

Habeck, M.; Rieping, W.; Nilges, M. Bayesian Estimation of Karplus Parameters and Torsion Angles from Three-Bond Scalar Couplings Constants. *J. Magn. Reson.* 2005, 177 (1), 160–165.

Table 1

Mean values and precision of the six Karplus curves (due to the twofold degeneracy in B we calculated the statistics for its absolute value)

	A (Hz)	$ B $ (Hz)	C (Hz)
$^3J(C'-C')$	1.30 ± 0.12 (1.36)	0.93 ± 0.06 (0.93)	0.64 ± 0.03 (0.60)
$^3J(C'-H^a)$	3.84 ± 0.14 (3.72)	2.19 ± 0.10 (2.18)	1.20 ± 0.11 (1.28)
$^3J(C'-C^b)$	2.52 ± 0.33 (1.74)	0.49 ± 0.33 (0.57)	0.51 ± 0.12 (0.25)
$^3J(H^N-C')$	4.19 ± 0.30 (4.29)	0.99 ± 0.18 (1.01)	0.03 ± 0.05 (0.00)
$^3J(H^N-H^a)$	7.13 ± 0.34 (7.09)	1.31 ± 0.13 (1.42)	1.56 ± 0.34 (1.55)
$^3J(H^N-C^b)$	3.26 ± 0.23 (3.06)	0.87 ± 0.24 (0.74)	0.10 ± 0.08 (0.13)

The values reported in the PDB restraint file 1d3z are indicated in brackets.

Ludvigsen, S.; Andersen, K. V; Poulsen, F. M. Accurate Measurements of Coupling Constants from Two-Dimensional Nuclear Magnetic Resonance Spectra of Proteins and Determination of Phi-Angles. *J. Mol. Biol.* 1991, 217 (4), 731–736.

COZY

Schmidt, J. M.; Blümel, M.; Löhr, F.; Rüterjans, H. Self-Consistent 3J Coupling Analysis for the Joint Calibration of Karplus Coefficients and Evaluation of Torsion Angles. *J. Biomol. NMR* 1999, 14 (1), 1–12.

Table 1. Self-consistent Karplus coefficients for the angular dependence of polypeptide ϕ -related 3J couplings based on flavodoxin data

Coupling type	Karplus coefficients ^a			Torsion phase increments ^b			
	A (Hz)	B (Hz)	C (Hz)	l	$\Delta\phi$ (deg)	ξ	ϑ
$^3J(H^N, H^a)$	7.90 ± 1.02	-1.05 ± 0.54	0.65 ± 0.58	5	-60	$-\xi$	$-\vartheta$
$^3J(H^N, C')$	4.41 ± 0.81	-1.36 ± 1.03	0.24 ± 0.37	3	± 180	$-\xi$	
$^3J(H^N, C^b)$	2.90 ± 0.80	-0.56 ± 0.52	0.18 ± 0.37	1	+60	$-\xi$	-2ϑ
$^3J(C'_{i-1}, H^a)$	3.76 ± 1.05	-1.63 ± 0.56	0.89 ± 0.60	2	+120		$-\vartheta$
$^3J(C'_{i-1}, C'_i)$	1.51 ± 0.86	-1.09 ± 1.11	0.52 ± 0.39	0	± 0		
$^3J(C'_{i-1}, C^b_i)$	2.72 ± 0.80	-0.31 ± 0.52	0.39 ± 0.37	4	-120		-2ϑ

^aCoefficients given are for use with the equation $^3J(\theta) = A \cos^2 \theta + B \cos \theta + C$ where $\theta = \phi + \Delta\phi + \xi + \vartheta$ according to Figure 1. In the basic study $\xi = 0$ and $\vartheta = 0$. The definition chosen warrants that the dihedral angle $\theta(C'_{i-1}-N-C^a-C'_i)$ is always identical to ϕ . Previously established procedures (Schmidt et al., 1996, 1997a, and references cited therein) were applied to obtain $\pm 1\sigma$ confidence boundaries on the Karplus coefficients. Tests were made against the critical Fisher variance ratio $F = 1.137$, given the numbers of observables and adjustables, $n = 705$ and $p = 140$, respectively. Variances σ_m^2 of the Fourier coefficients C_m translate into the usual representation of Karplus parameters according to $\sigma_A^2 = 2\sigma_2^2$, $\sigma_B^2 = \sigma_1^2$ and $\sigma_C^2 = |\sigma_2^2 - \sigma_0^2|$.

^bDihedral angle increments $\xi = \theta(H^N-C^a-N-C'_{i-1}) - 180^\circ$ and $2\vartheta = \theta(C^b-N-C^a-C'_i) - 120^\circ$ account for possible distortion of ideal planar and tetrahedral bond geometry at N and C^a sites, respectively.

Smith, L. J.; Sutcliffe, M. J.; Redfield, C.; Dobson, C. M. Analysis of 3J and 1J Torsion Angles for Hen Lysozyme in Solution from Proton NMR Spin-Spin Coupling Constants. *Biochemistry* 1991, 30 (4), 986–996.

COZY

Vuister, G. W.; Bax, A. Quantitative J Correlation: A New Approach for Measuring Homonuclear Three-Bond JHN,H Coupling Constants in 15N-Enriched Proteins. *J. Am. Chem. Soc.* 1993, 115 (17), 7772–7777.

