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# Relation between peptide backbone solvation and the energetics of peptide hydrogen bonds☆

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**Abstract**

The H-bond inventory approach is used commonly to interpret data involving changes in the number or types of protein hydrogen bonds. I point out here that this approach gives an incorrect answer either for the standard free energy or enthalpy of the reaction between simple amides and water. On the other hand, an electrostatic solvation approach fits almost within error the polar solvation free energies of small molecules, including amides. The electrostatic solvation approach is used here to discuss the relation between peptide backbone solvation and the enthalpy change for forming an alanine helix.


*Keywords:* Electrostatic solvation; H-bond inventory; Enthalpy change; Alanine helix

## 1. Introduction

For a long time, it has been controversial how much peptide hydrogen bonds contribute to protein stability and even whether they are stabilizing or destabilizing. A commonly used approach is to take an inventory of protein–water H-bonds made by the unfolded protein and of peptide H-bonds made by the folded protein, and then to use auxiliary data to deduce the difference between their H-bond energies, or else to argue that the difference is small and can be neglected. A newer approach is based on calculating the solvation free energy of the peptide backbone, both in the folded and unfolded forms of the protein, by using an electrostatic algorithm and parameters that have been calibrated from experimental polar solvation free energies of small molecules. The two approaches are compared here first by applying them to experimental data for the standard free energy and enthalpy of polar solvation of amides, and then to interpret the enthalpy of alanine helix formation.

## 2. Early studies of models for the peptide H-bond in water

John Schellman's 1955 model [1] for the stability of an α-helix in aqueous solution proposes that

☆ This paper is dedicated to John Schellman, my close friend and long-time scientific counsellor.

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helix formation should be driven by the net enthalpy of the peptide H-bond, with the net ΔH being approximately −6.3 kJ/mol per H-bond. This value is based on interpreting heat of dilution data for urea solutions in terms of putative urea dimers, and assuming that the CO and NH groups also form competing H-bonds to water. The change in backbone conformational entropy, which opposes helix formation, lies between −13 and −29 J/mol K per residue in Schellman's model, based on low and high estimates for the number of backbone conformations available in the coil form. The model includes an end effect, a stability deficit, arising primarily from 4 unmade peptide H-bonds at the helix ends, plus a backbone entropy bonus equivalent to one unconstrained residue, arising from a free backbone bond at each end of the helix. The overall result is that short peptide helices are predicted to be either marginally stable or else unstable in water. Specific interactions between pairs of side chains were found later to be an important factor contributing to helix stability [2,3]; they are not included in the model.

Schellman's proposal that helix formation is driven by peptide H-bonds was adopted in both the Zimm–Bragg [4] and Lifson–Roig [5] treatments of helix formation by statistical mechanics. Klotz and Franzen [6] later used the dimerization of *N*-methylacetamide as a model system for determining the strength of the peptide H-bond. They found that the dimerization reaction is too weak to measure and afterwards the energetic status of the peptide H-bond in protein folding has been obscure and controversial [7–9].

The discovery by Marqusee et al. [10] that alanine-based peptides form moderately stable helices in water without the aid of helix-stabilizing side chain interactions gave hope that alanine helices would reveal the energetic role of peptide H-bonds. Of the 20 naturally occurring amino acids, only alanine forms a stable helix in water without the aid of side chain interactions [11]. Some polar residues are needed to make alanine helices water soluble, but as few as two ornithine residues at either end of a 13-residue alanine sequence will suffice [12]. Because alanine has only a $–CH_3$ group for a side chain, its ability to form a stable helix in water indicates that the helix backbone itself is stable, in agreement with Schellman's model. Moreover, alanine helix formation is enthalpy-driven with $\Delta H = -3.8 \pm 0.4$ kJ/mol per residue [13]. The favorable enthalpy change has been measured recently as a function of temperature by isothermal titration calorimetry; the results show that ΔCp is small and ΔH is essentially independent of temperature [13]. The ΔH found by isothermal titration calorimetry [13] agrees satisfactorily with values from some older studies by differential scanning calorimetry [14,15] in which it was not possible to separate the heat effects arising from ΔCp vs. ΔH.

