DIRECT OBSERVATION OF C^{α}-$H^{\alpha}\cdots O=C$ HYDROGEN BONDS IN PROTEINS BY INTERRESIDUE $^{h3}J_{C\alpha C'}$ SCALAR COUPLINGS

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SUPPORTING INFORMATION

A. Experimental Details

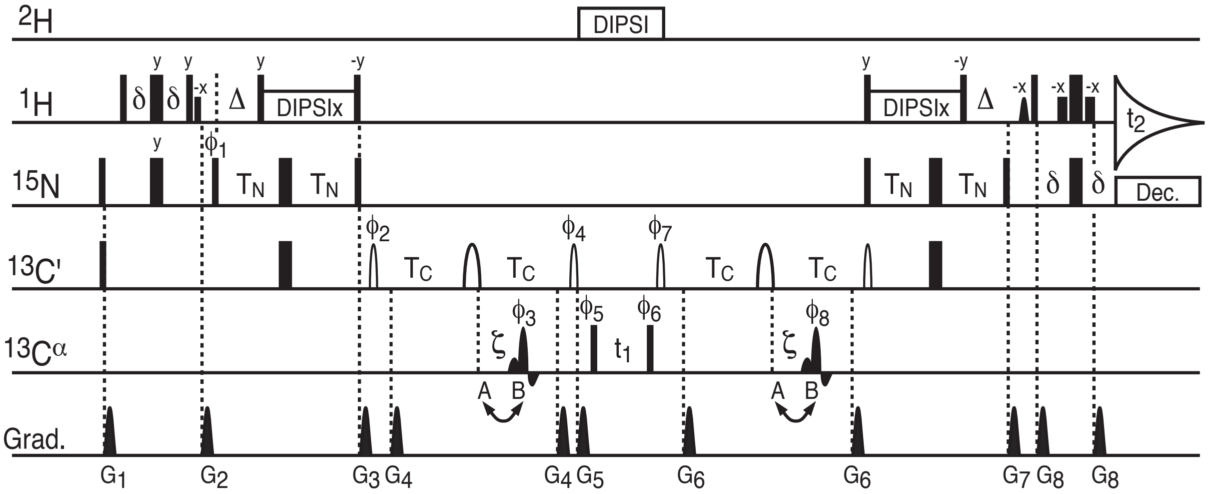

Figure S1. Pulse sequence of the long-range quantitative $^{h3}J_{C\alpha C'}$ H(NCO)CA experiment. Narrow and wide pulses denote 90° and 180° flip angles, respectively, and unless indicated the phase is x. The carrier frequencies are set to water (^{1}H, ^{2}H), 116.5 ppm (^{15}N), 177 ppm (^{13}C′) and 56 ppm (^{13}C$^{\alpha}$). DIPSI$_x$ refers to the ^{1}H decoupling, using the DIPSI-2 modulation scheme with the RF field ($\gamma_H B_1$ = 5 kHz) applied along the x-axis. The ^{2}H decoupling (DIPSI-2) is applied at a field strength of 1.8 kHz. Rectangular low power ^{1}H pulses are applied using $\gamma_H B_1$ = 200 Hz. The shaped 90°$_{-x}$ pulse has a sine-bell amplitude profile and a duration of 3.32 ms. The regular ^{15}N pulses are applied at an RF field strength $\gamma_N B_1$ = 6.25 kHz, whereas the ^{15}N decoupling is applied at an RF field strength of $\gamma_N B_1$ = 1.25 kHz. Rectangular ^{13}C′(^{13}C$^{\alpha}$) 180° and 90° pulses denoted by black rectangles are applied at an RF field strength of 3.1 and 4.7 kHz, respectively. Open shaped pulses indicate ^{13}C′ 90° and 180° with a sine-bell amplitude profile and a duration of 150 and 300 µs, respectively. Shaped ^{13}C$^{\alpha}$ 180° pulses have a g3 amplitude profile with a duration of 1.43 ms corresponding to an inversion bandwith of ± 7 ppm. Note, that these band-selective ^{13}C$^{\alpha}$ inversion pulses were used in order to minimize magnetization losses to sidechain ^{13}C$^{\beta}$ and ^{13}C$^{\gamma}$ nuclei. Delay durations: δ = 2.25 ms; Δ = 5.4 ms; T_N = 15.5 ms; T_C = 58 ms (note that T_C is optimized for minimal transfer by $^{1}J_{C'C\alpha}$ couplings and deviates from the nominal value of $6/^{1}J_{C'C\alpha}$ due to the finite length of the ^{13}C 180° pulses). Phase cycling: ϕ_1= 4(x),4(-x); ϕ_2 = x,-x; ϕ_3= 16(x),16(-x); ϕ_4= y (compensated for Block-Siegert shift by –8°); ϕ_5= 2(x),2(-x); ϕ_6= 8(x),8(-x); ϕ_7= y (compensated for Block-Siegert shift by +8°); ϕ_8= 32(x),32(-x); receiver = R,-R,-R,R, with R=x,2(-x),x. Gradients (sine bell shaped; 25 G/cm at center): $G_{1,2,3,4,5,6,7,8}$ (in z direction) = 1, 1, 1.5, 1.5, 2, 1, 1 and 0.4 ms. Quadrature detection in the t_1 dimension is obtained by altering ϕ_5 in the States-TPPI manner.

