Supplementary Material

4D APSY-HBCB(CG)CDHD experiment for automated assignment of aromatic amino acid side chains in proteins

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The 4D APSY-HBCB(CG)CDHD experiment: Matching APSY side chain correlations to the backbone assignment

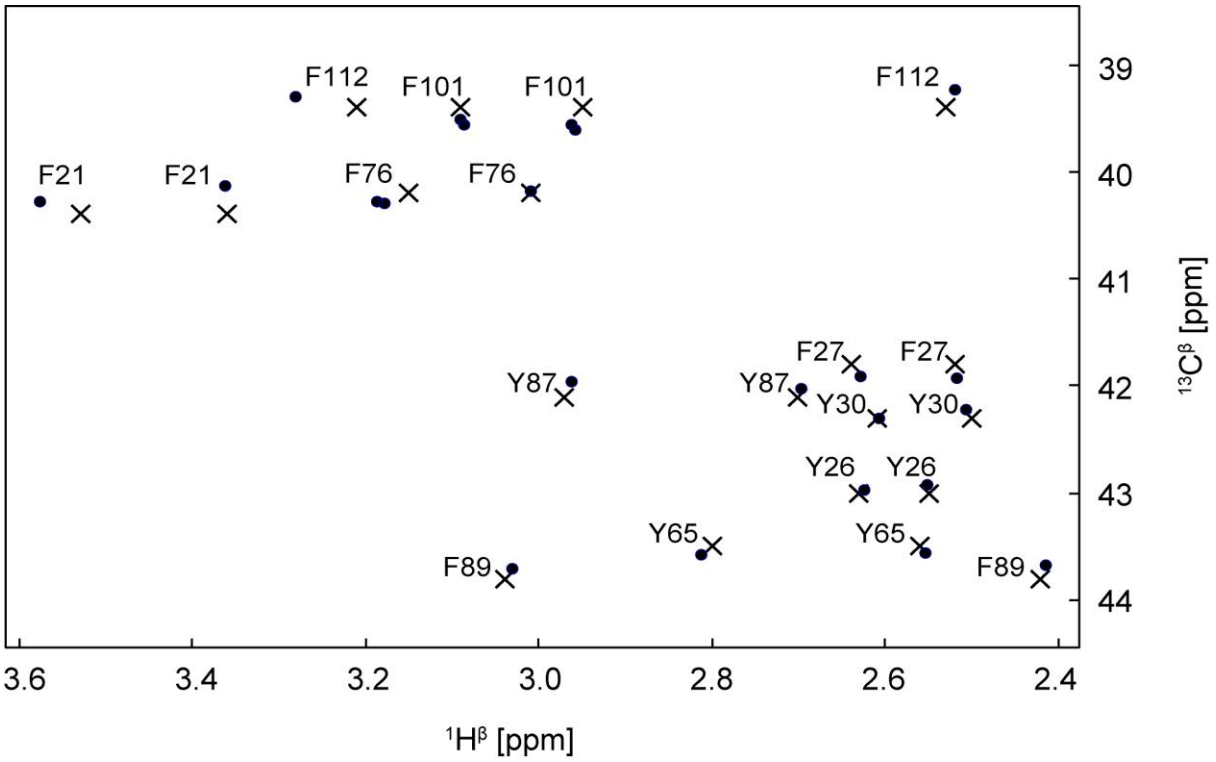

Fig. S1 Plot of tyrosine and phenylalanine $^{1}H^{\beta}$ and $^{13}C^{\beta}$ chemical shifts in β-CH_2 groups in TM1290 generated with the MATLAB script described in the main text. The values from the backbone assignment (Etezady-Esfarjani et al. 2003) are indicated with crosses, the two corresponding dimensions from the experimental 4D APSY-HBCB(CG)CDHD peak list measured on a 700 MHz spectrometer are indicated with black dots. The sequence-specific resonance assignments are indicated. The $^{1}H^{\beta}$–$^{13}C^{\beta}$ cross peaks of the backbone assignment can be inspected and possibly visually complemented, whereby the aromatic $^{1}H^{\delta}$ and $^{13}C^{\delta}$ are sequence-specifically assigned via the 4D APSY correlations. For Phe 101 (F101) and Phe 76 (F76), correlations to ε-

