11. NMR chemical shifts: theory and experiment

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A review of reprinted paper [B54]:
Medium effects in proton magnetic resonance. I. Gases
W.T. Raynes, A.D. Buckingham and H.J. Bernstein
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Nuclear magnetic resonance spectroscopy is a powerful technique that is used very widely in the characterization of systems ranging from simple molecules in low-density gases to molecules in biological systems, whole tissues or even whole animals, as well as materials complex and heterogeneous, such as polymer blends and catalysts. The NMR parameter which permits the dispersion of nuclear resonance frequencies into separate signals at separations proportional to the strength of the applied magnetic field is the nuclear magnetic shielding. The difference between nuclear shielding values in two different nuclear sites is called the NMR chemical shift. The extreme sensitivity of the nuclear shielding to the electronic environment gives rise to the dispersion of resonances; for example the ^{13}C nuclei of the alpha carbons of the various alanine residues in a protein all have different resonance frequencies each of which is different from the free amino acid.

In a series of papers [B40, B41, B54], Buckingham introduced the idea of additive contributions to NMR chemical shifts arising from molecular interactions with solvent molecules. The individual chemical-shift contributions identified are as follows: bulk susceptibility σ_b, magnetic anisotropy σ_a, electric-field effects σ_E, and van der Waals σ_W. These papers constitute the framework on which nearly all attempts at the interpretation of the relation of the proton chemical shifts in proteins to the secondary

structure are based. In the recent past, the powerful multidimensional NMR methods of determining protein structure in solution made no use at all of the chemical shift information which is a natural byproduct of the resonance frequency assignment step. All connectivities and the complete secondary structure were derived using the so-called NOEs (cross-peaks between two protons depending on their through-space separation), and the through-bond spin–spin couplings. Now, chemical-shift-based methods are to be employed in assignment techniques that determine secondary structure directly after the signal assignment step. Such procedures are likely to become indispensable for larger proteins (molecular weights $\geq$ 20 kDa) where many of the key NOE connectivities may be missing, and where spin diffusion is a limiting factor. I am sure that in 1960 David did not foresee this particular application of his NMR papers [B40, B41, B54].

Unlike the NOE which depends on through-space ^1H–^1H separation or the coupling constants which have well-defined values according to the through-bond pathway, essentially independent of environment, "chemical shifts involve summations of contributions from a variety of sources". The phrase in quotes is a way of thinking about chemical shifts that was introduced by Buckingham in 1960. Three NMR papers [B40, B41, B54], of which only the third is reprinted here, have influenced the work of a vast number of scientists in many disciplines in the past 36 years, as evidenced by their number of citations. (Paper [B40] is a "Science Citation Classic".) In this essay we look at the context of the related papers, compare with recent advances, and occasionally give some recent examples of verification of early Buckingham predictions. I can mention in passing only a few of the applications in which these papers have been indispensable. The ideas from these papers have permeated our thinking to such an extent that many publications apply the ideas and insight offered in these papers without even citing them.

Magnetic contributions to NMR chemical shifts had already been invoked by Pople in 1956 [1], when he considered the Pauling model of a magnetic dipole placed at the center of the benzene hexagon producing a local magnetic field at the position of the proton. This model provided a chemical shift of the correct sign to account crudely for the difference in proton chemical shift between ethylene and benzene. Waugh and Fessenden in 1957 [2] constructed a model of two loops of wire parallel to the benzene ring plane above and below, separated by an empirically adjusted distance, producing local magnetic fields at proton positions. Only a minor correction factor (found also by Waugh and Fessenden) was later inserted into their equations by Johnson and Bovey [3]. (It is regrettable that the

Waugh–Fessenden model has been attributed entirely to Johnson and Bovey in most of the literature of the past 40 years.)

