Dipoles localized at helix termini of proteins stabilize charges

( electrostatic energy/dielectric effect in proteins/helix termini dipole)

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ABSTRACT The charge-stabilization effect associated with α-helices in proteins has been reexamined by microscopic calculations without any a priori assumptions about the dielectric constant of the protein. The calculations reproduce the observed charge stabilization effect of a helix in two well-defined test cases: a histidine residue situated at the C-terminal end of a helix in barnase and the sulfate ligand located near the N-terminal end of a helix of the sulfate-binding protein. They also show that the effective dielectric constant for helix-charge interactions is much larger than previously assumed and that the stabilizing effect of the helix is not associated with the helix macrodipole but rather with a few localized dipoles confined mostly to the first turn of the helix. It is predicted that mutations at one end of the helix should have very small effects on the stabilization of charges at the opposite terminus. It is pointed out that the relatively short-ranged effect of the helix is essentially similar to other cases in which localized dipoles play key roles in electrostatic stabilization.

The fact that α-helices of typical lengths found in proteins possess a substantial permanent dipole moment attracted the attention of scientists some time ago (1, 2), because it pointed toward possible functional roles of the helix dipole field. It has been suggested that the electrostatic field that results from the dipole moments of the individual peptide groups in a helix can be approximated by the field produced by two charges of opposite sign separated by the length of the helix (1, 2) (namely, the dipole pole). A subsequent study by Ward (3) demonstrated that α-helices did frequently occur so as to favor the interaction with ionic species.

The suggestion that the helix macrodipole can play a major role in ligand binding and catalysis has been widely accepted perhaps because of the structural significance of α-helices. However, it is difficult to see why the effect of a giant helix dipole would not be largely shielded by the dielectric of the surrounding environment as is the case with many charge–charge interactions in proteins (4–7). In particular, the magnitude of the charge stabilization caused by helix dipoles cannot be assessed by using Coulomb’s law and a small dielectric constant since this will lead to unrealistically large effects (4). It is thus important to determine the magnitude and range of the effect of helix dipoles without relying on any a priori assumptions regarding protein dielectric constants.

A quantitative examination of charge stabilization due to interaction with helices was reported by Fersht and coworkers (8) in a study of the small enzyme barnase. In this case, it was observed that the pKₐ of His-18, situated at the C terminus of a helix, was shifted upward to 7.9, which corresponds to stabilization of the protonated form (relative to water) by about 2.1 kcal/mol (1 cal = 4.184 J). Similar energetics were obtained in related experiments (9).

In light of these studies, the effect of the helix appears much smaller than that expected from a corresponding macrodipole in a low-dielectric medium. For example, in the pioneering study of ref. 2, an effective dielectric constant (ɛₑₗ) of 2 was considered to be a reasonable approximation for estimating the helix effect. Using this dielectric constant in barnase would give 20 kcal/mol for the stabilization of His-18 rather than 2 kcal/mol. Furthermore, the helix effect may be due only to the amide dipoles at the end of the helix (e.g., the H bonds in the last turn) rather than to the entire macrodipole. It seems that previous model calculations have drastically overestimated the helix effect (e.g., refs. 1, 2, 6, and 10–12) and that its origin cannot be conclusively deduced from these studies. In fact, the main issue is not whether the effect of the amide dipoles can be represented by a macrodipole, but how to model the dielectric properties of the system correctly. Assuming a particular value for the effective dielectric “constant” in a macroscopic continuum model may lead to incorrect conclusions, if this value does not reflect the actual situation on the molecular level (4), which we will refer to as the microscopic situation.

Here we reexamine the macrodipole concept by microscopic calculations. As test cases we have chosen barnase, mentioned above, and sulfate-binding protein (SBP), which binds SO₄⁻ in a buried site with a binding energy of 9.3 kcal/mol (13). Also in SBP, helix–charge interactions are likely to be important, as three α-helices have their N termini oriented toward the SO₄⁻ binding site (13, 14). For both of these proteins, the overall stability of the relevant charge is fairly well reproduced by our calculations. Thus we can examine the origin of the stabilization by considering the actual magnitude of the separate contributions to this effect (these contributions are not available experimentally).

Our study demonstrates that in both cases only the first one or two turns of the helix are responsible for the electrostatic effect of the helix. As an additional probe of the range of the helix effect, we calculate the influence of a charge mutation at the distant end of an helix on the charge stabilization at the other terminus. Such a mutation is predicted to have a nearly negligible effect on the stability of the charged group.

