

High Accuracy of Karplus Equations for Relating Three-Bond J Couplings to Protein Backbone Torsion Angles

Fang Li, Jung Ho Lee, Alex Grishaev, Jinfa Ying, and Ad Bax

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Supporting Information

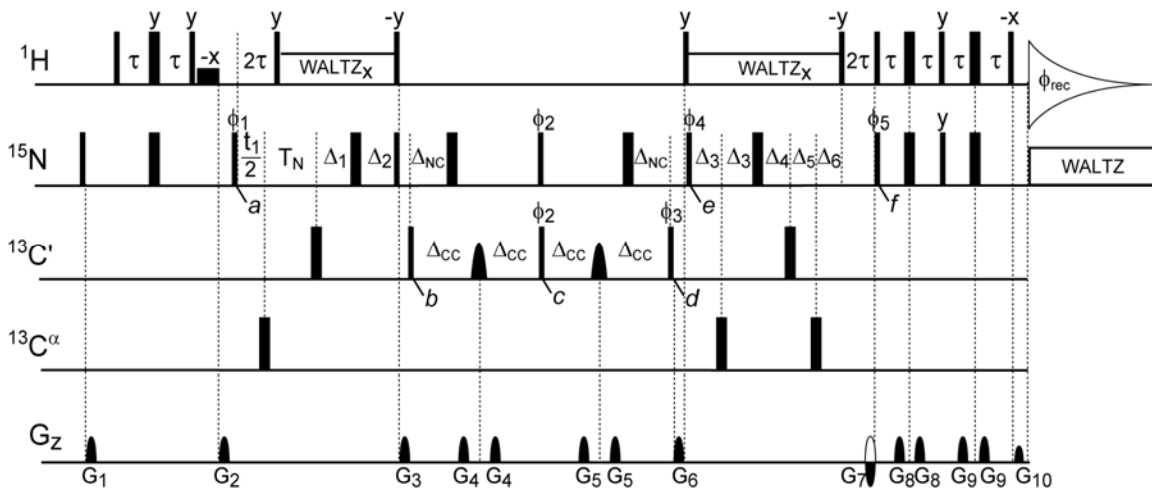


Figure S1. Pulse scheme of the HN(COCO)NH COSY experiment. The carriers of $^{13}\text{C}'$ and $^{13}\text{C}^\alpha$ are at 177 and 48 ppm. Narrow and wide bars indicate non-selective 90° and 180° pulses, respectively. Rectangular low-power ^1H pulse are applied using $\gamma_{\text{H}}B_1 = 167$ Hz. ^1H decoupling is applied using a WALTZ16 scheme ($\gamma_{\text{H}}B_1 = 2.5$ kHz; phase x). The $^{13}\text{C}'$ shaped pulses are of the ReBURP type,^[1] and have a duration of 800 μs . Unless specified, all pulse phases are x . Phase cycling: $\phi_1 = x$; $\phi_2 = x, -x$; $\phi_3 = x, x, -x, -x$; $\phi_4 = x, -x$; $\phi_5 = x$; and $\phi_{\text{rec}} = x, -x, -x, x$. Delay durations: $\tau = 1/(4J_{\text{NH}}) \approx 2.7$ ms; $T_{\text{N}} = 15$ ms; $\Delta_1 = \max(0, 0.5 \times t_1 - T_{\text{N}})$; $\Delta_2 = \max(0, T_{\text{N}} - 0.5 \times t_1)$; $\Delta_{\text{NC}} = 1/(4J_{\text{NC}'}) \approx 16$ ms; $\Delta_{\text{CC}} = 50$ ms; $\Delta_3 = \max(0, 0.5 \times T_{\text{N}} - 0.25 \times t_2)$; $\Delta_4 = \max(0, 0.5 \times t_2 - T_{\text{N}})$; $\Delta_5 = \min(0.5 \times T_{\text{N}} + 0.25 \times t_2 + \tau, T_{\text{N}})$; $\Delta_6 = (0.5 \times T_{\text{N}} + 0.25 \times t_2 + \tau, T_{\text{N}} > 0.5 \times t_2; T_{\text{N}} + 0.5 \times t_2 + 2\tau, T_{\text{N}} < 0.5 \times t_2)$. Gradient pulses $G_{1, \dots, 10}$ (z-axis) have durations of 1.3, 0.5, 0.6, 1.7, 1.9, 1.1, 1.0, 0.2, 0.2 and 0.1 ms, and strengths of 21, 35, 35, 11.9, 11.9, 28, 30.1, 10.5, 10.5, and -30.1 G/cm. Quadrature detection in the t_1 dimension is achieved by increasing ϕ_1 in the regular States-TPPI manner; quadrature in the t_2 dimension is obtained by the Rance-Kay method, inverting G_7 and ϕ_5 . Cross peak to diagonal peak intensity ratios correspond to $\tan^2(\pi J_{\text{C}'\text{C}^\alpha} T)$, with the $^{13}\text{C}'$ - $^{13}\text{C}^\alpha$ de-/re-phasing interval $T = 2 \times \Delta_{\text{CC}}$ set to 100 ms.

