# Quantum-Chemical Characterization of Nuclear Spin−Spin Couplings Across Hydrogen Bonds

C. Scheurer and R. Brüschweiler*

*Gustaf H. Carlson School of Chemistry*
*Clark University, Worcester, Massachusetts 01610*

*Received May 26, 1999*
*Revised Manuscript Received August 5, 1999*

The recent discovery of spin−spin couplings between nuclei across hydrogen bonds by Grzesiek and co-workers[1,2] offers an important new way to directly establish the presence of a large number of hydrogen bonds in isotopically labeled biomolecules.[1−5] While these parameters represent a valuable addition to other NMR parameters for the characterization of structure and dynamics of biomolecules in solution, a detailed theoretical understanding is still missing. Theoretical insight into the nature of these couplings is important, since it would assist their proper interpretation, allow their prediction in new systems, and provide a better understanding of hydrogen bonds in biomolecules in general. Clearly, dipole-CSA cross-correlation-induced couplings[6,7] are generally too weak to cause these effects[1] and residual dipolar couplings can also be excluded as their origin.[3] While the experimental NMR data are consistent[1−5] with the notion that these couplings are electron mediated and thus scalar *J* couplings, a quantum-chemical analysis would help to resolve this question.

Here, we report a quantitative investigation of the trans-hydrogen $^{3h}J_{NC'}$ coupling involved in polypeptide N−H···O═C hydrogen bonds[2,4,5] and scalar $^{2h}J_{NN}$ couplings[1,3] across N−H···N hydrogen bonds of Watson−Crick and Hoogsteen base pairs using density functional theory (DFT). The calculations, which adequately reproduce magnitudes and correlations with $H^N$ isotropic shifts, provide a theoretical explanation of these couplings that corroborates their quantum-chemical origin. The usefulness of DFT has recently been shown for the interpretation of trans-hydrogen bond *J* couplings in strong, low-barrier F−H···Base hydrogen bonds.[8]

For all *J*-coupling calculations, the sum-over-states density functional perturbation theory (SOS-DFPT),[9] as implemented in the *deMon NMR* program,[10] was used. All major *J*-coupling contributions were calculated including the Fermi contact (FC), the paramagnetic spin−orbit (PSO), and the diamagnetic spin−orbit (DSO) term, while the spin−dipolar (SD) term that is usually only a small fraction of the leading FC term was neglected. The couplings were calculated using the Loc.1 SOS-DFPT approximation,[9] with the molecular orbitals localized by the method of Boys.[11] Following ref 9a the Perdew−Wang exchange functional with the Perdew correlation functional was used.[12] Numerical quadrature was carried out on FINE RANDOM angular grids[9c,10] with 64 radial shells. All calculations used the IGLO-III basis sets.[13] Isotropic shifts were calculated by methods described previously[14,15] using the *deMon NMR* program.

**Table 1.** Intramolecular $^1J_{NH}$ Couplings in Hertz between $^{15}N$ and $^1H$ of *N*-Methlyacetamide (NMA)

| molecule | $^1J_{NH}^{FC}$ | $^1J_{NH}^{PSO}$ | $^1J_{NH}^{DSO}$ | $^1J_{NH}^{tot}$ [b] |
|---|---|---|---|---|
| NMA[a] | −79.1 | −2.5 | −0.4 | −82.0 |

[a] The N−H distance was set to 1.02 Å. [b] Experimental protein backbone $^1J_{NH}$ coupling constants typically vary between 90 and 100 Hz.

To assess the accuracy of the computations of scalar *J* couplings in biomolecular fragments, first the isotropic intramolecular $^1J_{NH}$ coupling constant was calculated for *N*-methylacetamide (NMA). The results given in Table 1 show that the dominant contribution is due to the FC term. The magnitude of the coupling is roughly 10% smaller than the corresponding experimental value found for protein backbone $^1J_{NH}$. This and other calculations of $^1J$ and $^2J$ coupling constants in NMA and in the amidated alanine-alanine dipeptide (Ala-Ala) suggest that the computational error generally does not exceed about 10−15%.