## 3. Enthalpy balance for the alanine helix found from the hydrogen bond inventory

The enthalpy change for forming an isolated peptide H-bond in the gas phase is −28 kJ/mol, according to a recent ab initio quantum mechanics study [16]. Other quantum mechanics calculations indicate that the enthalpies of amide–amide, amide–water, and water–water H-bonds are close to each other, within ±4 kJ/mol [17]. Thus, the modest enthalpy change found for unfolding the alanine helix (3.8 kJ/mol residue) might reasonably be attributed to the success of the hydrogen bond inventory approach [18], which predicts there should be only a small free energy difference when peptide H-bonds are made. In using the H-bond inventory approach, the exchange of H-bond partners on forming the helix is written

$$NH\bullet\bullet\bullet W + CO\bullet\bullet\bullet W \rightleftharpoons NH\bullet\bullet\bullet OC + W\bullet\bullet\bullet W \qquad (1)$$

where W represents water and the peptide CO group is assumed to make only one H-bond to water. The enthalpy of the water–water H-bond in liquid water at 25 °C is −22 kJ/mol, as estimated from dividing the heat of vaporization of water by two, for two H-bonds per water molecule [19]. Using this value in the H-bond inventory together with the measured enthalpy of alanine helix formation (−3.8 kJ/mol), gives the sum of the two enthalpies of forming the NH•••W and CO•••W H-bonds as −28−22+4=−46 kJ/mol. This estimate agrees satisfactorily with a quantum mechanics study indicating that the individual H-

bond energies of the NH•••Wand CO•••W H-bonds lie between −20 and −25 kJ/mol [17].

## 4. Standard free energy and enthalpy of polar interaction of *N*-methylacetamide with water

There is a standard approach in physical chemistry for measuring the solvation free energy of a solute. Equilibrium is established as the solute is transferred from the vapor phase to the liquid, and the Gibbs free energy of transfer is obtained from the distribution coefficient of the solute between the gas and liquid phases. The transfer enthalpy can be measured calorimetrically. There is a large difference in the free energy of liberation of the solute in the gas phase vs. the liquid phase. It affects particularly the entropy of vaporization of the solute, and it opposes transfer from the gas to the liquid phase; it must be taken into account. There is also a small difference in the enthalpy of liberation of the solute between the two phases. The thermodynamics of solute liberation can be eliminated, when using the transfer energetics to obtain the energetics of solvation of the polar groups, by using standard states of 1 mol/l in both the gas and liquid phases [20]. In doing this, it is convenient to treat the gas as being ideal in the 1 M standard state [21]—see equations 11 and 13 of [21].

When determining the energetics of the interaction between water and the solute's polar groups, i.e. the polar solvation, from the transfer energetics, two other terms must be taken into account. The first term is the cavity term, which arises from the work required to make a cavity in water for the solute; it is chiefly entropic—see [21]. The second term, arising from the van der Waals interactions between the solute and water, is enthalpic.

The sum of the cavity and van der Waals terms has been evaluated approximately by using data for uncharged hydrocarbons, whose polar solvation is zero, by Sitkoff et al. [22] and also independently by Makhatadze and Privalov [23]. The combined correction term for the sum of the cavity and van der Waals terms is taken from the transfer free energy of an alkane with the same solvent-accessible surface area (ASA) as the polar molecule. (Interpolation is made in a linear plot of transfer free energy vs. ASA.) Thus, the combined correction term is assumed to depend only on ASA. Likewise, the enthalpy of polar solvation is obtained from the calorimetrically measured transfer enthalpy by subtracting a combined correction term [24] given by transfer data for alkanes. The error in assuming that the cavity term depends only on ASA is believed to be small [24], whereas the error involved in approximating the van der Waals term by this assumption has not yet been evaluated.

Table 1
Data for the standard free energy and enthalpy of polar solvation of amides, and comparison with calculated free energies

| Amide | $-\Delta G^{oa}$ | $-\Delta H^{a}$ | $-\Delta G_{ESF}{}^{a}$ |
|---|---|---|---|
| Acetamide | 48.7 | 48.7 | 49.2 |
| *N*-methyl″ | 51.2 | 48.1 | 51.0 |
| *N*,*N*-dimethyl″ | 45.4 | 43.1 | ND |
| Propionamide | 48.1 | 50.4 | 48.4 |

[a] Experimental values of standard free energy ($\Delta G^{o}$), enthalpy ($\Delta H$) and calculated values of standard free energy ($\Delta G_{ESF}$), in units of kJ/mol, for polar solvation, the interaction between water and the polar groups of the amide. Values are reprinted from [24] and refer to 25 °C. Experimental values have been obtained from the measured transfer free energy or enthalpy by correcting to a standard state of 1 mol/l in both the gas and liquid phases [20,21], and by subtracting the cavity and van der Waals terms with the use of a combined correction term based on hydrocarbon data at the same value of ASA [22].

The overall transfer free energy of an amide can be measured directly in a distribution experiment by using isotopic labeling to provide sensitive detection of the solute in the gas phase [25]. The transfer enthalpy is obtained by combining results from two experiments, in which the heat of vaporization of the liquid amide and its heat of solution in water are measured [26]. Polar solvation enthalpies for 4 amides in water, obtained previously [24], are given in Table 1. The polar solvation enthalpy for *N*-methylacetamide (NMA) is −48.1 kJ/mol and the average enthalpy of polar solvation for the 4 amides in Table 1 is $-47.6 \pm 3.1$ (S.D.) kJ/mol.