Microscopic Evaluation of Electrostatic Energies

To examine the effect of helices on stability of charged groups, the electrostatic contributions to the free energy of the relevant groups should be evaluated. These contributions may be estimated, in principle, by several strategies ranging from free-energy perturbation methods (15, 16) to the simplified microscopic protein dipoles/Langevin dipoles (PDLD) model (4, 17) and discretized macroscopic models (6, 7, 18). The main point is, however, that one should try to

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include the effect of the entire environment (protein and solvent) in the calculations.

Since the study of the helix effect involves very large systems with complicated boundaries, it is important that a large region of the protein comprising the relevant helices be described by the surrounding medium. Furthermore, since we wish to examine long-range electrostatic effects, it is not warranted to use any cutoff radius beyond which the interactions are truncated. This makes free-energy perturbation approaches exceedingly time consuming, making it necessary to use simplified solvent representations, such as in the PDLG (17) or discretized continuum methods (6, 7, 18). The calculations reported in this paper were carried out using the PDLG approach as implemented in the program POLARIS (19).

As the PDLG model has been described elsewhere (17), only its main features will be pointed out here. The model takes as a starting assumption that the electrostatic energy calculated for the crystallographic (average) structure is a good approximation for the electrostatic free energy of the system. The contributions from groups of the protein to the electrostatic energy are determined on a microscopic level, taking both permanent and induced protein dipoles into account. The solvent around the protein is represented by a grid of dipoles whose average polarization follows the Langevin formula. The polarization energy and orientation of the induced protein dipoles, as well as of the Langevin dipoles, are calculated and updated consistently. Since the present problem involves large permanent dipole moments, it is important to include the field from the Langevin dipoles when calculating the induced polarization energy. The effect of structural relaxation upon charging in a given group (20) is taken into account by considering the corresponding change in interaction among the protein dipoles (in addition to the interaction between these dipoles and the charge). The total electrostatic free energy is thus obtained as the sum of contributions from the permanent (\(V_{Q\text{p}}\)), induced (\(V_{Q\text{d}}\)), and solvent (\(V_{\text{Igm}}\)) dipoles, a possible relaxation term (\(V_{\text{reld}}\)), and a bulk term (\(V_{\text{bulk}}\)).

In the calculations on barnase, a Langevin dipole grid with a 25-Å radius centered on His-18 was used, whereas for SBP we used a larger sphere of 35 Å centered 7 Å from the SO\(^{3}\) ion on the axis of the \(\alpha_{s}\)-helix. In both cases the grid spacing was 3 Å and Boltzmann averaging over 50 slightly different grid origins was performed.

**His-18-\(\alpha_{s}\)-Helix Interaction in Barnase**

The x-ray structure of barnase (21) is shown in Fig. 1, where the side chain of His-18 is located at the C terminus of the \(\alpha_{s}\)-helix, which is approximately 20 Å long. Both the helix and the His-18 ring are fairly exposed to the solvent. This reemphasizes the point that a proper representation of the solvent polarization is essential for modeling the energetics of the system. The shift in pK\(_{a}\) of His-18 relative to its value in solution is approximately given by (4)

\[-2.3RT(\Delta pK_{a}) = \Delta \Delta G_{\text{sol}}^{\omega} = \Delta \Delta G_{\text{sol}}^{\omega}(\text{His}^{\text{+}}) = \Delta \Delta G_{\text{sol}}^{\omega}(\text{His} \rightarrow \text{His}^{\text{+}}) = \Delta \Delta G_{\text{sol}}^{\omega}(\text{His} \rightarrow \text{His}^{\text{+}}), \]