For 86 of the residues with precisely determined $^3J(\text{H}^{\text{N}}\text{H}^{\alpha})$ values, including 5 glycines, the backbone angle ϕ is known from previous crystallographic work.²⁴ Comparing these J values with the values predicted by the Karplus equation

$$J(\phi) = A \cos^2(\phi - 60) + B \cos(\phi - 60) + C \quad (5)$$

results in a rmsd of 0.76 when the parametrization of Pardi et al.⁶ ($A = 6.4$, $B = -1.4$, $C = 1.9$) is used. When the values of Ludvigsen et al.⁷ ($A = 6.7$, $B = -1.3$, $C = 1.5$) are used a larger rmsd of 0.82 is obtained. Optimization of the A , B , and C parameters to yield a best fit results in $A = 6.51$, $B = -1.76$, and $C = 1.60$, with a rmsd of 0.73. A plot of the measured values and the best fitted curve is shown in Figure 4. In order to gain insight into the range each of the three parameters can adopt, 12 J values were randomly omitted and a least-squares fitting was performed to obtain optimal A , B , and C values for the remaining 79 J couplings. This procedure was repeated 10 000 times. The resulting distributions of the A , B , and C parameters are shown in Figure 5. Maxima in the three distributions clearly center around the values calculated with use of the complete set ($A = 6.51$, $B = -1.76$, $C = 1.60$). Although the errors in the individual values of 3J are neither expected to be uncorrelated nor normally distributed, the spread of the A , B , and C parameters observed in Figure 5 correlates well with the calculated variances (0.14, 0.03, and 0.08, respectively).

Both Lys70 and Lys78 deviate significantly (ca. +3 and -3 Hz, respectively) from the Karplus curve for any of the three parametrizations. The $J(\text{H}^{\text{N}}\text{H}^{\alpha})$ values measured for these two residues are nearly identical with those measured previously by Kay et al.,¹³ and error in the measurement can therefore be excluded. Both these residues are involved in crystal contacts and their ϕ angles are less certain than for most other residues; they changed by +14° and -13° for Lys70 and Lys78, respectively, during the final stage of crystallographic refinement.⁴² Because the ϕ angles of Lys70 and Lys78 are near the region where the Karplus equation has its strongest dependence on ϕ , an error of only 25° in ϕ results in a ~3 Hz deviation from the Karplus curve.

Hu, J.-S. H.; Bax, A. Determination of ϕ and ψ Angles in Proteins from ^{13}C – ^{13}C Three-Bond J Couplings Measured by Three-Dimensional Heteronuclear NMR. How Planar Is the Peptide Bond? *J. Am. Chem. Soc.* 1997, 119 (27), 6360–6368.

Table 2. Coefficients of Karplus Equations, $J = A \cos^2(\phi + \theta) + B \cos(\phi + \theta) + C^a$

	θ (deg)	A	B	C	ϕ	rmsd ^d
$^3J_{\text{H}^{\text{N}}\text{H}^{\text{a}}}$	-60	+6.47	-1.43	+2.03	X-ray ^b	0.70
	-60	+7.09	-1.42	+1.55	solution ^c	0.39
$^3J_{\text{H}^{\text{e}}\text{C}^{\text{e}}}$	120	+3.63	-2.10	+1.29	X-ray	0.29
	120	+3.72	-2.18	+1.28	solution	0.24
$^3J_{\text{H}^{\text{N}}\text{C}^{\beta}}$	60	+2.71	-0.35	+0.05	X-ray	0.30
	60	+3.06	-0.74	+0.13	solution	0.21
$^3J_{\text{H}^{\text{N}}\text{C}^{\text{e}}}$	180	+4.10	-1.08	+0.07	X-ray	0.45
	180	+4.29	-1.01	0.00	solution	0.32
$^3J_{\text{C}^{\text{e}}\text{C}^{\text{e}}}$	0	+1.35	-0.91	+0.61	X-ray	0.17
	0	+1.36	-0.93	+0.60	solution	0.13
$^3J_{\text{C}^{\text{e}}\text{C}^{\beta\text{e}}}$	-120	+1.61	-0.66	+0.26	X-ray	0.24
	-120	+1.74	-0.57	+0.25	solution	0.16

^a Karplus coefficients determined from singular value decomposition analysis. ^b Karplus coefficients derived using the ϕ (X-ray) angles.¹⁰ ^c Karplus coefficients derived using the ϕ (solution) angles that yield best agreement between the 3J values measured for a given residue and those predicted by the Karplus equations (derived using ϕ (XRAY)). For deriving a $^3J_{\text{XY}}$ Karplus relation from ϕ (solution) angles, $^3J_{\text{XY}}$ values were not used for deriving ϕ (solution). For 45 residues five 3J couplings were used to determine ϕ (solution); for an additional 12 residues only four 3J couplings were used. ^d Root-mean-square difference between measured J values and those predicted by the Karplus equation derived using either ϕ (XRAY) or ϕ (solution). ^e $^3J_{\text{CC}^{\beta}}$ values have not been

Pardi, A.; Billeter, M.; Wüthrich, K. Calibration of the Angular Dependence of the Amide Proton-C Alpha Proton Coupling Constants, 3JHN Alpha, in a Globular Protein. Use of 3JHN Alpha for Identification of Helical Secondary Structure. J. Mol. Biol. 1984, 180 (3), 741–751.

TABLE 2
Coefficients for the Karplus equation (1) evaluated from different experiments with BPTI

n.m.r. $^3J_{\text{HN}\alpha}$ (from Table 1)	Crystal structure ϕ (from Table 1)	A	B	C	Root-mean-square deviation†
36°C	Deisenhofer & Steigemann (1975)	6.4	1.4	1.9	0.87
68°C	Deisenhofer & Steigemann (1975)	6.6	1.4	1.8	0.85
36°C	Walter & Huber (personal communication)	6.5	1.4	1.8	0.68
68°C	Walter & Huber (personal communication)	6.7	1.4	1.8	0.63

† The root-mean-square of the distances along the $^3J_{\text{HN}\alpha}$ axis between the Karplus curve and the experimental points (see Fig. 3).