TROSY APSY-HN(CO)CACB experiment (BANH)

Spectrometer: Bruker Avance III 700 MHz with cryogenic probe

Temperature: 50°C

Total experiment time: 33 h

Nr. of projections: 29

Interscan delay: 1.5 s

GAPRO parameters: $S_{min,1} = S_{min,2} = 8$, $R_{min} = 30.0$ Hz, $\Delta\upsilon = 5.0$ pt, S/N = 3.8 (Hiller et al. 2005)

(parameters are usually variable by approximately ±20-30% without a significant impact on the result)

Table S7 Acquisition parameters of the 4D TROSY APSY-HN(CO)CACB experiment

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]	Maximal evolution time [ms]
ω_1	CB	80.0	14086	42.0	8.0
ω_2	CA	60.0	10565	42.0	11.2
ω_3	N	35.0	2484	118.0	19.7
ω_4	H	14.3	10000	4.7	102.5

Table S8 Angles and parameters for 2D projections of the 4D TROSY APSY-HN(CO)CACB experiment

α [°]	β [°]	Time [min]	# of complex points in indirect dimension	Maximal acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	0	23	48	19.3	8	N
0	90	52	112	8.0	8	CB
90	0	55	118	11.2	8	CA
90	±35.7	75	162	8.0 / 11.1	8	CB / CA
0	±22.1	57	122	7.9 / 19.6	8	CB / N
±29.5	0	59	126	11.0 / 19.5	8	CA / N
±29.5	±19.5	79	170	8.0 / 11.1 / 19.7	8	CB / CA / N
90	±55.2	59	126	7.9 / 5.5	8	CB / CA
±15.8	0	36	76	5.5 / 19.6	8	CA / N
0	±11.5	35	74	4.0 / 19.5	8	CB / N
±90	±19.8	60	130	4.0 / 11.2	8	CB / CA
±48.5	0	56	120	11.2 / 9.9	8	CA / N
±15.8	±21.4	138	136	8.0 / 5.6 / 19.6	16	CB / CA / N
0	±39.1	54	114	7.9 / 9.7	8	CB / N

Orthogonal projection spectra of the 4D TROSY APSY versions of a HNCACO, a HNCOCA, a HNCACB and a HN(CO)CACB experiment

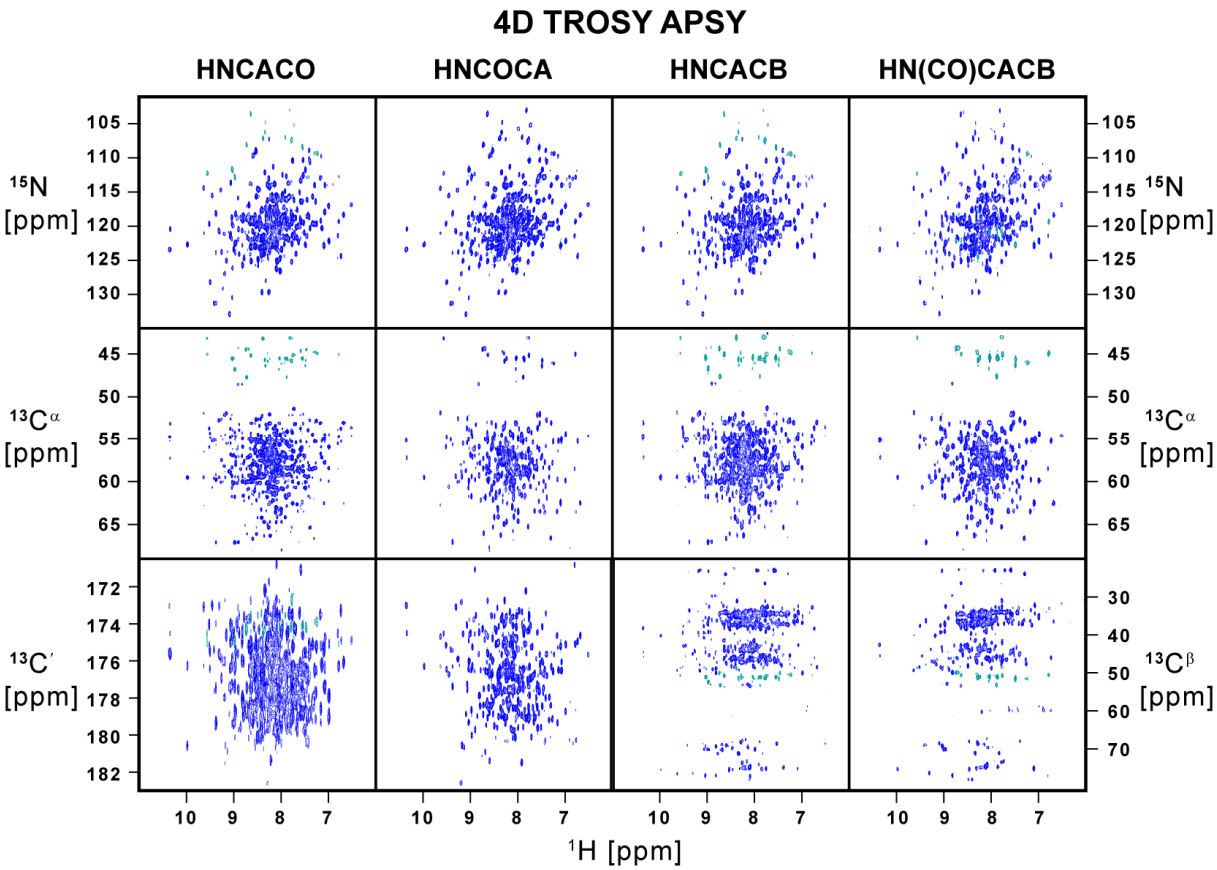

Fig. S5 Orthogonal 2D projection spectra of a set of TROSY APSY experiments for large proteins. The orthogonal projections are the 2D projection spectra which include the frequencies of only one nucleus in the indirect dimension. They provide a valuable basis to estimate the sensitivity and completeness of APSY experiments. They were all measured with 8 scans per FID, and a relaxation delay of 1.5 s. All other parameters are listed in tables S1-S8.

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