where \(\Delta \Delta G_{\text{sol}}^{\omega}(\text{His} \rightarrow \text{His}^{\text{+}})\) is the difference in solvation free energy between the charged and uncharged forms in the given medium. The calculated value of \(\Delta \Delta G_{\text{sol}}^{\omega}\) is \(-53.8\) kcal/mol, which is in reasonable agreement with experimental estimates (4, 22). The corresponding quantity for His-18 in barnase, decomposed as \(\Delta \Delta G_{\text{sol}}^{\omega} = \Delta V_{Q\text{p}} + \Delta V_{Q\text{d}} + \Delta V_{\text{Igm}} + \Delta V_{\text{reld}}\), is \(-38.9\) \(-0.5\) – 10.5 – 6.0 kcal/mol = \(-55.9\) kcal/mol. This gives a relative stabilization, compared to water, of the charged His-18 of \(\Delta \Delta G_{\text{sol}}^{\omega}(\text{His}^{\text{+}}) = -2.1\) kcal/mol, which is in good agreement with the experimental observation (8). Note that the contribution from the \(\alpha_{s}\)-helix backbone to \(\Delta V_{Q\text{p}}\) amounts to about 90% of the total value, which suggests that almost all of the stabilizing effect is due to the interaction with the helix.

In considering the range of the helix interaction, we start with an oversimplified approach that will illustrate the problems associated with the definition of the helix effect. In this procedure we neutralize successively the partial charges of the helix backbone. The corresponding results are given in Fig. 2a, where the enumeration, \(x\), of the abscissa denotes the number of helix half-turns, starting from the N terminus (here two peptide groups are equal to half a turn), that have been neutralized. Thus \(x = 0\) corresponds to the intact helix, and with \(x = 5.5\) the entire backbone dipole is neutralized. The resulting curve in Fig. 2a (solid squares) may at first seem somewhat surprising since it might suggest that the overall effect of the helix dipole moment is about 9 kcal/mol, which is much greater than the observed value of 2.1 kcal/mol. However, the artificial procedure of replacing the backbone dipoles of the helix in a rigid protein by a hydrophobic medium does not correspond to a realistic experimental operation (e.g., by protein engineering) and cannot provide the missing stabilization of the His\(^{\text{+}}\) (see below). Nevertheless, it is instructive to estimate the effective dielectric constant for the helix–H\(^{\text{+}}\) interaction in this unrealistic system. This can be done by dividing \(\Delta \Delta V_{Q\text{p}}(x = 0 \rightarrow x = 5.5)\) by \(\Delta \Delta G_{\text{sol}}^{\omega}(x = 0 \rightarrow x = 5.5)\). The resulting dielectric constant represents only the effect of the helix partial charges on the stability of the protonated histidine, assuming that one could “turn off” these dipoles without producing any other changes in the system. With this unphysical definition, we obtain the value \(\varepsilon_{\text{eff}} = 4\) (where \(\varepsilon\) corresponds to the above “hydrophobic” substitution), which indeed is a rather low number. This does not mean that the pK\(_{a}\) of His-18 would be shifted.

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**Fig. 1.** Stereoview of the crystallographic structure of barnase (21). The side chain of His-18 can be seen at the C terminus of the \(\alpha_{s}\)-helix.
Fig. 2. (a) Calculated electrostatic stabilization of the charged His-18 as a function of the length of the α1-helix relative to the intact helix. Solid squares, case where the helix backbone partial charges are neutralized; solid triangles, case where the helix and its side chains are deleted from the protein. x is number of helix half-turns neutralized or deleted, starting from the N terminus. (b) Decomposition of the total electrostatic free energy (solid squares) of the charged histidine into different contributions, $\Delta V_{\text{tot}}$ (solid triangles) is the contribution from the permanent dipole-charge interaction, $\Delta V_{\text{lev}}$ (open triangles) is the Langevin dipole energy term, $\Delta V_{\text{ind}}$ (open squares) is the induced polarization energy, and $\Delta V_{\text{bulk}}$ is the solvation term due to the surrounding (infinite) solvent with a dielectric constant of $\varepsilon = 80$.

by 7 pK_a units if the "hydrophobic helix" should somehow be created but, rather, indicates that the corresponding protein would not be stable. We are hence missing an energy term related to the relaxation or unfolding of the "hydrophobic helix" structure.