Protein backbone hydrogen bonds for which a large number of $^{3h}J_{NC'}$ couplings were observed[2,4,5] are modeled here by two suitably spatially arranged NMA molecules (Figure 1). The $^{3h}J_{NC'}$ coupling constants were computed as a function of the hydrogen bond length $r_{NO}$ and the out-of-plane tilt angle $\theta(OH^NN)$using the method outlined above. As can be seen in Figure 1, the couplings are all negative, as found experimentally,[5] with absolute values that are largest for a short $r_{NO}$ distance and a linear H-bond geometry $\theta(OH^NN) = 180°$. For constant $\theta$, the $r_{NO}$ distance dependence of $^{3h}J_{NC'}$ is in good approximation exponential in agreement with the empirical parametrization of ref 5. For hydrogen-bond lengths and angular distributions that are typically found in globular proteins, the calculated $^{3h}J_{NC'}$ coupling constants range between −0.2 and −1.5 Hz, which covers the range observed experimentally.[2,5] The calculated couplings, however, tend to be systematically larger in their absolute value as compared to experiment. Two likely reasons are (i) motional processes that are omnipresent in proteins and that may cause distortions of the hydrogen-bonding geometry and (ii) the limitation of the NMA molecules to realistically represent the protein backbone. In fact, a computation for an Ala-Ala dimer with $r_{NO} = 2.9$ Å and $\theta(OH^NN) = 180°$ yields $^{3h}J_{NC'} = -0.99$ Hz, which differs by 8.3% from the corresponding value of the NMA dimer (−1.08 Hz), indicating that remote substituents can have a nonnegligible effect also on trans-hydrogen bond couplings. The major contribution to the $^{3h}J_{NC'}$ coupling stems from the FC (96%) term, which is dominated by two orbitals, each of them involving all peptide-bond atoms of either one of the Ala-Ala monomers.

The previously reported correlation between isotropic $H^N$ shifts and $^{3h}J_{NC'}$ couplings[2] can be directly examined using DFT. A 2D surface for the isotropic $H^N$ shift $\delta(H^N)$ was calculated for the

(1) Dingley, A. J.; Grzesiek, S. *J. Am. Chem. Soc.* **1998**, *120*, 8293−8297.
(2) Cordier, F.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, *121*, 1601−1602.
(3) Pervushin, K.; Ono, A.; Fernández, C.; Szyperski, T.; Kainosho, M.; Wüthrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14147−14151.
(4) Cornilescu, G.; Hu, J.-S.; Bax, A. *J. Am. Chem. Soc.* **1999**, *121*, 2949−2950.
(5) Cornilescu, G.; Ramirez, B. E.; Frank, M. K.; Clore, G. M.; Gronenborn, A. M.; Bax, A. *J. Am. Chem. Soc.* **1999**, *121*, 6275−6279.
(6) Brüschweiler, R. *Chem. Phys. Lett.* **1996**, *257*, 119−122.
(7) Tjandra, N.; Grzesiek, S.; Bax, A. *J. Am. Chem. Soc.* **1996**, *118*, 6264−6272.
(8) Golubev, N. S.; Shenderovich, I. G.; Smirnov, S. N.; Denisov, G. S.; Limbach, H.-H. *Chem. Eur. J.* **1999**, *5*, 492−497. Shenderovich, I. G.; Smirnov, S. N.; Denisov, G. S.; Gindin, V. A.; Golubev, N. S.; Dunger, A.; Reibke, R.; Kirpekar, S.; Malkina, O. L.; Limbach, H.-H. *Ber. Bunsen-Ges. Phys. Chem.* **1998**, *102*, 422−428.
(9) (a) Malkin, V. G.; Malkina, O. L.; Salahub, D. R. *Chem. Phys. Lett.* **1994**, *221*, 91−99. (b) Malkina, O. L.; Salahub, D. R.; Malkin, V. G. *J. Chem. Phys.* **1996**, *105*, 8793−8800. (c) Malkin, V. G.; Malkina, O. L.; Salahub, D. R. *Chem. Phys. Lett.* **1996**, *261*, 335−345. (d) Malkin, V. G.; Malkina, O. L.; Casida, M. E.; Salahub, D. R. *J. Am. Chem. Soc.* **1994**, *116*, 5898−5908.
(10) St-Amant, A.; Salahub, D. R. *Chem. Phys. Lett.* **1990**, *169*, 387−392. Salahub, D. R.; Fournier, R.; Mlynarski, P.; Papai, I.; St-Amant, A.; Ushio, J. In *Density Functional Methods in Chemistry*; Labanowski, A., Andzelm, J., Eds.; Springer: New York, 1991; p 77.
(11) Foster, S.; Boys, S. *Rev. Mod. Phys.* **1960**, *32*, 303−304.
(12) Perdew, J. P.; Wang, Y. *Phys. Rev. B* **1986**, *33*, 8800−8802. Perdew, J. P. *Phys. Rev. B* **1986**, *33*, 8822−8824.
(13) Kutzelnigg, W.; Fleischer, U.; Schindler, M. *NMR Basic Principles and Progress* **1991**, *23*, 165−262.
(14) Malkin, V. G.; Malkina, O. L.; Eriksson, L. A.; Salahub, D. R. In *Modern Density Functional Theory: A Tool for Chemists*; Seminario, J. M., Politzer, P., Eds.; Elsevier: Amsterdam, 1995; Vol. 2, p 273. Bühl, M.; Kaupp, M.; Malkina, O. L.; Malkin, V. G. *J. Comput. Chem.* **1999**, *20*, 91−105.
(15) Scheurer, C.; Skrynnikov, N. R.; Lienin, S. F.; Straus, S. K.; Brüschweiler, R.; Ernst, R. R. *J. Am. Chem. Soc.* **1999**, *121*, 4242−4251.