As described in the text, the ^{13}C$^{\alpha}$ 180° pulses are either (A, ζ = 10 µs) directly following the ^{13}C′ 180° pulses in the middle of the C'-C$^{\alpha}$ INEPT and reverse INEPT periods ("long range $J_{C\alpha C'}$ experiment") or (B, ζ = 4.83 ms) shifted relative to these pulses by a delay of $1/(4\ ^{1}J_{C'C\alpha})$ ("$^{1}J_{C\alpha C'}$ reference experiment"). The size of the $^{h3}J_{C\alpha C'}$ coupling constants can then be determined from the intensity ratio of the $^{h3}J_{C\alpha C'}$ correlation (A) and the $^{1}J_{C'C\alpha}$ correlation (B): $|^{h3}J_{C\alpha C'}| = 1/(2\pi\ T_C)\ \mathrm{atan}((I_A/I_B)^{1/2})$.

Data were acquired on a Bruker DRX-600 spectrometer, equipped with a triple resonance, 3-axis pulsed field gradient probe. Spectra were recorded as 86* (^{13}C$^{\alpha}$) × 700* (^{1}H^{N}) complex data matrices with acquisition times of 6 and 76 ms respectively. The total experimental times for the H-bond (A) and reference (B) experiments were 141 h and 2.8 h.

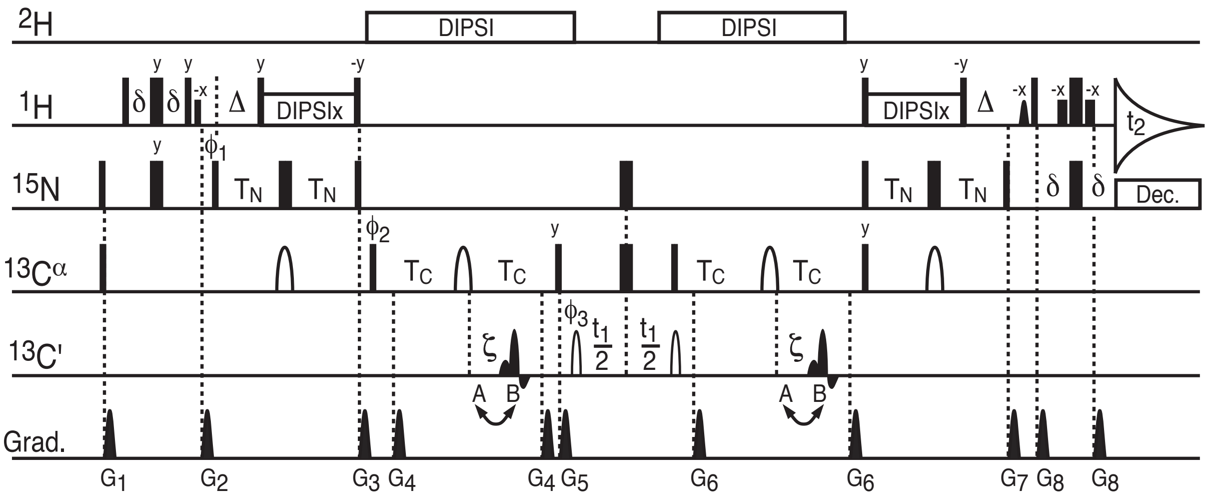

Figure S2. Pulse sequence of the long-range quantitative ${}^{h3}J_{C\alpha C'}$ selective H(NCA)CO experiment. Unless indicated otherwise, parameter settings and symbols are as reported in Figure S1. Rectangular ${}^{13}C^{\alpha}$ 180° and 90° pulses denoted by black rectangles are applied at an RF field strength of 4.7 kHz. Shaped, open ${}^{13}C^{\alpha}$ 180° pulses have a sine-bell amplitude profile and a duration of 2.4 ms. Shaped ${}^{13}C'$ 180° pulses have a g3 amplitude profile with a duration of 2.86 ms corresponding to an inversion bandwidth of ± 3.5 ppm. Delay durations: δ = 2.25 ms; Δ = 5.4 ms; T_N = 22 ms; T_C is optimized to minimize the unwanted transfer through ${}^{1}J_{C\alpha C'}$ (typically ≈ 67.6 – 70.6 ms). Gradients (sine-bell shaped; 25 G/cm at center): $G_{1,2,3,4,5,6,7,8}$ (direction) = 1(-y), 1(z), 1.5(-y), 2(x), 1(z), 1.4(y), 1(-y) and 0.4(x) ms. Phase cycling: ϕ_1= 2(x),2(-x); ϕ_2 = 4(x),4(-x); ϕ_3= x,-x; receiver = x,2(-x),x, -x,2(x),-x. Quadrature detection in the t_1 dimension is obtained by altering ϕ_3 in the States-TPPI manner.