In view of the above discussion and previous studies (4, 5), it seems clear that the value of the effective dielectric constant for a given property depends on the operational definition of the given system and its surroundings. When exploring the spatial range of the helix interaction, one faces a major problem in defining the helix effect and the corresponding $\varepsilon_{\text{eff}}$. Many such definitions are possible but only a few will correspond to a physically measurable situation. Perhaps the simplest consistent definition corresponds to an experiment where the helix is being gradually deleted. In the absence of such an experiment, we may try to perform it by a computer simulation approach, expecting that the energy loss upon removal of the helix should be similar to the observed stabilization of the charge near the helix edge. This is a reasonable approximation if the stabilization effect is really due to the helix. Thus, we will now define the effect of the helix by a actually shortening it, deleting not only the partial charges but also the atoms of the helix. In this way the helix (including side chains) is successively being cut out from the rest of the protein allowing water molecules to penetrate to the newly formed cavities. Realistic calculations should also consider the subsequent protein relaxation to accommodate the modified helix (see below) but as a first step one may start with a rigid protein. The result of such calculations are depicted in the lower curve (solid triangles) of Fig. 2a. As expected, the free-energy difference, $\Delta \Delta G_{\text{tot}}(x = 0 \rightarrow x = 5.5) \approx 3.9$ kcal/mol, now becomes smaller than for the "hydrophobic helix" case yielding $\varepsilon_{\text{eff}} \approx 9$. This result is more reasonable but it does not still converge to the value of 2.1 kcal/mol. Again, this should be interpreted as a consequence of missing relaxation energy. However, the relative contributions to $\Delta \Delta G_{\text{tot}}$ as a function of the helix length are similar for both procedures [as we shall see below, they are also similar to those obtained with a macroscopic model (2)]. The last turn accounts for 70–80% of the overall charge stabilization and, with the last two turns intact, about 95% of the total effect is retained.

Fig. 2b depicts the various energy components as a function of the helix length for the case where only the dipole charges are neutralized. It can be seen that $\Delta V_{\text{tot}}$ is almost entirely due to the helix backbone dipoles. Since this quantity (with the proper dielectric screening) is the source for stabilizing the charged histidine relative to water, we conclude that it is the $\alpha$-helix or, more precisely, the last one to two turns that cause the pK_a shift of His-18. Fig. 2b also demonstrates how the loss in $\Delta V_{\text{tot}}$ ($\approx 35$ kcal/mol) is to large extent compensated by increased water solvation as the helix charges are turned off. However, a more complete compensation yielding a larger $\varepsilon_{\text{eff}}$ is provided when the helix is actually being deleted rather than just neutralized.

SO$_4^{2-}$-$\alpha_{14}$-Helix Interaction in SBP

Part of the crystal structure of SBP (14) is shown in Fig. 3 where the three helices, $\alpha_{14}$, $\alpha_{14}$, and $\alpha_{1}$, can be seen with their N termini pointing toward the ion-binding site. In this

Fig. 3. Stereoview of the SO$_4^{2-}$ binding site in SBP (14).
case, not only the three α-helices but also the side chains and the NH groups (14) provide dipoles that stabilize the buried oxydianion. The SBP system is nevertheless interesting for probing the influence of helix dipoles, especially since the α_{4ν}-helix is unusually long, extending almost 30 Å away from the SO_{4}^{2-} binding site.

As a first check of the SBP–SO_{4}^{2-} interaction, we calculated the binding free energy of the ion, \( \Delta G_{\text{SBP}}^{\text{bind}} = \Delta G_{\text{SBP}}^{\text{sol}}(\text{SO}_{4}^{2-}) - \Delta G_{\text{HB}}^{\text{SO}}(\text{SO}_{4}^{2-}) \). For small multiply-charged ions such as SO_{4}^{2-}, unrealistically small atomic radii are required to reproduce the observed solvation energies with a Langevin dipole model. This is due to the difficulties of using dipole models for the first solvation shell of multiply-charged ions. We therefore evaluated the solvation energy of SO_{4}^{2-} in water by including a shell of 16 explicit molecules around the ion and surrounding this system by the PDLG grid (in the protein the ion is completely buried and the above problem does not arise). The orientations of these water molecules were obtained by energy minimizations. The nonbonded parameters for the water–oxygen interactions were taken from ref. 20. The calculated value for \( \Delta G_{\text{sol}}^{\text{SO}}(\text{SO}_{4}^{2-}) = -292.0 \) kcal/mol [in reasonable agreement with experimental estimates (22)]. The corresponding solvation energy in the protein was \( \Delta G_{\text{SBP}}^{\text{sol}}(\text{SO}_{4}^{2-}) = -305.1 \) kcal/mol. Thus, the calculated binding energy for SO_{4}^{2-} is \( \Delta G_{\text{SBP}}^{\text{bind}}(\text{SO}_{4}^{2-}) = -13.1 \) kcal/mol and is in reasonable agreement with the observed energy \( \Delta G_{\text{obs}} = -9.3 \) kcal/mol (13).