In the same year (1957), Buckingham and Pople considered the electric-field effects on the magnetizability of the hydrogen atom [B22]. Subsequently, in an analysis closely following this work, Marshall and Pople (1958) [4] considered the electric-field effects on the shielding of a hydrogen atom. By symmetry, there is no linear response of the shielding to the electric field in the case of an atom. In paper [B40] Buckingham presented the general theory of the response of the nuclear shielding in a molecule to a uniform electric field $\boldsymbol{F}$, in the expansion:

$$\sigma_{\alpha\beta} = \sigma_{\alpha\beta}^{(0)} + \sigma_{\alpha\beta\gamma}^{(1)} F_\gamma + \tfrac{1}{2}\, \sigma_{\alpha\beta\gamma\delta}^{(2)} F_\gamma F_\delta + \ldots. \qquad (1)$$

Paper [B40] delineates the symmetry properties of the derivatives such as $\sigma_{xxz}^{(1)}$ and $\sigma_{zzxx}^{(2)}$ (sometimes referred to as shielding polarizabilities and hyperpolarizabilities[1]) for a cylindrically symmetric X–H bond, in which the dominant first-order electric-field effect is considered for the first time. The response to an electric-field gradient and a cage of electrostatic charges was considered separately [B44]. Also proposed in paper [B40] were the typical magnitudes of the response of the isotropic shielding to an electric field, for a proton in a cylindrically symmetric X–H bond: $\sigma = 2 \times 10^{-5} - 2 \times 10^{-12}$ $F_z - 1 \times 10{-}18\, F_z^2$, in c.g.s. units. For comparison with recent results, let us put the Buckingham values in terms of the A and B parameters that were introduced later in the reprinted paper [B54] and explicitly in the form of the following equation [6, B166]:

$$\sigma_{iso} = \sigma_{iso}^{(0)} \quad - A_z F_z - B_{zz} F_z^2 - B_{xx} (F_x^2 + F_y^2), \qquad (2)$$

where the z direction points in the direction from X to the proton, in an isotropic average over all magnetic-field directions. Buckingham estimated 2×10^{-12} e.s.u. or 34.3 ppm au (in modern units) for $A_z = -(1/3)[\sigma_{zzz}^{(1)} + 2\sigma_{xxz}^{(1)}]$ and 1×10^{-18} esu or 290 ppm au for $B_{zz} = -(1/3)[\frac{1}{2}\sigma_{zzzz}^{(2)} + \sigma_{xxzz}^{(2)}]$. The current best value for ^{1}H in a cylindrically symmetric C–H bond (in HCN) is $A_z = 55.2$ ppm au and $B_{zz} = 76.3$ ppm au, and for HCCH the values are 65.6 and 25.4 ppm au respectively [7]. The signs corresponding to a decrease in shielding for the electric field in the X–H direction were correctly

1 This terminology coined by C.E. Dykstra *et al.* [5] is somewhat ambiguous, but has become commonly used. David prefers the more explicit linear and quadratic electric-field coefficients of nuclear magnetic shielding.

predicted. The order of magnitude of A was correct. Recent calculations show that B values are more variable, and either sign of B_{zz} for ^{1}H in X–H bonds is found, depending on X [8].

For a dipolar molecule or a molecule containing polar groups dissolved in a liquid, Buckingham considered "internal" and "reaction" fields. Bond dipoles within the molecule provide a source of electric field ("internal", the same as in the isolated molecule). When a polar molecule is dissolved it polarizes the surrounding medium, and this polarization leads to an electric field, a "reaction field" at the solute. To calculate this field Buckingham invoked the Onsager model of a spherical solute molecule containing a point dipole at its center in a solvent represented by a continuum with a dielectric constant ε [B40]. Electric fields arising from the induced charges on the solvent molecules near the highly polar groups of a non-dipolar solute molecule lead to a non-uniform electric field at the nucleus of interest. Contributions to shielding from the reaction field and reaction-field gradients were expressed in terms of the dielectric constant of the solvent [B40]. Earlier, Stephen [9] had briefly considered shielding changes arising from distortion of the electron distribution in a molecule by strong electric fields such as those in liquids of strongly polar molecules and molecules which form strong hydrogen bonds, but he was primarily concerned with magnetic anisotropy contributions.