Quantum calculations

DFT calculations were carried out using Gaussian 09 [2] on the NIH Biowulf Cluster. A dipeptide analogue (Ac-Ala-NH-CH₃) with four water molecules linearly hydrogen bonded (d_{OO} or d_{NO} of 2.85 Å) was first built as described in the main text. To facilitate the investigation of coupling dependence on hydrogen bonding and local geometry, the internal coordinates of the above model dipeptide in the format of a Z-matrix were used in the DFT calculations. The O–O or N–O distance was independently varied from 2.65 to 3.15 Å in steps of 0.1 Å for each hydrogen bond except that the water molecule H-bonded to the C-terminal N-methyl group was fixed at $d_{NO} = 2.85$ Å. For the impact of the local geometry on the couplings under study, the N–C^α–C' bond angle was altered from 106.6° to 113.6° in 1° steps, while all H-bonds were kept at 2.85 Å. In addition, the contribution of each H-bond to the couplings was also studied by removing one or more water molecules. To evaluate the impact of amino acid residue type, fully hydrogen bonded Ac-Gly-NH-CH₃ and Ac-Ser-NH-CH₃ with all three χ_1 configurations were also built with the default geometries adopted by Molden.

A partial geometry optimization was first performed using the B3LYP/6-311++G** density functionals^[3-6] to optimize the hydrogen positions with all the non-hydrogen atoms frozen. The coupling calculations were carried out at the same level of theory on the resulting geometries in two steps using the NMR=Mixed keyword in Gaussian 09.^[7] In the first step, the Fermi contact (FC) term was computed using the uncontracted basis set. In the second step, calculation of the additional terms including the spin-dipolar (SD), paramagnetic and diamagnetic spin-orbit (PSO and DSO) contributions with the unmodified basis set was then performed. The sum of all four terms is reported below in Tables S2-S4 of the Supporting Information. The results indicate that hydrogen bonding, and the distribution of the hydrogen bond distance and the local geometry (*e.g.* the N–C^α–C' bond angle) seen in the refined structure of GB3, as well residue type and sidechain χ_1 can impact $^3J_{CC'}$ and $^3J_{HNH\alpha}$, causing small deviations of these couplings from the values defined by their respective Karplus equations.

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Table S1. Root-mean-square difference (Å) between the backbone atom (N, C^α, C') of various GB3 structures.

Structure	1IGD ^[8]	REDO ^[9]	Joint ^[10]	2OED ^[11]
REDO	0.046			
Joint	0.040	0.026		
2OED	0.314	0.319	0.317	
New ^a	0.595	0.596	0.595	0.383

^a Coordinates of the newly RDC-refined NMR structure can be downloaded from http://spin.niddk.nih.gov/bax/structures/GB3_RDC_refinement_2014.zip.

Table S2. $^3J_{CC'}$ couplings obtained from 3D HN(COCO)NH spectra of GB3.

