

SUPPORTING INFORMATION FOR:

Protein resonance assignment at MAS frequencies approaching 100 kHz: A quantitative comparison of J-coupling and dipolar-coupling-based transfer methods

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Experimental Parameters

SI-Table 1: Experimental parameters of 3D experiments: Pathways and time

Experiments (Transfer pathways)	Correlations	1. Transfer step	2. Transfer step	3. Transfer step	4. Transfer step	5. Transfer step	Number of scans	Measurement time
(H)CONH	H(i)N(i)CO(i-1)	H-CO CP	CO-N CP	N-H CP			4	2 hrs 16 min
(HN)CONH	H(i)N(i)CO(i-1)	H-N CP	N-CO-N INEPT	N-H CP			4	2 hrs 23 min
(H)CO(CA)NH with DREAM	H(i)N(i)CO(i)	H-CO CP	CO-CA DREAM	CA-N CP	N-H CP		4	2 hrs 19 min
(H)CO(CA)NH with INEPT	H(i)N(i)CO(i)	H-CO CP	CO-CA INEPT	CA-N CP	N-H CP		4	2 hrs 21 min
(H)CANH	H(i)N(i)CA(i)	H-CA CP	CA-N CP	N-H CP			4	5 hrs 11 min
(HN)CANH	H(i)N(i)CA(i,i-1)	H-N CP	N-CA-N INEPT	N-H CP			4	5 hrs 30 min
(H)CA(CO)NH	H(i)N(i)CA(i-1)	H-CA CP	CA-CO DREAM	CO-N CP	N-H CP		4	5 hrs 15 min
(HCO)CA(CO)NH	H(i)N(i)CA(i-1)	H-CO CP	CO-CA-CO INEPT	CO-N CP	N-H CP		4	5 hrs 15 min
(HCA)CB(CA)NH	H(i)N(i)CB(i)	H-CA CP	CA-CB-CA INEPT	CA-N CP	N-H CP		4	9 hrs 47 min
(HNCA)CB(CA)NH	H(i)N(i)CB(i,i-1)	H-N CP	N-CA-N INEPT	CA-CB-CA INEPT	N-H CP		4	10 hrs
(HCA)CB(CACO)NH	H(i)N(i)CB(i-1)	H-CA CP	CA-CB-CA INEPT	CA-CO INEPT	CO-N CP	N-H CP	4	10 hrs 55 min
(HCOCA)CB(CACO)NH	H(i)N(i)CB(i-1)	H-CO CP	CO-CA-CO INEPT	CA-CB-CA INEPT	CO-N CP	N-H CP	4	10 hrs 55 min

SI-Table 2: Experimental parameters of 3D experiments: CP and INEPT transfers

Transfers	Specification	RF-field ¹ H (kHz)	RF-field ¹³ C (kHz)	RF-field ¹⁵ N (kHz)	Contact time (ms)	Shape	Carrier (ppm) (if changed)	Transfer time (ms)
H-N CP	DQ (n=1)	78	-	15	1	40-60 tangential	-	-
N-H CP	DQ (n=1)	78	-	15	1	60-40 tangential	-	-
H-CA CP	ZQ (n=1)	130.8	37.5	-	4	tangential	55 (¹³ C)	-
H-CO CP	ZQ (n=1)	130.8	37.5	-	6.5	tangential	170 (¹³ C)	-
CA-N CP	DQ (n=1)	-	68	25	10	tangential	55 (¹³ C)	-
CO-N CP	DQ (n=1)	-	68	25	10	tangential	170 (¹³ C)	-
CA-CO DREAM		-	47	-	9	55-45 tangential	91.4 (¹³ C)	-
CO-CA DREAM			47	-	9	tangential	91.4 (¹³ C)	-
CA-CB DREAM			47	-	5	tangential	31.3 (¹³ C)	-
CB-CA DREAM			47	-	5	tangential	28.2 (¹³ C)	-
N-CA-N INEPT	out-and- back, simple INEPT	-	-	-	-	-	-	11
CO-CA-CO INEPT	out-and- back, simple INEPT	-	-	-	-	-	-	4.5
CA-CB-CA INEPT	out-and- back, simple INEPT	-	-	-	-	-	-	6
CA-CO INEPT	refocused INEPT	-	-	-	-	-	-	4.5
CO-CA INEPT	refocused INEPT	-	-	-	-	-	-	4.5