resonances were also observed, leading to two independent observations of the $^{1}H^{\beta}$ and $^{13}C^{\beta}$ chemical shift pairs (two black dots very close by). The signal detected on $^{1}H^{\varepsilon}$ is significantly weaker than on $^{1}H^{\delta}$, which makes their differentiation easy. The chemical shifts of His 48 are not shown, since only the APSY peak list contained β-values for histidine, but not the backbone assignment. However, the $^{13}C^{\beta}$ and $^{13}C^{\delta}$ chemical shifts are characteristic for histidine, the two 4D APSY cross peaks could thus be directly assigned to His 48

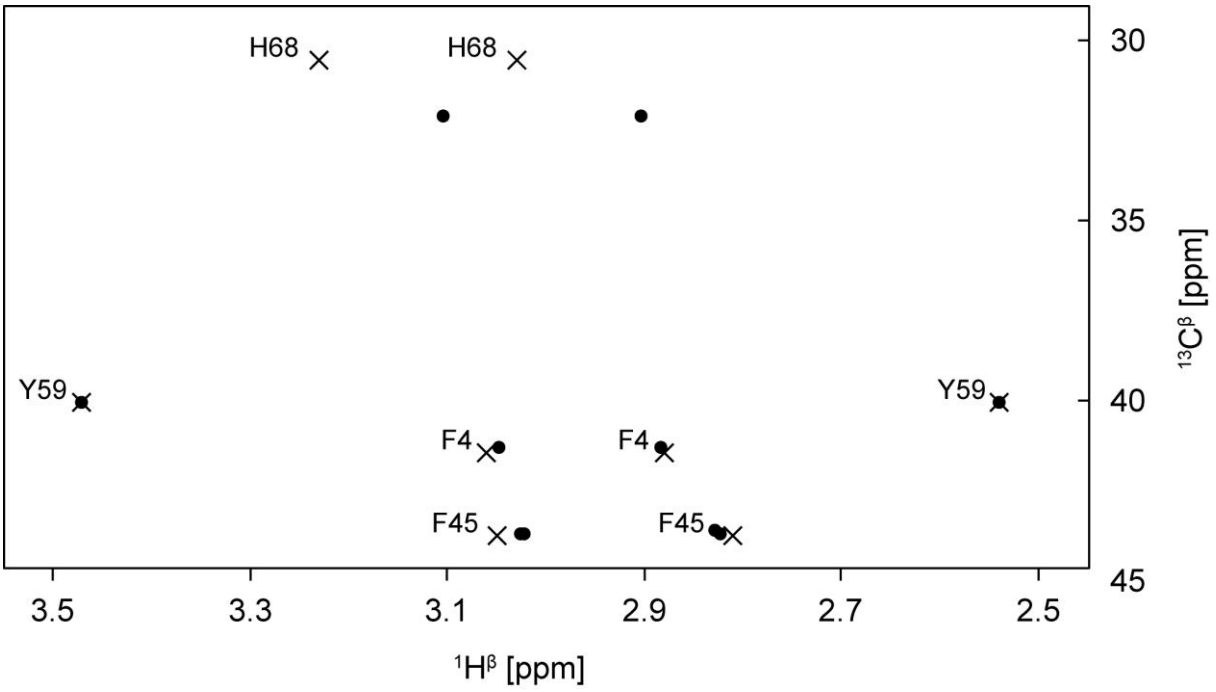

Fig. S2 Plot corresponding to Fig. S1 for ubiquitin. The His 68 (H68) signals in the APSY peak list are shifted with respect to the backbone assignment since the samples differ in pH as described in the discussion. This prevents automated matching, visual matching is nonetheless straightforward. All other resonances can be matched both visually and automatically. Due to correlations to $^{1}H^{\varepsilon}$ for Phe 45 (F45) there are two entries in the APSY peak list for each $^{1}H^{\beta}$, which leads to two black dots very close by for the β-CH for Phe 45