10.1021/ja9917417 CCC: $18.00 
Published on Web 09/08/1999

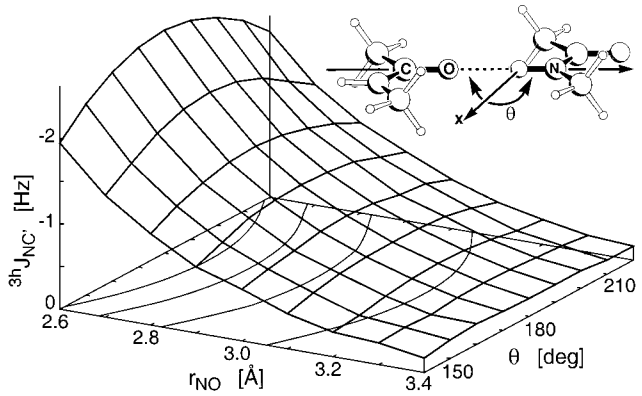


**Figure 1.** Dependence of the trans-hydrogen bond $^{3h}J_{NC'}$ coupling constant in a *N*-methylacetamide (NMA) dimer (upper right) as a function of the hydrogen bond length $r_{NO}$ and the out-of-plane tilt angle $\theta(OH^NN)$, which describes a rotation of the right NMA molecule about the *x* axis. $\theta = 180°$ corresponds to a linear hydrogen bond. The 2D surface is displayed as both a mesh and a contour plot (vertical projection with contour lines drawn at −0.5, −1, −1.5, −2 Hz).

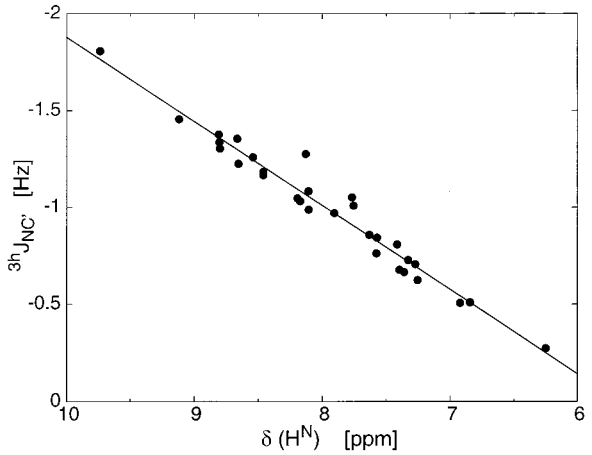


**Figure 2.** Correlation between the trans-hydrogen bond $^{3h}J_{NC'}$ coupling constant and the isotropic $H^N$ chemical shift predicted from the X-ray structure of ubiquitin[17] and DFT computations. After addition of $H^N$ protons to the X-ray structure, the backbone hydrogen bond lengths $r_{NO}$ and the out-of-plane tilt angles $\theta(OH^NN)$ were extracted. Using these parameters the $^{3h}J_{NC'}$ coupling constant and the $H^N$ chemical shift were determined from the NMA-NMA model of Figure 1 and plotted against each other. The calculated $H^N$ shifts are given with respect to absolute shieldings of TMS protons obtained at the same level of theory.[18]

NMA dimer using the methods described in ref 15 (based on the Perdew−Wang exchange-correlation functional[16] and IGLO-III basis functions[13]) as a function of the $r_{NO}$ distance and the angle $\theta(OH^NN)$. The close connection of this surface to that of Figure 1 is illustrated in Figure 2: for pairs of $r_{NO}$ and $\theta(OH^NN)$ values found in the X-ray structure of ubiquitin[17] the $\delta(H^N)$ and $^{3h}J_{NC'}$ parameters were extracted from the corresponding 2D surfaces and plotted against each other. The linear correlation ($r = 0.98$) is in good agreement with the experimental findings reported previously (ref 2, Figure 3).

(16) Perdew, J. P.; Wang, Y. *Phys. Rev. B* **1992**, *45*, 13244−13249.
(17) Alexeev, D.; Bury, S. M.; Turner, M. A.; Ogunjobi, O. M.; Muir, T. W.; Ramage, R.; Sawyer, L. *Biochem. J.* **1994**, *299*, 159−163 (PDB file 1UBI).
(18) Kaupp, M.; Malkina, O. L. *J. Chem. Phys.* **1998**, *108*, 3648−3659.