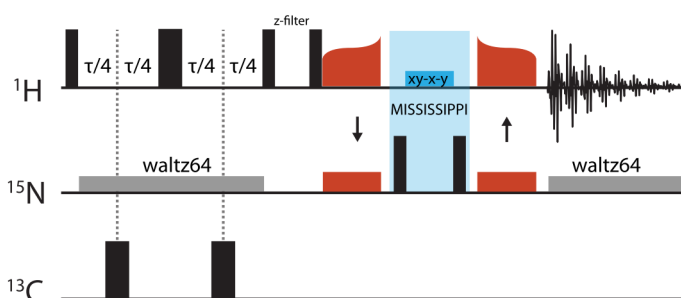
SI-Table 3: Experimental parameters for 3D experiments: Water suppression and decoupling

Decoupling/Water suppression	¹ H decoupling	¹⁵ N decoupling	Solvent suppression
Scheme	Frequency-swept TPPM	WALTZ64	MISSISSIPPI
RF-field (kHz)	10	5	15
Pulse length (μs)	48	102	60 000
Placement (timing)	indirect dimensions/ J-coupling evolution	acquisition	-

SI-Table 4: Experimental parameters for 3D experiments: Dimensions and pulses

Dimensions	¹ H (t3)	¹⁵ N (t2)	¹³ CA/ ¹³ CO/ ¹³ Cali (t3)
Spectral widths (ppm)	46.6	35	30/13/55
Acq. times (ms)	70	10	5
Data points	5550	64	64/28/118
Phase-sensitive detection	DQD	States-TPPI	States-TPPI
Carrier (ppm)	4.6	117.5	55/170/42.5
RF-fields, hard pulses (kHz)	100	50	83.3

Relaxation times



SI-Figure 1: Pulse sequence for measuring T_2' of the amide 1H

SI-Table 5: Relaxation times at 93 kHz MAS (bulk)

Nucleus	T_1	T_2'
¹ H	0.78 s	16.3 ms
¹³ CA	9.3 s	~ 25 ms
¹³ CO	12.9 s	48 ms
¹⁵ N	not measured	59.0 ms

H-CA and H-CO CP efficiency calculation

The transfer efficiency of the H-CA and H-CO CP was found by comparing a ¹³C direct pulse spectrum with the selective H-CA or H-CO ¹³C spectrum, taking into account T_1 relaxation times (0.78 s for ¹H, 12.9 s for CO, and 9.3 s for CA) and the initially chosen recycle delay d_1 (1.0 s for CP spectrum, 13 s for ¹³C spectrum). The following equation shows the calculation of the H-CA CP efficiency. The calculation for the H-CO CP efficiency is analogous.

$$\epsilon(HCA) = \frac{I(HCA)}{I(CA)} \cdot \frac{1 - \exp\left\{-\frac{d_1(^{13}C)}{T_1(CA)}\right\}}{1 - \exp\left\{-\frac{d_1(^1H)}{T_1(^1H)}\right\}} \cdot \frac{\gamma(^{13}C)}{\gamma(^1H)}. \quad (1)$$

Summary of transfer efficiencies

SI-Table 6: Summary of the efficiencies and information content for the assignment spectra as concluded from the previous sections. Unassigned resonances are classified by their H_i, N_i pair and include resonances from 1-71 only. The average SNR considers assigned resonances only. Experiments with best total transfer efficiency and information content in comparison are marked in red.