The equation:

$$\sigma_{\text{solvent}} = \sigma_b + \sigma_a + \sigma_W + \sigma_E, \tag{3}$$

eq. (2) in paper [B41], appears in many textbooks of NMR, and is invoked not only for solvent effects but more generally for shielding changes in all sorts of systems, in wide-ranging studies such as conformational analysis of steroids, aggregation studies of zwitterionic amphiphiles, thermometry in tissues during magnetic resonance imaging, monitoring of DNA melting or phase transitions in liquid crystals or protein folding, etc. The shielding contribution σ_b proportional to the bulk magnetic susceptibility of the medium was derived here, leading to the well-known result first deduced by Dickinson [10]. The shielding contribution from the magnetic anisotropy σ_a was derived and an explicit formula was provided for an axially symmetric solvent molecule. This is the same result arrived at independently first by McConnell [11] and then by Stephen [9]. McConnell used Ramsey's equations to derive the shielding at a given nucleus arising from a group of electrons which can be regarded as insulated from the immediate region of the molecule where the nucleus is located. For a group which is at long range from the nucleus, the largest non-zero term is the magnetic

dipole term. For an axially symmetric group, the shielding contribution in McConnell's theory is the same as that derived later in paper [B41]. McConnell suggested that this could be important in intermolecular shielding in liquids, particularly aromatic liquids. The list of references in paper [B41] show no indication that the authors had seen McConnell's paper. Later, Buckingham and Stiles generalized the magnetic anisotropy term to include higher-order multipoles [B132]. The σ_E term draws from Buckingham's earlier work, including the reaction field and reaction-field gradients for solute molecules having polar groups [B40].

Paper [B41] introduces σ_W for the first time, predicting (at constant density) a temperature independent part and a temperature dependent part. According to this paper, the first comes from the solvent in its equilibrium configuration causing a distortion of the electronic environment of the nucleus, leading to a decrease in diamagnetic shielding. The second comes from what was called "buffeting" of the solute by solvent molecules as the solvent departs from equilibrium configuration leading to a time-dependent distortion of the electronic structure. The first part has been directly observed in the ^{129}Xe chemical shift of a single Xe atom trapped inside a rigid zeolite cage (solvent at its equilibrium configuration) [12]. The Xe signal from the single Xe atom in the small side pocket in Na mordenite is temperature independent since the cage is small enough that the Xe atom position within the cage is essentially invariant. In this same paper [B41], Buckingham predicted the sign of the second part and the sign of its temperature coefficient. He predicted also that ^{129}Xe in Xe would show the largest temperature-dependent negative σ_W at constant density, that is, an increased shift to high frequency as temperature increases at constant density. This has been directly observed for a fixed number of Xe atoms trapped in a rigid zeolite cage (constant density). For example, the ^{129}Xe chemical shift of 7 Xe atoms trapped in a cage of zeolite NaA is 228.3 ppm (relative to the isolated Xe atom) at room temperature and increases linearly with increasing temperature, with a temperature coefficient of 0.133 ppm/K in the range 180–400 K [13].

The theoretical model of additive contributions to intermolecular chemical shifts was originally presented in a form suitable for the interpretation of solvent shifts in the liquid phase [B41]. In the reprinted paper [B54], the theory is articulated in detail, with general expressions from which each of the four contributions may be calculated for gases. The concept of the virial expansion of any equilibrium electromagnetic property in the gas phase had been proposed by Buckingham and Pople in 1956 [B27]. For shielding this is given as eq. (1) in the reprinted paper:

$$\sigma = \sigma_0 + (\sigma_1/V_m) + (\sigma_2/V_m^2) + \ldots , \quad (4)$$

where V_m is the molar volume. In the dilute gas, only the first two terms are usually significant, that is, a linear density dependence is observed over a wide range of densities. In dense gases, non-linear density dependence has been observed [14]. In this theoretical framework, the concept of a second virial coefficient of any equilibrium electromagnetic property is explicitly defined in terms of the molecular electronic property for the interacting pair integrated over all configurations of the pair, the $\exp(-U/k_BT)$ term in the intermolecular potential energy explicitly appearing in the integral. In paper [B54] the very first measurements of the second virial coefficient of nuclear shielding were reported. At the same time, the various contributions to the nuclear magnetic shielding for an interacting pair of molecules were explicitly derived. The magnetic anisotropy is as previously presented in paper [B41] with the collision partner at a variable distance and orientation relative to the molecule of interest. The electric field and field squared at the location of the nucleus, arising from permanent electric moments on the collision partner, are expressed explicitly. Each contribution is taken all the way through to the second virial coefficient of nuclear shielding, by averaging over a sample potential form (Stockmayer) that includes the anisotropy arising from permanent electric moments. In other words a complete theory (albeit with some assumptions) is presented for the intermolecular shifts in mixtures of gases, and the first gas-phase experimental data on intermolecular effects on NMR chemical shifts were interpreted with this theory.

In this paper [B54], the $\sigma_{pair,W}$ was approximated by assuming that the deshielding is brought about by the fluctuating electric field whose non-vanishing square leads to dispersion forces. The quadratic response of the shielding to a static electric field is then used together with an expression for the mean-square field. If the response of the shielding to a static electric field is to be used in this context, an effective static mean-square electric field that is equivalent to the mean-square fluctuating field would be more appropriate. In the Drude model, which leads to the same result as the London formula for the dispersion energy, the effective *static* mean-square field at atom 1 due to atom 2 is [15]:

$$\langle F^2 \rangle = (3/2)\, [IP_1\, IP_2/(IP_1 + IP_2)]\, \alpha_2(0)\, R^{-6}, \quad (5)$$

where IP_1 and IP_2 are the ionization potentials of the atoms. For identical atoms, this expression differs by a factor of four from eq. (19) in paper

[B54]. This factor is of little consequence so long as the magnitude of the quadratic response to an electric field is relatively unknown and B is taken to be an empirical parameter. The van der Waals contribution to shielding is clearly the dominant term for Xe atom in a gas. So it was particularly disappointing to find that empirical values of B_{ave} obtained by fitting experimental values of $\sigma_I(T)$ of ^{129}Xe in mixtures of Xe and other gases to $-B_{ave}\langle F^2\rangle$ following the formula in paper [B54] ranged from 9×10^4 to 25×10^4 ppm au [14]. Clearly, the empirical parameter is not a constant for Xe with an arbitrary collision partner, and problems also arose later in attempting to account for the temperature dependence $\sigma_I(T)$ of ^{129}Xe in pure xenon gas and in mixtures of rare gases. The magnitude of the quadratic electric-field coefficient of ^{129}Xe shielding in Xe atom was unknown until Bishop and Cybulski carried out large basis set calculations at the SCF and MP2 level, finding $B_{ave} = (1/3)[\frac{1}{2}\sigma^{(2)}_{zzz} + \sigma^{(2)}_{zzxx}] = 4404$ ppm au [16]. This is about 50 times too small to account for the observed $\sigma_I(T)$. Whether or not the mean-field model is a good model for the dispersion contributions to shielding remains to be seen. What is clear is that we all failed to pay proper attention to the footnote included in paper [B54], which reads: "The term in $-B\langle F^2\rangle$ represents the longest range contribution to $\sigma_{pair,W}$, but in molecular collisions, shorter-range effects may be appreciable. The latter could arise from the kind of overlap that leads to repulsive forces." *Ab initio* calculations including second-order electron correlation give results that are only very slightly different from calculations at the Coupled Hartree–Fock level for the ^{39}Ar $\sigma_{pair}(R)$ shielding surface in Ar–Ar [17]. This means that to the extent that the second-order electron correlation values include dispersion contributions to shielding, we have found out that these contributions are small compared to the total intermolecular shielding in the range of distances (0.5–2) times the characteristic r_{min} of the intermolecular potential function. Overlap and exchange account for nearly all the intermolecular shielding in rare gas pairs [17,18]. Short range effects are indeed appreciable, just as the footnote in paper [B54] stated! Unfortunately, the misuse of $-B\langle F^2\rangle$ for the entire van der Waals shift persists even after this revelation.