Res#	AA type	$^3J_{CC'}$ (Hz)
2	GLN	0.65
3	TYR	1.31
4	LYS	1.30
5	LEU	1.30
6	VAL	0.88
7	ILE	0.77
8	ASN	1.10
9	GLY	0.64
10	LYS	0.42
11	THR	1.20
12	LEU	1.17
13	LYS	1.54
14	GLY	2.82
15	GLU	2.40
16	THR	2.60
17	THR	2.01
18	THR	2.54
19	LYS	0.90
20	ALA	2.61
23	ALA	0.37
24	GLU	0.43
27	GLU	0.48
28	LYS	0.43
29	ALA	0.52
30	PHE	0.40
31	LYS	0.40
32	GLN	0.53
33	TYR	0.52
34	ALA	0.47
35	ASN	0.51
39	VAL	1.06
40	ASP	2.32
41	GLY	2.59
42	VAL	0.62
43	TRP	0.92
44	THR	1.94
45	TYR	2.26
46	ASP	1.24
47	ASP	0.36
48	ALA	0.40
49	THR	0.92
50	LYS	0.71
51	THR	1.62
52	PHE	0.62
53	THR	1.41
54	VAL	1.38
55	THR	1.32

Table S3. Variation of ${}^3J_{C'C'}$, ${}^3J_{HNH\alpha}$, and ${}^1J_{NH}$ couplings calculated for fully hydrogen bonded dipeptide analogue Ac-Ala-N-CH₃ as a function of the H-bond distance and the N-C^α-C' bond angle, as calculated by DFT.

dO _{i-1} O _w ^a (Å)	dN _i O _w ^b (Å)	dO _i O _w ^c (Å)	τ ^d (°)	α -helix (Hz)		β -sheet (Hz)	
				${}^3J_{C'C'}$	${}^3J_{HNH\alpha}$	${}^3J_{C'C'}$	${}^3J_{HNH\alpha}$
no HB ^e	no HB ^e	no HB ^e	no HB ^e	0.458	0.863	1.029	10.864
2.65	2.85	2.85	111.6	0.642	1.436	1.318	10.522
2.75	2.85	2.85	111.6	0.638	1.426	1.309	10.510
2.85	2.85	2.85	111.6	0.628	1.406	1.300	10.498
2.95	2.85	2.85	111.6	0.619	1.389	1.293	10.486
3.05	2.85	2.85	111.6	0.612	1.374	1.286	10.477
3.15	2.85	2.85	111.6	0.605	1.363	1.280	10.468
2.85	2.65	2.85	111.6	0.639	1.069	1.361	9.999
2.85	2.75	2.85	111.6	0.636	1.277	1.327	10.272
2.85	2.85	2.85	111.6	0.628	1.406	1.300	10.498
2.85	2.95	2.85	111.6	0.621	1.503	1.281	10.811
2.85	3.05	2.85	111.6	0.615	1.574	1.265	10.920
2.85	3.15	2.85	111.6	0.609	1.625	1.252	11.007
2.85	2.85	2.65	111.6	0.627	1.411	1.307	10.483
2.85	2.85	2.75	111.6	0.627	1.407	1.303	10.489
2.85	2.85	2.85	111.6	0.628	1.406	1.300	10.498
2.85	2.85	2.95	111.6	0.627	1.405	1.297	10.503
2.85	2.85	3.05	111.6	0.628	1.405	1.295	10.513
2.85	2.85	3.15	111.6	0.629	1.408	1.293	10.520
2.85	2.85	2.85	106.6	0.717	1.162	1.394	10.365
2.85	2.85	2.85	107.6	0.697	1.218	1.372	10.388
2.85	2.85	2.85	108.6	0.679	1.271	1.354	10.413
2.85	2.85	2.85	109.6	0.660	1.313	1.335	10.442
2.85	2.85	2.85	110.6	0.643	1.360	1.318	10.472
2.85	2.85	2.85	111.6	0.628	1.406	1.300	10.498
2.85	2.85	2.85	112.6	0.614	1.451	1.283	10.528
2.85	2.85	2.85	113.6	0.601	1.494	1.264	10.714

^a dO_{i-1}O_w: the distance between the acetyl carbonyl oxygen and the oxygen atom from the water molecule that is hydrogen bonded to the carbonyl group.