Experiments [Transfer pathways]	Correlations as 3D	Average SNR	Missing signals (*)	Total transfer efficiency
(H)CANH [H-CA ^{CP} , CA-N ^{CP} , N-H ^{CP}]	H(i), N(i), CA(i)	67	1Met, 19Pro, 24Glu, 37Pro, 38Pro, 53Gly	0.11±0.01
(HN)CANH [H-N ^{CP} , N-CA-N ^{INEPT} , N-H ^{CP}]	H(i), N(i), CA(i)	75	1Met, 9Thr, 19Pro, 24Glu, 25Asn, 37Pro, 38Pro, 40Gln, 45Phe, 50Leu, 52Asp, 53Gly, 64Glu	0.115±0.006
	H(i), N(i), CA(i-1)	36	1Met, 2Gln, 9Thr, 10Gly, 11Lys, 19Pro, 23Ile, 24Glu, 25Asn, 37Pro, 38Pro, 40Glu, 45Phe, 52Asp, 53Gly	
(H)CA(CO)NH [H-CA ^{CP} , CA-CO ^{DREAM} , CO-N ^{CP} , N-H ^{CP}]	H(i), N(i), CA(i-1)	42	1Met, 19Pro, 24Gly, 37Pro, 38Pro, 53Gly	0.065±0.007
(HCO)CA(CO)NH [H-CO ^{CP} , CO-CA-CO ^{INEPT} , CO-N ^{CP} , N-H ^{CP}]		61	1Met, 7Thr, 9Thr, 10Gly, 13Ile, 19Pro, 24Glu, 25Asn, 37Pro, 38Pro, 52Asp, 53Gly, 62Glu, 68His	0.071±0.004
(H)CONH [H-CO ^{CP} , CO-N ^{CP} , N-H ^{CP}]	H(i), N(i), CO(i-1)	64	1Met, 19Pro, 24Glu, 37Pro, 38Pro, 53Gly	0.139±0.007
(HN)CONH [H-N ^{INEPT} , N-CO-N ^{INEPT} , N-H ^{CP}]		110	1Met, 9Thr, 19Pro, 23Ile, 24Glu, 25Asn, 37Pro, 38Pro, 53Gly	0.214±0.005
(H)CO(CA)NH with DREAM [H-CO ^{CP} , CO-CA ^{DREAM} , CA-N ^{CP} , N-H ^{CP}]	H(i), N(i), CO(i)	23	1Met, 9Thr, 19Pro, 24Glu, 37Pro, 38Pro, 53Gly, 71Leu	0.041±0.004
(H)CO(CA)NH with INEPT [H-CO ^{CP} , CO-CA ^{INEPT} , CA-N ^{CP} , N-H ^{CP}]		19	1Met, 7Thr, 8Leu, 9Thr, 10Gly, 19Pro, 22Thr, 24Glu, 37Pro, 38Pro, 51Glu, 52Asp, 53Gly, 71Leu	0.035±0.003
(HCA)CB(CA)NH with out-and-back INEPT [H-CA ^{CP} , CA-CB-CA ^{INEPT} , CA-N ^{CP} , N-H ^{CP}]	H(i), N(i), CB(i)	56	1Met, 10Gly, 19Pro, 35Gly, 37Pro, 38Pro, 47Gly, 52Asp, 53Gly	0.046±0.005
(HCA)CB(CA)NH with double DREAM [H-CA ^{CP} , CA-CB-CA ^{DREAM} , CA-N ^{CP} , N-H ^{CP}]	H(i), N(i), CB(i)	13	1Met, 10Gly, 19Pro, 35Gly, 37Pro, 38Pro, 47Gly, 53Gly	< 0.023 (**)
(HNCA)CB(CA)NH [H-N ^{CP} , N-CA-CB-CA-N ^{INEPT} , N-H ^{CP}]	H(i), N(i), CB(i)	57	1Met, 9Thr, 10Gly, 19Pro, 23Ile, 24Glu, 25Asn, 35Gly, 37Pro, 38Pro, 47Gly, 52Asp, 53Gly	0.048±0.004
	H(i), N(i), CB(i-1)	27	1Met, 9Thr, 10Gly, 11Lys, 19Pro, 23Ile, 24Glu, 25Asn, 35Gly, 36Ile, 37Pro, 38Pro, 48Lys, 53Gly, 54Arg	
(HCOCA)CB(CACO)NH [H-CO ^{CP} , CO-CA-CB-CA-CO ^{INEPT} , CO-N ^{CP} , N-H ^{CP}]	H(i), N(i), CB(i-1)	24	1Met, 8Leu, 9Thr, 10Gly, 11Lys, 19Pro, 24Glu, 25Asn, 36Ile, 37Pro, 38Pro, 48Lys, 52Asp, 53Gly, 54Arg, 62Glu, 65Ser, 70Val, 71Val	0.030±0.002
(HCA)CB(CACO)NH [H-CA ^{CP} , CA-CB-CA ^{INEPT} , CA-CO ^{INEPT} , CO-N ^{CP} , N-H ^{CP}]		42	1Met, 8Leu, 9Thr, 10Gly, 11Lys, 19Pro, 23Ile, 24Glu, 25Asn, 35Gly, 36Ile, 37Pro, 38Pro, 48Lys, 52Asp, 53Gly, 54Arg, 59Thr, 62Gln, 64Glu, 65Ser, 70Val	0.030±0.003

(*) assignment refers to the H_i, N_i pair (** see text for details)