The ${}^{13}C'$ 180° pulses are either (A, ζ = 10 μs) directly following the ${}^{13}C^{\alpha}$ 180° pulses in the middle of the C^{α}- C' INEPT and reverse INEPT periods ("long range $J_{C'C\alpha}$ experiment") or (B, ζ = 4.83 ms) shifted relative to these pulses by a delay of $1/(4\ {}^{1}J_{C'C\alpha})$ ("${}^{1}J_{C\alpha C'}$ reference experiment"). The size of the ${}^{h3}J_{C\alpha C'}$ coupling constants can then be determined from the intensity ratio of the ${}^{h3}J_{C'C\alpha}$ correlation (A) and the ${}^{1}J_{C\alpha C'}$ correlation (B): $|{}^{h3}J_{C\alpha C'}| = 1/(2\pi\ T_C)\ \mathrm{atan}((I_A/I_B)^{1/2})$.

Data were acquired on a Bruker DRX-600 spectrometer, equipped with a triple resonance, 3-axis pulsed field gradient probe. Spectra were recorded as 50* (${}^{13}C'$) × 768* (${}^{1}H^{N}$) complex data matrices with acquisition times of 30 and 83 ms respectively. As described in the main text, spectra were recorded separately for several C^{α}-H^{α}···O=C H-bonds with the ${}^{13}C^{\alpha}$ carrier set on resonance to the respective ${}^{13}C^{\alpha}$ frequencies. The total experimental times varied between 32 and 214 h for the H-bond experiments (A) and were 12 min for the reference (B) experiments.

B. Computational Methods

Sequences containing the donor-acceptor residues were extracted from the 1IGD crystallographic structure for protein G.[18] In order to reduce the computational demands, the side chains were selectively pruned[22] to yield peptide fragments containing 32 – 37 atoms. Except for the G46 glycine residue of G46/T60, sidechains were replaced by methyl groups at the C^{β}-positions. The donor C-H^{α} and N-H hydrogen atom positions were optimized (at the B3PW91/6-31G** level using the Gaussian 98 codes[1s]) while all other atoms were constrained to the positions derived from the crystallographic structure. Entered in Table 1s are relevant structural data in the C^{α}-H^{α}···O=C moieties of the six peptide sequences.

Table 1s. Structural data[1)] in the C^{α}-H^{α}···O=C regions of the peptide sequences.

Donor C^{α}-H^{α}	L10	E20	T22	G46	W48	F57
Acceptor O=C	F57	L10	Y8	T60	T58	T49
$r_{H\alpha O}$	2.346	2.193	2.440	2.240	2.235	2.306
θ_1, $\angle C^{\alpha}\cdots O{=}C$	143.4	135.6	124.9	122.4	144.1	135.4
θ_2, $\angle H^{\alpha}\cdots O{=}C$	126.1	149.9	139.4	113.8	147.8	135.1

[1)] All distances are in Angstroms and all angles are in degrees. Data were obtained via DFT calculations (B3PW91/6-31G**) for peptide fragments extracted from the 1IGD crystal structure.[18,1s]

The Fermi contact contributions to $^{h3}J_{C\alpha C'}$ were obtained for the six sequences using methods of density functional theory[2s,3s] (DFT) and finite perturbation theory[4s] (FPT) at the unrestricted UB3PW91/6-311G** triple-split level with polarization functions on hydrogen and heavier atoms. All scalar couplings are based on the FC output of the FIELD option of Gaussian98,[2s,5s,6s] and are entered in the fifth row of Table 1.

Footnotes and References

(1s) Gaussian 98, Revision A.11.1, Frisch, M. J., Trucks, G. W.; Schlegel, H.B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G., Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G,; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W. M.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A.

(2s) Hohenberg, P.; Kohn, W. *Phys. Rev. B.* **1964**, *136*, 864-871. Kohn, W., Sham, L. J. *Phys. Rev. A.* **1965**, *140*, 1133-1138.

(3s) For a recent comparison of DFT and ab initio calculations of hydrogen-bond-transmitted indirect nuclear spin-spin couplings see Pecul, M.; Sadlej, J.; Helgaker, T. *Chem. Phys. Lett.* **2003**, *372*, 476-484.

(4s) Pople, J. A.; McIver, Jr. J. W.; Ostlund, N. S. *J. Chem. Phys.* **1968**, *49*, 2960-2964, 2965-2970.

(5s) Onak, T.; Jaballas, J.; Barfield, M. *J. Am. Chem. Soc.* **1999**, *121*, 2850-2856.

(6s) All scalar-coupling computations were performed with λ = 0.01 in eq 3 of reference 5s, and the *tight* SCF convergence option of G98.