There have been significant advances in the calculations of electric-field effects on shielding in molecules. Raynes and Ratcliffe [6] extended the earlier work of Buckingham and Malm [B129] to derive the symmetry properties of $\sigma_{\alpha\beta\gamma}^{(1)}$ and $\sigma_{\alpha\beta\gamma\delta}^{(2)}$ for all nuclear site symmetries. Very little was done in the 1970s and 80s on theoretical calculations of these quantities, but the field has been very active recently with several groups doing calculations (Raynes in the U.K., Dykstra in the U.S.A., Bishop in Canada,

and groups collaborating in Denmark and Norway) [5,7,8,16,19–26]. The electric-field coefficients of the nuclear magnetic shielding have been calculated in a large number of molecules, by various *ab initio* methods [27]. We compare here the results using various methods for ^{1}H in the HF molecule: Buckingham and Day [B166] calculated the values $A_\parallel = A_z = -(1/3)\,[2\sigma^{(1)}_{xxx} + \sigma^{(1)}_{zzz}] = 77.3$ ppm au and $B_\perp = +20.9$ ppm au, $B_\parallel = -170.8$ ppm au. Recently reported values are not that different: $A_\parallel = 83.5$ ppm au, $B_\perp = 40.1$ ppm au, $B_\parallel = -157.4$ ppm au [8], $A_\parallel = 81.5$, and $B_\parallel = -164.3$ ppm au [21], $A_\parallel = 79.4$ ppm au, $B_\perp = 51.9$ ppm au, $B_\parallel = -162.5$ ppm au (at the SCF level) and $A_\parallel = 79.1$ ppm au, $B_\perp = 75.5$ ppm au, $B_\parallel = -134.6$ ppm au (at the MP2 level) [23]. Recent *ab initio* calculations of the linear and quadratic electric-field coefficients of shielding using various methods of including electron correlation [7,25] reveal substantial contributions from electron correlation especially for the quadratic coefficients. Shielding derivatives with respect to the electric field have sufficiently large sensitivity to molecular geometry that vibrational averaging has also been carried out [24].

It turns out that the $[\sigma_{pair}(R) - \sigma(\text{free atom})]$ function for a rare gas pair is non-monotonic (unlike $-BR^{-6}$) and might have looked like the function $[\alpha_{pair}(R) - \alpha(\text{free atom})]$ first sketched out in Fig. 1.17 of paper [B170], except that in these cases $[\sigma_{pair}(R) - \sigma(\text{free atom})]$ did not have the outer positive hump that electric–dipole pair polarizability does [17]. The R-dependence of the longest range contribution for two rare gas atoms is R^{-6} but the short-range contributions need not be of this form, in fact, we should expect terms in R^{-8}, R^{-10}, etc. Indeed, the *ab initio* shielding functions $[\sigma_{pair}(R) - \sigma(\text{free atom})]$ for various rare-gas pairs Ar–Ar, Ar–Ne, Ne–Ne, Ne–He as well as Xe–Xe have this non-monotonic shape and can be fitted by a sum of terms in inverse even powers of R [18,28]. Furthermore, *ab initio* calculations of $[\sigma_{pair}(R) - \sigma(\text{free Xe atom})]$ for the Xe–CO_2, and Xe–N_2 collision pairs provide surfaces that can be fitted to expansions of the form:

$$\left[\sum_n A_n^{(6)}\, P_n(\cos\theta)\right] R^{-6} + \left[\sum_n A_n^{(8)}\, P_n(\cos\theta)\right] R^{-8} + \left[\sum_n A_n^{(10)}\, P_n(\cos\theta)\right] R^{-10} + \ldots ,$$

where $n = 0,2,4, \ldots$; Xe–CO requires odd n also [28]. When integrated with $\exp(-U(R\theta)/k_B T)$ using reasonable anisotropic potential functions, these

pair shielding surfaces account for the observed $\sigma_1(T)$ for mixtures of Xe with CO_2, CO and N_2, giving the correct shape of the temperature dependence [28].