The 4D APSY-HCCH-TOCSY experiment

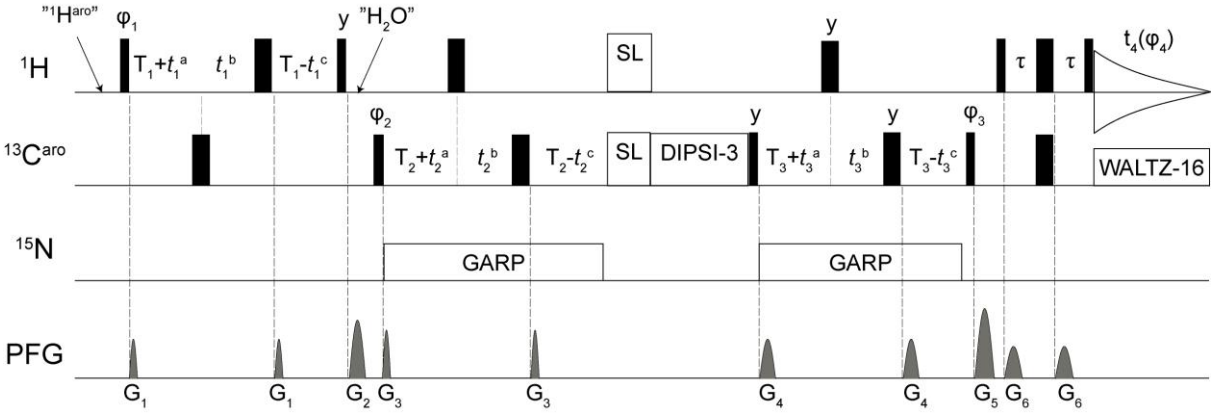

Fig. S3 Pulse sequence of the 4D APSY-HCCH-TOCSY experiment. Black thin and wide rectangular bars represent 90° and 180° high-power pulses, respectively. The carrier frequency on the proton channel (line ^{1}H) was first set to the aromatic region at 7.3 ppm, and after the first INEPT step to the water frequency at 4.7 ppm, as indicated by "$^{1}H^{aro}$" and "H_2O", respectively. During both ^{13}C evolution periods (t_2 and t_3), ^{15}N was decoupled with GARP (Shaka and Keeler 1987). Spin-lock pulses (SL) on both the proton and the carbon channel are applied prior to the TOCSY (Hartmann and Hahn 1962; Braunschweiler and Ernst 1983) mixing sequence. Isotropic mixing was performed with DIPSI-3 (Shaka et al. 1988) at an *rf* field strength of 7.14 kHz for 15.2 ms; additional time periods: T_1 = 1.5 ms, T_2 = 1.1 ms, T_3 = 1.1 ms, and τ = 1.5 ms. All pulses were applied along the *x*-axis unless indicated otherwise above the pulse symbol. The following phase cycle was used: φ_1= 2(*x*), 2(-*x*); φ_2 = *x*, -*x*; φ_4 = -*x*, *x*, *x*, -*x* (receiver phase). States-TPPI (Marion et al. 1989) quadrature detection for the indirect dimensions was achieved with the phases φ_1, φ_2, and φ_5 for t_1($^{1}H^{aro}$), t_2($^{13}C^{aro}$) and t_3($^{13}C^{aro}$), respectively, and 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). All three indirect dimensions were measured in a semi-constant time manner (Logan et al. 1993; Grzesiek and Bax 1993). The sine-bell shaped pulsed field gradient (PFG) pulses were applied with the following durations and strengths: G_1: 400 µs, 15 G/cm; G_2: 800 µs, 22.5 G/cm; G_3: 390 µs, 20 G/cm; G_4: 800 µs, 15 G/cm; G_5: 1000 µs, 40 G/cm; G_6: 800 µs, 9 G/cm

