There is interest in understanding how much secondary shielding mechanisms contribute to the chemical shielding anisotropy in backbone nuclei of proteins, including its site variability, and the orientation of the shielding tensor. In a pair of circular current loops of radius (the shielding tensor of a nucleus, local), the modulus 4f contribution from ring currents and 4g local is obtained via DFT computations using a model with CYH replacing the benzene ring and with 4h local calculated via eqs 1 and 2 (both local and 4i local were obtained in the same reference frame). For computations of 4j local we assume that a CYH molecule provides an olefinic analogue for benzene. A series of models were calculated in two different ways: (i) directly from DFT calculations using the atomic model with the benzene ring and (ii) as 4k local + 4l where 4m local is obtained via DFT computations using a model with CYH replacing the benzene ring and with 4n local were obtained in the same reference frame. For computations of 4o local we assume that a CYH molecule provides an olefinic analogue for benzene.

\[
\sigma_{zz}(\text{ppm}) = \frac{ID(z)}{(1 + \rho(z) + z^2)^{1/2}} \left[ K(k) + \frac{1 - \rho(z)^2 + z^2}{(1 - \rho(z)^2 + z^2)^{1/2}} E(k) \right]
\]

(1)

\[
\sigma_{zz}^{\text{loc}}(\text{ppm}) = \frac{ID(z)}{\rho((1 + \rho(z)^2 + z^2)^{1/2})} \left[ -K(k) + \frac{1 + \rho(z)^2 + z^2}{(1 - \rho(z)^2 + z^2)^{1/2}} E(k) \right]
\]

(2)

\[
\sigma_{zz} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}
\]

(3)

Figure 1. The initial geometry of the models used for calculating the shielding tensor, 4p. The rings are in the xy plane with the center of mass of the carbons on the z-axis. For the models in (A) the NMA is positioned so that the 4q H and N atoms are aligned on the z-axis with a distance of 3.65 Å between the 4r H and the center of the ring. For the models in (B) the 4s H and N atoms and the origin are aligned along an axis inclined at 54.7° to the z-axis with a distance of 4 Å between the 4t H and the center of the ring. The geometry of the models was modified by applying the rotation 4u as described in the text and no significant van der Waals violations occur with the molecular separations described above. The DFT based shielding calculations used the hybrid B3LYP exchange-correlation functional 4v,4w the 6-311++G(2d,p) basis set 4x,4y and gauge-including atomic orbitals 4z,4a. Equations 1 and 2 used \( l = 1 \) and \( a = 1.39 \). The eigenvalues of the traceless symmetric part of the 4b shielding tensor were ordered with \( \sigma_{zz} \leq \sigma_{xy} \leq \sigma_{zz} \). \( \Delta \sigma \) was calculated using the convention described by Grant 4b, so that \( \Delta \sigma \) changes sign as \( \sigma_{zz} \) crosses the midpoints between \( \sigma_{yy} \) and \( \sigma_{zz} \). Panels (C) and (D) display the variation of \( \Delta \sigma \) with change in model geometry starting from the fragments in (A) and (B), respectively. The plots show \( \Delta \sigma \) from the DFT computations using the NMA–CYH model (open squares, dashed line), the NMA–benzene model (filled circles, solid line) and \( \Delta \sigma \) from the summation of the NMA–CYH DFT results (\( \sigma_{\text{local}} \)) with the ring current contribution (\( \sigma_{rc} \)) from eqs 1 and 2 (open circles).
generated from those in Figure 1 by applying a rotation to the NMA molecule while holding the benzene or CYH ring fixed. The rotation is defined as: the rotation axis is parallel to the y-axis, and the pivot coincides with the H^8 atom. The amplitude of the rotation, Ω, is varied from 0° to 90° with a step size of 15°. For example a rotation of Ω = 90° applied to the model in Figure 1A results in the NMA peptide plane being oriented parallel to the plane of the ring, with the position of the H^8 atom and the ring unaltered.

