Application of H(N)CA,CO-E.COSY experiments for calibrating the $\phi$ angular dependences of vicinal couplings $J(C_{i-1}',H_i^\alpha)$, $J(C_{i-1}',C_i^\beta)$ and $J(C_{i-1}',C_i^\gamma)$ in proteins

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Summary
A triple-resonance NMR technique suitable for the determination of carbonyl-related couplings in polypeptide systems is introduced. The application of three novel pulse sequences to uniformly $^{13}$C/$^{15}$N-enriched proteins yields E.COSY-like multiplet patterns exhibiting either one of the $3J(C_{i-1}',H_i^\alpha)$, $3J(C_{i-1}',C_i^\beta)$ and $3J(C_{i-1}',C_i^\gamma)$ coupling constants in the indirectly detected $^{13}$C' dimension, depending on the passive spin selected. The experiments are demonstrated with oxidized flavodoxin from Desulfovibrio vulgaris. On the basis of the J-values measured and the backbone $\phi$-angles derived from a high-resolution X-ray structure of the protein, the three associated Karplus equations were reparametrized. The root-mean-square differences between the experimental coupling constants and those predicted by the optimized Karplus curves are 0.41, 0.33 and 0.32 Hz for $3J(C_{i-1}',H_i^\alpha)$, $3J(C_{i-1}',C_i^\beta)$ and $3J(C_{i-1}',C_i^\gamma)$, respectively. The results are compared with the Karplus parameters previously published for the same couplings.

Introduction
Information from homonuclear and heteronuclear $^3J$ coupling constants is now routinely included in protein structure determination (Bax et al., 1994; Biamonti et al., 1994; Case et al., 1994; Eberstadt et al., 1995). The conversion of coupling constants into local conformation is based on the empirical relation by Karplus (1959,1963):

$$3J(\theta) = A_k \cos^3 \theta + B_k \cos \theta + C_k$$

where the dihedral angle $\theta$ is subtended by three consecutive covalent bonds that connect the pair $k$ of coupled nuclei, and the coefficients $A_k$, $B_k$, and $C_k$ are given in Hz. Obviously, the utility of vicinal coupling constants in dihedral-angle calculation depends on both the quality of experimental data and the reliability of available Karplus coefficients. For peptide systems, parametrizations of Eq. 1 originally emerged from quantum-mechanical approaches or from experiments on either freely rotating or conformationally constrained small model molecules (Hansen et al., 1975; Bystrov, 1976; Fischman et al., 1980). Alternatively, empirical coefficients for vicinal $^1H,^1H, ^1H, ^13C$ and $^1H, ^15N$ couplings in peptides were calibrated based on known structures (DeMarco et al., 1978a,b,c; DeMarco and Llinás, 1979). Recent parametrizations of the dihedral-angle dependence of $3J(H^\alpha,H^\beta)$ in various proteins refer to high-resolution X-ray structures (Pardi et al., 1984; Ludvigsen et al., 1991; Vuister and Bax, 1993; Wang and Bax, 1996), taking advantage of the large number of data points simultaneously obtained from a single protein spectrum. The coefficients thus determined include the contributions from thermal torsional-angle fluctuations typically encountered in proteins (Hoch et al., 1985; Brüschweiler and Case, 1994).

According to Eq. 1, a particular J-value specifies up to four dihedral angles. Thus, the derivation of correct angular restraints requires that a set of coupling constants associated with the same torsion angle be measured in order to resolve the inherent degeneracy. In the following,
we will focus on vicinal couplings related to the backbone torsion angle \( \phi \), which is characterized by three \( ^1\text{H}, ^3\text{C}, \) a single \( ^1\text{H}, ^3\text{H} \) and two \( ^1\text{C}, ^13\text{C} \) three-bond couplings. Usually, a reduced set made up of the homonuclear \( ^1\text{J}(^1\text{H}, ^1\text{H}) \) interaction and the heteronuclear coupling constants \( ^1\text{J}(^1\text{H}, ^3\text{C}), ^1\text{J}(^3\text{C}, ^3\text{C}) \) and \( ^1\text{J}(^3\text{C}, ^1\text{H}) \), which were all parametrized by Wang and Bax (1995,1996) using human ubiquitin as a model protein, serves as the experimental basis. In contrast, the circumjacent carbon–carbon couplings have only very recently been addressed (Hu and Bax, 1996a,b; Löhr and Rüterjans, 1996), although approximate values for \( ^1\text{J}(^3\text{C}, ^3\text{C}) \) in alanine residues in calmodulin.