NMR spectroscopy

Setup of 4D APSY-HBCB(CG)CDHD experiments

The NMR experiments were carried out on Bruker Avance III spectrometers, which are operated with the software Topspin 2.1 (Bruker, Karlsruhe, Germany). Like for conventional 4D experiments, the frequency axes of the four dimensions are defined as F1 for $^{1}H^{\beta}$, F2 for $^{13}C^{\beta}$, F3 for $^{13}C^{\delta}$, and acquisition dimension F4 for $^{1}H^{\delta}$. The automated setup of the projections is carried out with an AU program (provided on www.apsy.ch), which proposes an angle file based on the sweep widths, and creates the 2-dimensional projection experiments accordingly. Starting from the setup for a direct projection of a single indirect dimension, the number of scans is doubled for each additionally active indirect dimension, in order to compensate for sensitivity differences. For the 4D APSY-HBCB(CG)CDHD experiment, the number of scans was also doubled for projections which included the $^{1}H^{\beta}$ dimension, since there are two $^{1}H^{\delta}$–$^{1}H^{\beta}$ cross peaks for each aromatic amino acid, and additional relaxation during $^{1}H^{\beta}$ semi-constant time evolution. Further acquisition parameters are summarized in tables S1 to S6. The parameters of the measurement on TM1290 (Tables S1 and S2) can serve as starting point for further applications of the experiment. The measurements on ubiquitin were performed for pulse sequence development and are therefore not optimized with respect to time efficiency.

The projections which are set up and measured first are usually those which include the frequencies of only one nucleus type in the indirect dimension, and therefore correspond to "normal" 2-dimensional spectra, which are for the HBCB(CG)CDHD experiment the $^{1}H^{\beta}$–$^{1}H^{\delta}$, $^{13}C^{\beta}$–$^{1}H^{\delta}$ and $^{13}C^{\delta}$–$^{1}H^{\delta}$ spectra (Fig. 1). These projections can be used to estimate the specific sensitivity and the dispersion of the resonances of a particular protein.

Data analysis

Automated serial processing of the spectra was performed with Prosa 6.4 (Güntert et al. 1992), for which the input files were created by the AU program which also generated the APSY projections in the software Topspin. The FID in the acquisition dimension was measured with 1k data points, zero-filled to 2k data points, and multiplied with a 75°-shifted sine bell function prior to Fourier transformation. The indirect dimension was zero-filled to 128 data points and also multiplied with a 75°-shifted sine bell function. Linear prediction (Zhu and Bax 1990; Barkhuijsen et al. 1985) can be applied for improved resolution; in our applications the resolution was sufficient without linear prediction. Baseline corrections were applied to the transformed spectra in the acquisition dimension with a trigonometric iterative approach, and in the indirect dimension with a 2nd order polynomial. The analysis and calculation of the multidimensional peak list with GAPRO (available on www.apsy.ch) was carried out for TM1290 with $S_{min,1} = S_{min,2} = 6$, $R_{min} = 8.5$ pt, $\Delta\upsilon = 14.0$ Hz, S/N = 3.6 (discussion of GAPRO parameters in (Hiller et al. 2005)). The results were evaluated by overlaying the spectra with the back-projected multidimensional peak list in XEASY (Bartels et al. 1995; Eccles et al. 1991) as implemented in CARA 1.5.5 (cara.nmr.ch) as subroutine NEASY.

Parameters of 4D APSY-HBCB(CG)CDHD experiments

TM1290

Spectrometer: Bruker Avance III 700 MHz with cryogenic probe

Temperature: 20°C

Total experiment time: 13h 16min

Table S1 Acquisition parameters of the 4D APSY-HBCB(CG)CDHD experiment with TM1290

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]
ω_1	$^{1}H^{\beta}$	1.57	1100	2.85
ω_2	$^{13}C^{\beta}$	18.0	3169	36.5
ω_3	$^{13}C^{\delta}$	19.0	3346	127
ω_4	$^{1}H^{\delta}$	12.98	9091	4.7

Table S2 Projection angles and parameters of the 4D APSY-HBCB(CG)CDHD experiment with TM1290