By using the same procedures as for barnase, we examined the range of the α_{4ν}-helix–SO_{4}^{2-} interaction in SBP. Here again we started with the oversized approach, transforming the polar helix into a hydrophobic one by neutralizing the backbone dipoles. The results of these calculations are given by the solid-square curve in Fig. 4. The overall trend for the SBP α_{4ν}-helix is very similar to that found in barnase. Again, the first turn is responsible for about 80% of the total helix effect. Also for SBP, the apparent effect of neutralizing the backbone dipoles is much larger than the actual free energy of binding SO_{4}^{2-}. In this case, the effective dielectric constant associated with the transformation is \( \varepsilon_{\text{SBP}} = 2.2 \). The difference between the \( \varepsilon_{\text{SBP}} \) values in barnase and SBP is due to the helix in barnase being accessible to the solvent, whereas the one in SBP is shielded.

Next we repeated the helix-cutting procedure used for barnase for the α_{4ν}-helix in SBP. In this case, deleting the entire helix only produces a hole or “channel” inside the protein without exposing the ion-binding site to bulk solvent. Hence, only a small number of waters will be able to compensate for the missing helix (in contrast to the barnase case, where the charged group became more accessible to bulk solvent upon deleting the helix). Therefore, the result of deleting the entire SBP helix only increases \( \varepsilon_{\text{eff}} \) by a small amount. This indicates that the helix-depleted protein is still unstable and it is expected to undergo significant structural relaxation. To account in part for the missing relaxation, we carried out an energy minimization of the helix-depleted structure, in which the “helix channel” was filled with explicit water molecules. Of course, this type of calculation should not be regarded as an attempt to compute the actual conformation of a corresponding engineered protein, rather it serves solely as a check of the upper limit of the binding energies of the SO_{4}^{2-} in the relaxed helix-depleted protein. This procedure gave \( \Delta G_{\text{SBP}}^{\text{Helix depleted}}(\text{SO}_{4}^{2-}) \) of about \(-295 \) kcal/mol, leading to \( \varepsilon_{\text{eff}} = 11 \), which is a lower limit since the protein can be relaxed further.

**Effect of Mutations of Charged Residues at the End of Helices**

As an additional check of the range of helix–charge interactions, we introduced charge mutations at the distant ends of the helices in barnase and SBP. If the helix effect is long ranged and the effective “charge” that represents the macrodipole at the distant end is important, then a neutralization of this charge by an oppositely charged group would have a large effect on a charge at the other end. If, however, the conclusions of Figs. 2 and 4 are correct then the effect of a new charge will be small.

For barnase we calculated the pK_{a} of His-18 with and without Asp-8 being ionized. Asp-8 is located about 16 Å from His-18, close to the helix N terminus. Similarly, we examined the free energy of binding SO_{4}^{2-} to SBP with and without a net positive charge on His-147, which is located near the C terminus of the α_{4ν}-helix some 25 Å from the ion-binding site. The calculated effect of the charge state of Asp-8 on the pK_{a} of His-18 is \( \Delta G_{\text{SBP}}^{\text{Helix depleted}}(\text{His} \rightarrow \text{His}^+) - \Delta G_{\text{SBP}}^{\text{Helix depleted}}(\text{His} \rightarrow \text{His}^-) = -0.2 \) kcal/mol. For SBP, the calculated effect on ion binding produced by charging His-147 is \( \Delta G_{\text{SBP}}^{\text{Helix depleted}}(\text{SO}_{4}^{2-}) - \Delta G_{\text{SBP}}^{\text{Helix depleted}}(\text{SO}_{4}^{2-}) = -0.3 \) kcal/mol. These numbers are quite small indeed and agree with the proposal that the distant ends of helices do not significantly affect the stability of charged species at the other end. It might also be possible to examine these predictions experimentally.