The starting geometries of the models in Figure 1, A and B, were chosen so as to ensure that o_{local}, calculated via eqs 1 and 2 in the ring reference frame, consists solely of either o_{xyz} or o_{xyz}, respectively. Clearly, the magnitude of either o_{xyz} or o_{xyz} in the ring reference frame, do not change as a function of the specific rotation described above. However, the relative contributions of o_{local} and o_{xyz} vary with the rotation Ω, testing the approximation o = o_{local} + o_{xyz} under a variety of conditions. The results are illustrated in Figure 1, C and D, for Ω of the H^8 shielding tensor (see Figure caption for the definition of Ω). The crossed circles connected by the solid line show data for Ω from DFT computations of the NMA—benzene models. The open squares joined by the dashed line show Ω_{local} from the NMA—CYH models without including o_{xyz}. The open circles show the values for Ω from o = o_{local} + o_{xyz} where o_{local} was obtained from the NMA—CYH models with o_{xyz} calculated via eqs 1 and 2. These results indicate that, for the particular model system used here, including the additive term o_{xyz} improves agreement to the benchmark Δo values obtained from DFT computations of the NMA—benzene models. The isotropic shielding from the DFT computations of the NMA—benzene and NMA—CYH series of models differ by 0.99 ± 0.05 and -0.05 ± 0.14 ppm, which compare favorably to the values calculated from o_{local} of 1.04 and 0 ppm respectively.

Calculations were performed to assess the contribution of ring currents to the CSA of backbone 1H^8 nuclei in proteins using a structure of the second type-2 module of the protein fibronectin. To form a contribution from each aromatic amino acid after transforming the individual o_{xyz} generated in each local ring frame into the molecular frame. The data for [Δo] in Figure 2A were extracted from the shielding tensor (o_{local} + o_{xyz}). These data exhibit a maximum CSA of 16.6 ppm for the 1H^8 of G42. The large perturbation to Δo by the ring current term o_{xyz} for this residue is caused, almost exclusively, by the side chain of F19. The structure of this protein strongly suggests there is a N—H—π—π hydrogen bond involving the 1H^8 of G42 as the donor with F19 acting as the π-acceptor. The presence of an aromatic hydrogen bond is supported by the experimental chemical shift for this 1H^8 resonance at Δexp = 3.59 ppm. The large CSA extracted from the tensor (o_{local} + o_{xyz}) is supported by a DFT shielding calculation using the molecular fragment in Figure 2B. The DFT derived eigenvalues were Δ_{-9.8, -2.3, 12.1} ppm giving Δo = 18.2 ppm in reasonable agreement with the CSA from (o_{local} + o_{xyz}). The isotropic shielding from this DFT calculation of o_{calc} = 29.81 ppm, when converted to the shift scale, gives Δo = 3.63 ppm in good agreement with Δexp.

Using the approach described here the effect of ring currents upon the CSA can be assessed. A series of similar calculations suggests the method is also applicable to a fused ring system such as indole. For protons there is predicted to be a significant contribution from the ring currents of aromatic amino acids to the proposed CSA site variation. The contribution into the CSA of 13C^3 and 15N nuclei of the peptide backbone typically causes a rmsd variation of <1 ppm. For a N—H—π—π hydrogen bond the contribution of ring currents to the 1H^8 CSA is predicted to be pronounced.

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References
(14) Using definitions in ref 13a the parameters A, B, o_{xyz}, and θ have the values 0.71 Å, 2.37 Å, 17.7°, and 146° respectively. This set of values is consistent with a NH—π—π aromatic hydrogen bond interaction.
(15) (a) Jameson, C. J. In Encyclopedia of Nuclear Magnetic Resonance; Grant, D. M., Harris, R. K., Eds.; 1996; p 1273. (b) VanderHart, D. L. In Encyclopedia of Nuclear Magnetic Resonance; Grant, D. M., Harris, R. K., Eds.; 1996; p 2939. (c) o_{xyz} = o_{Δ} — o_{Δxyz}. The value for o_{Δxyz} was calculated using the absolute shielding for water protons (liquid, 307.7 K, sphere) of 25.79 ppm. The susceptibility correction for a superconducting solenoid is 3.9μT X 10^{-3} ppm = +1 ppm (ρH2O = 9.9 × 10^{-7} MKS units) and the shift of water protons (307.7 K relative to TMS protons is assumed to be 4.65 ppm giving Δo = 33.44 ppm.)