In the liquid phase, the reaction-field model for solvent shifts has prevailed in the literature up to the present time, but the variations on the original Buckingham theme have been strictly empirical, and most of the papers which adopted this reaction-field approach to solvent shifts in NMR fitted entire intermolecular shifts, rather than just the σ_E part, linearly to some function of ε or n^2. More realistic models are now being used. In one approach, the chemical shifts in polar and hydrogen-bonded solvents are calculated using canonical partition functions that include clusters of molecules, dimers up to hexamers in various configurations [29]. The shielding in the most significant clusters are calculated at a high level and the canonical ensemble average is computed. Another approach is to use classical molecular dynamics simulations of the liquid using some potential that tested well for some properties to generate typical configurations of the liquid. A large enough number of molecules is randomly selected from the central part of the simulation box to yield clusters of various sizes for quantum mechanical calculations of the shielding. The convergence of the shielding values reached at sufficiently large cluster sizes (*e.g.*, 13 molecules) provides the average shielding. This method was used to calculate the gas-to-liquid shifts of ^1H and ^{17}O in water [30].

A direct application of Buckingham's magnetic anisotropy contribution to chemical shift is the determination of isomers of fullerenes. The discovery of C_{60} excited the chemical community and earned the Nobel Prize in Chemistry in 1996. A number of higher fullerenes have been synthesized. Measurements of ^3He chemical shifts for He trapped inside fullerenes combined with calculations of magnetic contributions to shielding help in the assignments of isomers. ^3He NMR spectroscopy constitutes a clean analytical tool since only one signal is expected for each species and isomer. An *ab initio* shielding calculation at a point inside the fullerene, without basis functions on the He, provides a direct calculation of σ_a. Direct comparison with calculations including basis functions on the He atom proves that in this case the van der Waals contribution is negligible. David's notion of additivity also provides a physical picture: summing over the benzene and ethylene units present in a fullerene reproduces the endohedral ^3He shifts [31].

What David Buckingham has provided in the series of papers culminating in paper [B54] is a way of thinking about complex, even heterogeneous systems. The effects of the parts of the system farther out have to be taken

into account, but this can be done by using additive contributions. In fact, new hybrid electronic structure computations are currently being investigated, [32,33] in which a local fragment is done at a high *ab initio* level, perhaps with a locally dense basis set, the immediate neighbors are done *ab initio* with smaller basis sets or else semiempirically, and the far out parts are treated by molecular mechanics or replaced by constellations of point charges. In a parallel example, one might calculate the linear response to the electric field by a very high level *ab initio* method for a neutral fragment including the bond to the nucleus of interest, in a geometry appropriate to that *in situ*. Yet the electric field itself could be calculated from point charges located at atomic positions outside the fragment. The whole approach to the interpretation of chemical shifts in proteins or other biopolymers is based on this kind of thinking [34].