α [°]	β [°]	Time [min]	# of complex points in indirect dimension	Acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	0	10	13	3.9	16	$^{13}C^{\delta}$
0	90	30	22	20.0	32	$^{1}H^{\beta}$
90	0	12	17	5.4	16	$^{13}C^{\beta}$
90	±70.9	60	22	14.2/4.9	64	$^{1}H^{\beta}/^{13}C^{\beta}$
0	±71.8	49	18	11.6/3.8	64	$^{1}H^{\beta}/^{13}C^{\delta}$
±46.5	0	22	16	3.6/3.4	32	$^{13}C^{\beta}/^{13}C^{\delta}$
±64.7	±79.1	60	22	17.4/3.0/1.4	64	$^{1}H^{\beta}/^{13}C^{\beta}/^{13}C^{\delta}$
90	±80.2	60	22	17.9/3.1	64	$^{1}H^{\beta}/^{13}C^{\beta}$
±27.8	0	21	14	2.0/3.7	32	$^{13}C^{\beta}/^{13}C^{\delta}$
0	±56.7	41	14	5.7/3.7	64	$^{1}H^{\beta}/^{13}C^{\delta}$

Ubiquitin

Spectrometer: Bruker Avance III 750 MHz with conventional probe

Temperature: 20°C

Total experiment time: 12h 43min

Table S3 Acquisition parameters of the 4D APSY-HBCB(CG)CDHD experiment with ubiquitin

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]
ω_1	$^{1}H^{\beta}$	4	3001	2.5
ω_2	$^{13}C^{\beta}$	26	4904	38
ω_3	$^{13}C^{\delta}$	30	5659	128
ω_4	$^{1}H^{\delta}$	8.42	6313	4.7

Table S4 Projection angles and parameters of the 4D APSY-HBCB(CG)CDHD experiment with ubiquitin

α [°]	β [°]	Time [min]	# of complex points in indirect dimension	Acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	0	17	24	4.2	16	$^{13}C^{\delta}$
0	90	43	64	21.3	16	$^{1}H^{\beta}$
90	0	21	30	6.1	16	$^{13}C^{\beta}$
90	±58.5	75	57	9.5/5.8	32	$^{1}H^{\beta}/^{13}C^{\beta}$
0	±62.1	64	48	8.0/4.2	32	$^{1}H^{\beta}/^{13}C^{\delta}$
±49.1	0	38	28	2.9/2.5	32	$^{13}C^{\beta}/^{13}C^{\delta}$
90	±73	82	62	13.8/4.2	32	$^{1}H^{\beta}/^{13}C^{\beta}$
±30	0	35	26	1.8/3.1	32	$^{13}C^{\beta}/^{13}C^{\delta}$
0	±42.3	48	36	4.0/4.2	32	$^{1}H^{\beta}/^{13}C^{\delta}$

GB1

Spectrometer: Bruker Avance III 750 MHz

Temperature: 25°C

Total experiment time: 3h 59min

Table S5 Acquisition parameters used of the 4D APSY-HBCB(CG)CDHD experiment with GB1

Dimension	Nucleus	Sweep width [ppm]	Sweep width [Hz]	Carrier frequency [ppm]
ω_1	$^{1}H^{\beta}$	0.867	650	3.0
ω_2	$^{13}C^{\beta}$	24.02	4530	40.0
ω_3	$^{13}C^{\delta}$	20.14	3800	126.5
ω_4	$^{1}H^{\delta}$	13.33	10000	4.7

Table S6 Projection angles and parameters of the 4D APSY-HBCB(CG)CDHD experiment with GB1

α [°]	β [°]	Time [min]	# of complex points in indirect dimension	Acquisition time [ms]	Nr. of scans	Indirect dimension frequencies
0	0	5	14	3.7	8	$^{13}C^{\delta}$
0	90	12	19	29.2	16	$^{1}H^{\beta}$
90	0	12	37	8.2	8	$^{13}C^{\beta}$
90	±81.8	17	27	29.3/4.2	16	$^{1}H^{\beta}/^{13}C^{\beta}$
0	±80.3	13	20	21.8/3.7	16	$^{1}H^{\beta}/^{13}C^{\delta}$
±40.0	0	13	20	3.1/3.7	16	$^{13}C^{\beta}/^{13}C^{\delta}$
±40.0	±77.4	31	25	22.2/3.2/3.8	32	$^{1}H^{\beta}/^{13}C^{\beta}/^{13}C^{\delta}$

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