^b dN_iO_w: the distance between the amide nitrogen atom of Ala and its hydrogen bond acceptor from the water molecule.

^c dO_iO_w: the distance between Ala's carbonyl oxygen and the oxygen atom from the hydrogen bonded water molecule.

^d τ refers to the Ala N-C^α-C' bond angle.

^e no HB: no groups in Ac-Ala-N-CH₃ are hydrogen bonded to a water molecule.

Table S4. DFT-calculated impact of removal of H-bonding water molecules on ${}^3J_{C'}$ and ${}^3J_{HNH\alpha}$ couplings in Ac-Ala-N-CH₃.

hydrogen bonds ^a	α -helix (Hz)		β -sheet (Hz)	
	${}^3J_{C'}$	${}^3J_{HNH\alpha}$	${}^3J_{C'}$	${}^3J_{HNH\alpha}$
no water	0.458	0.863	1.029	10.864
w4	0.512	1.796	1.055	10.986
w14	0.573	1.879	1.115	11.198
w24	0.545	1.222	1.216	10.482
w34	0.506	1.727	1.083	10.969
w123	0.623	1.441	1.272	10.354
w124	0.616	1.363	1.284	10.663
w134	0.584	1.921	1.146	11.182
w234	0.543	1.228	1.229	10.320
w1234	0.628	1.406	1.300	10.498

^a This column indicates the water molecule(s) H-bonded to the amide C=O and/or N-H groups. The O-O/N-O distances for all H-bonds were kept at 2.85 Å and the N-C^α-C' bond angle at 111.6°.

The numbering of the water molecules is as shown below:

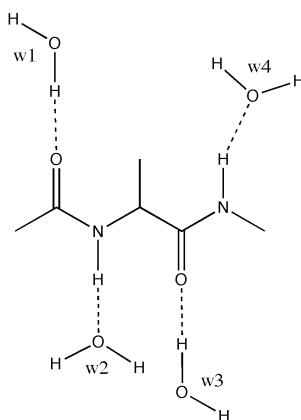


Table S5. DFT-calculated $^3J_{C'}$ and $^3J_{HNNH\alpha}$ couplings for the fully H-bonded dipeptide analogues Ac-Ala-N-CH₃, Ac-Gly-N-CH₃, and Ac-Ser-N-CH₃. The O-O/N-O distances for the H-bonds to the four water molecules were kept at 2.85 Å, and the N-C^α-C' bond angle at 111.6°.

residue (χ_1)	α -helix (Hz)		β -sheet (Hz)	
	$^3J_{C'}$	$^3J_{HNNH\alpha}$	$^3J_{C'}$	$^3J_{HNNH\alpha}$
Ala	0.628	1.406	1.300	10.498
Gly	0.771	1.387/8.012	1.221	10.773/2.366
Ser (g-)	0.739	1.842	1.272	10.349
Ser (g+)	0.594	1.645	1.223	10.356
Ser (trans)	0.679	1.491	1.226	10.669