The NMR chemical shift nonequivalences in proteins and nucleic acids, caused primarily by folding into their native conformation, spread out the NMR signals of a particular amino acid residue at different locations to give different resonance frequencies. Without such nonequivalences modern multi-dimensional NMR studies of protein structure would not be possible. The understanding of these chemical shift nonequivalences leads to new ways of determining and or refining protein structure. How does one think about the NMR shielding of a single nucleus in such a complex system? In a way, de Dios and Oldfield adopted the Buckingham viewpoint [34]. There is the short range contribution, very sensitive to the local geometry of the bonds to the nucleus in question (torsion angles, for example) which may be different from one site to another, and any hydrogen bonding at the nucleus in question or the next atom. Sites in helical or sheet segments of a protein normally have different torsion angles and the changes in the shielding due to these geometrical parameters are caused by the changes in the electronic wavefunctions near the nucleus of interest, that can best be evaluated through full *ab initio* calculation. Then there are the effects of all the rest of the protein plus any solvent molecules. These are usually viewed in Buckingham terms: magnetic anisotropy contributions, electric-field contributions, and van der Waals contributions from parts of the protein farther away in the through-bond pathway but in close proximity through space. The magnetic anisotropy contributions such as those arising from neighboring aromatic side chains and carbonyl groups can be treated classically, just as proposed in paper [B41], that is, they provide additive non-zero time-averaged local magnetic fields along the direction of the applied external field. The magnitude of the magnetic-anisotropy contribution is independent of the sensing nucleus, is small in proteins and therefore

becomes important only in the case of ^{1}H. Since the atoms that are sufficiently close to the nucleus of interest are included in the fragment subjected to full *ab initio* shielding calculations, any short-range (repulsive and exchange) van der Waals effect is already included. Dispersion contributions are neglected.

What about the electric-field effects from the rest of the protein? The rest of the protein is treated atomistically but only as partial fixed charges located at each atom position, by adding fixed-charge field terms to the Fock matrix with which the self-consistent-field calculations are done during the process of evaluating the nuclear shielding at the nucleus of interest. This, the charge-field-perturbation approach, is one way in which electric-field contributions due to remote parts of the protein are taken into account [34]. In a rigorous test, using fluorobenzene in the presence of up to five HF molecules, the charge-field-perturbation approach gave results that were very close to those obtained in full *ab initio* calculations of ^{19}F shielding in the (solute + *n*HF) clusters [35]. Another way, called a multipole shielding polarizability approach, is to use the Buckingham expansion of shielding in powers of the electric field and electric-field gradient [34,36]. ^{19}F shielding non-equivalencies at five Trp residues due to protein folding in a galactose binding protein are dominated by electrostatic fields. *Ab initio* calculations of the electric-field coefficients of ^{19}F shielding in [5-F]-tryptophan yield results that remain fairly constant in various Trp environments. The success of predicting chemical shift non-equivalencies on the basis of the Buckingham expansion therefore depends mainly on the accuracy with which the electric field and electric-field gradients at each ^{19}F nuclear site can be described. Internal electric fields are evaluated by using some previously tested model for internal electrostatic fields in a protein. Starting with the x-ray structure of the protein, then relaxing the structure by using low-temperature molecular-dynamic simulations of the ^{19}F-labeled protein, the average chemical shift over some 20-ps trajectory for each ^{19}F nuclear site of interest is calculated. It is found that the *A* term dominates and occasionally the electric-field gradient term in the shielding from paper [B44] becomes important. It has been shown that the *ca* 10 ppm spread in the otherwise identical Trp sites can be accounted for entirely by the electric-field effects [36]. The same results are obtained with the charge-field-perturbation approach described above. In another example, it has been shown that, by including electric-field effects from a lattice of point-charge fields from the neighboring molecules in the crystal, the 39 experimental ^{13}C shielding-tensor principal components for the zwitterionic threonine and tyrosine amino acids could be reproduced [37]. The

electric-field effects were particularly important for two of the shielding tensor components of the carboxyl ^{13}C sites.

There is a huge data base of proton chemical shift information in proteins. The additive contributions from magnetic anisotropy and electric-field effects described in paper [B41] constitute the framework on which all attempts at interpretation have been based, whether empirical-statistical [38–40], semiempirical quantum-mechanical [41], or partly *ab initio* [42]. The primary intermolecular contributions to ^{1}H shifts are assumed to be Buckingham's σ_a, the magnetic anisotropy from aromatic rings and carbonyl groups, and Buckingham's σ_E, the linear response to electric fields from distant polar groups (with partial charges taken from the Amber or CHARMM force field commonly used for proteins). ^{1}H secondary structure shifts are the differences between the corresponding chemical shifts in the native protein and its unstructured random coil reference state. The α and β proton shifts have been found to be generally useful in detecting helix formation, or identifying helical structures in isolated fragments of some proteins, and in characterizing protein-folding intermediates.