**Discussion**

Although the effect of an α-helix on a charge near its terminus is clearly real (see, e.g., ref. 3), the origin of this effect has been obscured by the difficulties in accounting for the relevant observed energies by theoretical models. The study in ref. 2 predicted effects of the order of 10–20 kcal/mol whereas the corresponding observed magnitudes are typically less than 2 kcal/mol. Similar problems appeared in a recent study (12) that examined the effect of the helix by a free-energy perturbation method without including the solvent. This work attempted to account for the effect of the solvent by a distance-dependent dielectric constant and drastically overestimated the helix interaction energy. It thus appears that the key question about the effect of the α-helix is associated with the value of the dielectric constant, which, of course, cannot be resolved by assuming it from the beginning. A more consistent study, which represented the solvent effect by the discretized continuum model, was reported in ref. 6. However, this model also overestimated the helix effect, giving 15 kcal/mol for a case where the helices appear to contribute about 2 kcal/mol. A similar overestimation has appeared in the calculations of the relevant pK_{a} shift.
It seems to us that the magnitude and range of the helix dipole effect must be examined by models that reproduce the observed energetics in well-defined test cases. Herein, we have considered the stabilization of charged species near helix termini in barnase and SBP. Our calculations, which were performed using microscopic concepts (and which do not require any assumptions about the dielectric properties of the system), reproduced the observed stability of the relevant charge groups. Thus, we may use the model to quantitatively assess the range of the helix dipole effect. The calculations demonstrate that the first turn of the helix accounts for about 80% of the overall effect of the helix whereas the first two turns account for 95% of this effect. This conclusion appears, in fact, to be rather independent of the approach used (see Fig. 4), and was also reached previously using a macroscopic dielectric constant (2). However, the unrealistically large interaction energies predicted in earlier theoretical studies of this effect made it difficult to assess its validity. In fact, judging from the literature, it appears that the short-range nature of the helix effect has not been fully realized nor appreciated, perhaps because the often used term "helix macrodipole" was given to the two turns of the helix which are believed to contribute significantly to the overall effect.

To further verify our conclusion about the nature of the helix effect, we calculated the effect of a charge mutation at the distant end of the helix and found it to be negligible. Such a mutation would have neutralized the "macrodipole charge" at the end of the helix, leading to a large change in the long range effect expected by some from the macrodipole model. Thus the negligible influence indicates that the helix effect is indeed rather short ranged. In this context, experimental examination of our predicted effects of charge mutations in barnase and SBP could serve as a convincing test.

In view of the above considerations, it is important to reconsider the hypothesis that the helix macrodipole provides a key catalytic advantage in enzymes. Apparently, the localized microscopic dipoles at the end of the helix are the main source of its charge stabilization effect (e.g., by providing H bonds). This "helix-edge" effect is basically the same type of effect associated with other localized dipoles, e.g., as in the oxyanion site of trypsin or related sites in other systems (4, 14, 23, 24). As argued previously, proteins can store significant electrostatic energy in preoriented dipoles (4, 23) and a helix edge is one of the ways to orient localized microscopic dipoles toward a given site.

The present study provides insight into the dielectric properties associated with helix dipoles. That is, the observed effect of an a-helix on a charged group near the terminus is rather small. However, if the effect of the backbone charge distribution is evaluated by using a nonpolar helix as a reference, the apparent effect is much larger. Evidently, this type of reference state does not provide a very useful definition of the helix effect (and the corresponding $\varepsilon_{eh}$), since it cannot be readily correlated with experiments. Probably our reference structure would become unstable as soon as the free energy associated with "turning off" the helix dipoles exceeds the folding free energy (for a related discussion, see ref. 4). To examine the dielectric effect associated with an actual deletion of the helix, it would be necessary to cut out the entire helix from the protein. This operation is likely to be accompanied by penetration of water into the newly formed cavity and/or partial unfolding of the structure; both effects would result in a larger effective dielectric constant than that deduced by holding the protein fixed and replacing the helix by vacuum or by a "hydrophobic cylinder."

In summary, it seems to us that the discussion of whether a macrodipole can represent the combined effect of the microscopic amide dipoles has detracted attention from the much more important question of what dielectric treatment can reproduce the observed helix effect. Models that severely overestimate the magnitude of the effect cannot really be considered reliable for structure-function correlation in proteins. For instance, a macrodipole in vacuum is basically an incorrect model for a helix inside a protein. More generally, a macrodipole without sufficiently compensating solvent effects is not a realistic model (e.g., since it will yield a large effect from charge mutations at the distant helix terminus). Models that do not consider the protein relaxation might also lead to incorrect conclusions. On the other hand, a model that involves a deletion of the helix and allows for a subsequent relaxation of the protein and the surrounding water can reproduce the observed helix effect. Using such a model has indicated that the last turn of the helix accounts for almost the entire observed effect.

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