Bruker pulse sequence code of the HN(COCO)NH experiment

```
;HN_CO_CO_NH.jhl  COSY mixing (2 x 100 ms)
;F3(CO) -> F2(15N) -> F1(1H)
;Coded by Jung Ho Lee on 16-May-2014; modified by Jinfa Ying on 20-July-2014
; Rance-Kay coherence selection

;prosol relations=<triple.jlb>

;prosol relations=<triple.jlb>

#include "/opt/topspin/exp/stan/nmr/lists/pp/user/bits.jhl"

#define N1_DIM
#define N2_DIM
#define RK      ; Toggle between ip13 and ip24/igrad

aqseq 321

;Increments
"in0=inf1*0.5"
"in10=in0"
"in11=in0"

"in20=inf2*0.25"
"in29=in20"
"in30=in20"
"in31=in20"

;Gradient Pulses
"PG1=1.3m"
"PG2=0.5m"
"PG3=0.6m"
"PG4=1.1m"
"PG5=1m"
"PG6=0.9m"
"PG7=1.7m"
"PG8=1m"
"PG9=101u"
"PG10=1.9m"
"PG13=0.7m"

;90 and 180 Pulses
"p2=p1*2"      ;H 180
"p12=p11*2"   ;N 180
;"p22=p21*2"  ;C' 180

;"p32=p21*2"  ;Ca 180
"p55=2m"      ;Spin Lock on C' (5.0 kHz)
"p9=90u"      ;DIPSI-3 on C'
"p6=1.2m"     ;WG
"p25=1.9m"    ;Shaped pulse before WG
"p15=1.5m"    ;Water suppression pulse
;"p61=100u"   ;p61: DIPSI-3 1H decoupling w/o isotropic mixing on C'
(100u) (2.5 kHz)
```

```
;"p62=200u" ;p62: DIPSI-3 1H decoupling w/ isotropic mixing on C'  
(200u) (1.25 kHz)
```

```
"d12=p12"  
"d21=p21"  
"d22=p22"  
"d32=p32"  
;"d33=(p32-p2)/2"  
"d41=p11/2-p1/2"
```

```
;Delays  
;"d3=2.5m" ;delta < 1/4JNH  
"d3=2.7m"  
"d16=200u" ; post gradient delay  
"d15=200u" ; buffer delay
```

```
"d33=d3-d16"  
"d34=PG9-0.637*p1"  
"d5=d3-100u-3u-PG6-d16-3u-p6-3u-3u"  
"d4=5.4m" ;epsilon = 1/2JNH  
"d44=d4-PG8-d16"
```

```
"d28=15m" ;TN < 1/4JC'N  
"d27=d28-3u-p1-d4"  
"d26=d28-PG7-3u"  
"d24=d28-PG10-3u"
```

```
"d0=3u"  
"d11=3u"  
"d10=d28"  
"d9=d10-d0-d32-d22-d11"
```

```
"d30=(d28-p32)*0.5"  
"d31=3u"  
"d20=(d28-d31-d22-d32-6u-p1-d4)*0.5"  
"d29=d20"
```

```
;13C frequency offsets. Set o2p=177ppm from command line.
```

```
"cnst22=177"  
"cnst32=48"  
"spoff21=0" ; C' 90  
"spoff22=0" ; C' 180  
"spoff23=bf2*((cnst32-cnst22)/1000000)" ; Ca 180
```

```
;"spoff11=0" ; C' 90  
;"spoff12=0" ; C' 180  
;"spoff13=bf2*((cnst32-cnst22)/1000000)" ; Ca 180
```

```
1 1m ze  
1m  
2 1m do:N  
1m LOCKH_OFF  
1m  
d15  
3 d15
```

```
4 d15*2
5 d15
6 d15
  d1
  1m UNBLKGRAD
  10u p11:H
  10u p111:N
  10u p10:C1 ;Ca
  10u p10:C2 ;C'

;Start pulse sequence
  (p11 ph0):N
  3u
  PG1:gp1
  d16

  (p1 ph0):H
  d3
  (center (p2 ph1):H (p12 ph0):N)
  d3
  (p1 ph1):H

  1u
  1u p115:H
  (p15 ph15:r):H
;goto 100

  3u
  PG2:gp2
  d16
  (p11 ph11):N

  (lalign
    (d4 p1 ph7 3u 3u p135 3u cpds1 ph27):H
    (d0 d32      d9 d22      d11 p12 ph0 d10):N
    (d0 d32      d9 p22:sp22 ph0 d11 d12      d10):C2
    (d0 p32:sp23 d9 d22      d11 d12      d10):C1
  )

  (lalign (p11 ph0):N (3u do 3u 3u p11 p1 ph8):H) ; end of 15N
evolution, 1H flip back