A comprehensive review of attempts to relate NMR chemical shifts in proteins to structure may be found in ref. [43]. Case has recently calculated the shielding of ^{1}H in a CH_4 molecule placed in various positions next to each of the aromatic amino acids, and CH_4 next to various nucleic acid bases and found that the ^{1}H shielding results can be fitted to the sum of ring-current magnetic anisotropy plus a linear response to the electric field [42]. The linear electric-field response parameter A which fits the results is 46.1–58.8 ppm au, depending on the model used for representing the ring current. This is the same order of magnitude as the *ab initio* value of A for a CH_4 molecule in a static electric field, 80.2 ppm au [8]. The two parameters, the ring-current intensity factor and the A value could have been determined independently from each other if he had also calculated the magnetic anisotropy separately (the shielding at the points where the protons are located without the CH_4 molecule) and subtracted out this value in each case to account for the linear electric-field response.

The major outcomes of these three papers [B40, B41, B54] are the insight and the theoretical framework in which all medium effects on shielding can be interpreted. All NMR chemical shifts are measured in systems where there are medium effects (except for the extremely few instances of molecular beam magnetic resonance experiments). Thus, the interpretation of observed chemical shifts is always inextricably linked with medium effects as described by these papers. Furthermore, the medium effects on the NMR chemical shift are used widely as probes to infer a multitude of environments,

to answer such questions as the following. Is the Na^+ ion inside or outside a biological cell? Is the molecule adsorbed inside a zeolite catalyst or on the outside surface? How big are the cavities in the microporous materials in which the Xe is adsorbed? Do the Xe chemical shifts indicate that the Pt atoms on the zeolite-support are covered with H_2 or not? Is a phase transition occurring? The chemical shift in a molecule dissolved in a thermotropic liquid crystal exhibits characteristic behavior, predictable from paper [B41], as it passes through the isotropic, nematic, and various smectic phases with decreasing temperature [44]. The routine use of NMR chemical shifts in structural studies, including the study of metal–ligand interactions, the analysis of drug-substrate binding and catalysis, the action of drugs on membranes [45], the study of folding/unfolding pathways, as well as the characterization of the three-dimensional structure of biopolymers, depend on an understanding of the effects of the environment on the NMR chemical shift. The answers to the questions (*i.e.*, the desired information) are intimately connected with the interpretation.

Nearly forty years later, these papers continue to provide the framework in which such chemical shifts are interpreted. Their gift to us is the general way of thinking about an observed molecular electronic property (especially a local property like NMR shielding) as a probe of the environment of the molecule. By using the Buckingham view of additive contributions, each of which is amenable to some level of calculation, using *ab initio* for some, semi-empirical, or classical models for others, the desired information about the environment may be obtained. These ideas have so permeated our way of thinking that sometimes we invoke them without being conscious of where they all started.

Finally, a few words about the co-authors of papers [B41] and [B54]. The work was done at the National Research Council of Canada in Ottawa, where David Buckingham was a regular Summer Visitor during his years in Oxford. W.G. Schneider and H.J. Bernstein are, of course, co-authors of the well-known book on NMR with John Pople. Bill Raynes performed the experiments and, after a post-doctoral period with G. Wilse Robinson at CalTech, joined David Buckingham's group in Oxford as a postdoc. He is very active in many aspects of NMR chemical shifts, in particular the electric-field effects. Ted Schaefer is professor at the University of Manitoba. Bill Schneider, who was a tennis partner of David's in the early 1960s, became President of NRC and is now living in Ottawa in retirement. Harold Bernstein (now deceased) performed important work on Raman spectroscopy (including resonant Raman effects), as well as NMR.

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