**Table 2.** Computed $^{2h}J_{NN}$ Couplings in Hertz between $^{15}N$ in Nucleic Acid Base Pairs

| donor[e] | acceptor | $^{2h}J_{NN}^{FC}$ | $^{2h}J_{NN}^{PSO}$ | $^{2h}J_{NN}^{DSO}$ | $^{2h}J_{NN}^{tot}$ |
|---|---|---|---|---|---|
| guanine[a,b] ($N_1-H_1$) | cytosine[a,b] ($N_3$) | 5.40 | −0.06 | 0.02 | 5.36 |
| uracil[a,c] ($N_3-H_3$) | adenine[a,c] ($N_1$) | 7.67 | −0.04 | 0.02 | 7.65 |
| thymine[d] ($N_3-H_3$) | adenine[d] ($N_7$) | 6.45 | −0.02 | 0.02 | 6.45 |

[a] Watson−Crick base pairs with coordinates corresponding to the 4th and 6th base pair of the Dickerson−Drew dodecamer CGCGAATTCGCG of ref 19b (for the calculation the T was replaced by U). [b] The 4th base pair (see footnote *a*) with $r(N_1-N_3) = 2.96$ Å and $\theta(N_1H_1N_3) = 164°$. [c] The 6th base pair (see footnote *b*) with $r(N_1-N_3) = 2.82$ Å and $\theta(N_3H_3N_1) = 174°$. [d] Hoogsteen base pair with coordinates taken from ref 20: $r(N_3-N_7) = 2.93$ Å and $\theta(N_3H_3N_7) = 179°$. [e] The N−H bond distance was set to 1.04 Å in all conputations in accordance with ref 20.

Trans-hydrogen bond couplings $^{2h}J_{NN}$ in nucleic acid base pairs were measured with magnitudes varying between 6 and 7 Hz.[1,3] We applied the computational protocol to Watson−Crick uracil-adenine (U-A) and cytosine-guanine (C-G) base pairs with relative arrangements as found in the Dickerson−Drew dodecamer[19] and also to the Hoogsteen adenine-thymine (A−T) base pair.[20] The results are summarized in Table 2. The computed couplings range between 5 and 8 Hz, and $^{2h}J_{NN}$ of U-A turns out to be larger than that of C−G, which is in agreement with experiment.[1,3] The underestimation of $^{2h}J_{NN}$ in the C-G pair is likely to be caused by the angular distortion of this hydrogen bond in the Dickerson−Drew dodecamer (see Table 2). For the Hoogsteen pair the calculated coupling is 6.45 Hz. For all three base pairs the dominant contribution arises from large FC terms with different sign which partially cancel each other. These FC terms originate from orbitals with $\sigma$ bond character across the hydrogen bond.

In conclusion, we have shown that the recently detected trans-hydrogen nuclear spin−spin couplings in proteins and nucleic-acid base pairs can be explained by quantum chemical calculations indicating that they have a predominantly scalar *J* coupling character. The nearly quantitative interpretation is based on recently developed DFT methods allowing a detailed analysis of their dependence on the hydrogen-bond geometry. The results demonstrate the power of modern quantum-chemical methods for the "ab initio interpretation" of scalar *J* couplings in biomolecules gradually overcoming the need of empirical parametrizations.

**Acknowledgment.** The authors thank Prof. D. Salahub for kindly providing the *deMon* program and Prof. V. G. Malkin and Dr. O. L. Malkina for helpful discussions and for providing a new version of the *deMon NMR* module. After submission of this manuscript the authors became aware of a paper in press[21] reporting DFT calculations of $^{1h}J_{HN}$ and $^{2h}J_{NN}$ couplings in DNA fragments.

**Supporting Information Available:** Two tables containing $^{3h}J_{NC'}$ coupling constants and $\delta(H^N)$ isotropic chemical shifts as a function of the hydrogen bond length $r_{NO}$ (Å) and the hydrogen bond angle $\theta$ (deg) shown in Figure 1 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA9917417

(19) (a) Wing, R.; Drew, H.; Takano, T.; Broka, C.; Takano, S.; Itakura, K.; Dickerson, R. E. *Nature* **1980**, *287*, 755−758. (b) Tereshko, V.; Minasov, G.; Egli, M. *J. Am. Chem. Soc.* **1999**, *121*, 470−471 (PDB file 436D).
(20) Frey, N.; Koetzle, T. F.; Lehmann, M. S.; Hamilton, W. C. *J. Chem. Phys.* **1973**, *59*, 915−924 (CSD REFCODE MTHMAD11).
(21) Dingley, A. J.; Masse, J. E.; Peterson, R. D.; Barfield, M.; Feigon, J.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, in press.