  3u
  PG3:gp3
  d16
  (p21:sp21 ph0):C2 ;start of 13C' transverse
  16m
  (p12 ph0):N
  31.3m
  PG7:gp7
  1m
  (p22:sp22 ph0):C2
  1m
  PG7:gp7
  47.3m
  d12 ;comp delay for p12
  (p21:sp21 ph30):C2 (p11 ph26):N ;13C' COSY mixing pulse, 15N
purge pulse
```

```
d12
47.1m
PG10:gp10
1m
(p22:sp22 ph0):C2
1m
PG10:gp10
31.1m
(p12 ph0):N
16m
(p21:sp21 ph12):C2 ; end of 13C' refocusing
3u
PG4:gp4
d16
;goto 100
(p1 ph7 1u):H
(1u pl35 1u cpds1 ph27):H ; 1H WALTZ-16 (100us)

; (p11 ph13):N
; (lalign
; (d30 d32 d30 p12 ph0):N
; (d30 d32 d30 d12 d31 p22:sp22 ph0):C2
; (d30 p32:sp23 ph0 d30 d12 d31 d22 d20 p32:sp23 ph0
d31):C1
; )
; d29
; (1u do):H
; (1u pl1 p1 ph8):H

(p11 ph13):N
(center
(d30 d32 d30 p12 ph0 d31 d22 d20 d32
d29):N
(d30 d32 d30 d12 d31 p22:sp22 ph0 d20 d32
d29):C2
(d30 p32:sp23 ph0 d30 d12 d31 d22 d20 p32:sp23
ph0 d29):C1
)
3u do:H
3u pl1:H
(p1 ph8):H
d44
PG8:gp8*EA
d16
#ifdef RK
(center (p1 ph0):H (p11 ph24):N)
d33 gron11
d16 groff
(center (p2 ph0):H (p12 ph0):N)
d33 gron11
d16 groff
(center (p1 ph1):H (p11 ph1):N)
d33 gron12
d16 groff
(center (p2 ph0):H (p12 ph0):N)
d33 gron12
```

```
d16 groff
d41
(p1 ph2):H
2u
PG9:gp9
;    100u

#else
    d4

    (p11 ph14):N
    3u
    PG5:gp5
    d16

98    3u pl0:H
    (p25:sp25 ph25:r):H
    3u
    3u pl1:H
    (p1 ph0):H

99    100u
    3u
    d5
    PG6:gp6
    d16
    3u pl6:H
    (p6 ph6:r):H
    2u
    1u pl1:H
    (center (p2 ph0):H (p12 ph0):N)
    2u
    1u pl6:H
    (p6 ph6:r):H
    3u
    d16
    PG6:gp6
    d5
    3u

#endif

100    10u
    10u pl30:N
    30u ;pl31:C2
    30u BLKGRAMP
    go=2 ph31 cpds3:N
    1m do:N
    1m LOCKH_OFF
    1m wr #0 if #0 zd

#ifdef N2_DIM
    d15*0.5 ip24*2
    d15*0.5 igrad EA
    lo to 3 times 2
    if "d30>in30+3u"
    {
        d15*0.3 dd30 ;note 2 increments here as there are 2 d30's
```

```
        d15*0.3 id20
        d15*0.2 id29
    }
else
    {
        d15*0.2 id31
        d15*0.2 id31
        d15*0.2 id29
        d15*0.2 id29
    }
d15*0.1 ip13*2
d15*0.1 ip31*2
lo to 4 times l3
d15*0.2 rd30
d15*0.2 rd31
d15*0.2 rd20
d15*0.2 rd29
d15*0.2
#else
    d15*3
#endif

#ifdef N1_DIM
    d15 ip11
    lo to 5 times 2
    d15*0.4 id0
    if "d10>in10+3u"
    {
        d15*0.3 dd10
    }
    else
    {
        d15*0.3 id11
    }
    d15*0.3 ip31*2
    lo to 6 times l4
#else
    d15*2
#endif

    1m do:N ;do:C2
    exit

;Phases
ph0=0
ph1=1
ph2=2
ph3=3
ph6=2
ph7=(360)90
ph8=(360)270
ph11=0
#ifdef RK
    ph13=0 2
period
    ph12=0 0 2 2
#else
    ;start of 2d 15N evolution
    ;13C' to 15N transfer pulse
```

```
ph13=0 2
ph14=0 0 2 2
ph12=0 0 0 0 2 2 2 2
#endif
ph17=0
ph19=2
ph15=2
ph24=0
ph25=2
ph26=0 2 ; 15N purge pulse
ph27=(360)1
ph30=0 2 ; 13C' COSY mixing pulse
ph31=0 2 2 0
```