Note the following two properties of the polar solvation of amides shown in Table 1. First, the enthalpy and free energy of polar solvation are nearly equal, as reported [24], so that the entropy

change when water interacts with an amide must be small. At first sight, this is surprising but it is known that the entropy changes accompanying the formation of hydrates of inorganic salts are small [27]. Moreover, the Born model, which interprets polar solvation by comparing the work of charging a sphere in vacuum vs. in water, predicts that the free energy of polar solvation should be almost entirely enthalpic if the solvent is water [28]. Second, the enthalpies of polar solvation are nearly the same for the 4 amides in Table 1, and so are the free energies of polar solvation. Neither the enthalpy nor the free energy is well correlated with the solvent-accessible surface area of the polar groups, which differs substantially among the 4 amides [24]. A main point from Table 1 is that the results for NMA are typical for amides and are not exceptional. Another main point is that the close agreement between the experimental enthalpy and standard free energy of polar solvation, which are measured by independent methods, indicates that the ASA-dependent correction for the sum of the cavity and van der Waals terms is reasonably reliable, because the correction is quite different for the enthalpy vs. the standard free energy [24]. As mentioned above, the Born equation can be used to show that the standard free energy is closely equal to the enthalpy if the solvent is water [28].

## 5. H-bond inventory for the interaction of *N*-methylacetamide with water

The measured enthalpy for the interaction of NMA with water may be compared with the value predicted from an H-bond inventory as follows. An exchange of H-bond partners occurs as the unsolvated amide (Am) in the gas phase (g) enters aqueous solution (aq) and forms H-bonds to water with its NH and CO groups. If liquid water at 25 °C is completely H-bonded, as it is in the random network model of water [29], and if the amide NH and CO groups both make only single H-bonds to water, then dissolving the amide in water disrupts one water–water H-bond and there is a net gain of one H-bond.

$$\mathrm{HN{-}Am{-}CO\ (g) + W{\bullet\bullet\bullet}W\ (aq)} \rightleftharpoons \mathrm{W{\bullet\bullet\bullet}HN{-}Am{-}CO{\bullet\bullet\bullet}W\ (aq)} \quad (2)$$

The two H-bonds between water and the NH and CO groups have a total enthalpy change of −46.0 kJ/mol, according to the enthalpy balance for the alanine helix (see above), and the enthalpy of the water–water H-bond is −22 kJ/mol (see above). Thus, the predicted enthalpy of polar solvation of NMA is $-46+22=-24$ kcal/mol, whereas the observed value is −48.5 kJ/mol, a two-fold error. This discrepancy indicates that something is seriously wrong with applying the H-bond inventory to the interaction between water and the polar groups of NMA. Whatever is wrong in this simple case is likely also to be wrong when the H-bond inventory is applied to the more complex problem of forming the alanine helix.

## 6. Electrostatic solvation of *N*-methylacetamide in water

Most of the problems and successes of using electrostatic models to interpret the polar solvation energetics of small molecules in water can be illustrated by using the Born model to interpret the solvation enthalpies of simple ions [28], which are reliably modeled as spheres. The solvation enthalpies can be measured easily and accurately by calorimetry, although a reference value must be assigned arbitrarily to one ion, usually the hydrogen ion. The measured enthalpy is huge for real ions, even monovalent ones: the solvation enthalpy of NaCl is −770 kJ/mol [28]. A major problem in applying the Born model to solvation is the treatment of water as a continuum solvent, which neglects both the polarization of water dipoles close to the ion (dielectric saturation) and hydrogen bonding between water and the ion. Nevertheless, the solvation enthalpies of simple anions and cations can be fitted with good success if the ionic radius is carefully defined and if the ions considered do not form chemical complexes with water [28].

For molecules more complex than simple ions, methods of performing the electrostatic calculation are still being developed and tested. They have in common the assignment of partial charges on the NH and CO groups as key parameters determining the polar solvation free energies of amides. The FDPB (finite difference Poisson–Boltzmann)

method of Honig and coworkers [22], which uses the DelPhi algorithm and PARSE parameter set together with the ASA-dependent correction for the cavity and van der Waals terms, reproduces the hydration free energies of 67 small molecules with an average error of 0.4 kcal/mol. Because the PARSE parameter set has been calibrated against a substantial experimental data base, it may be used to predict polar solvation free energies for new compounds of the same type. The partial charges assigned in this way should be regarded, however, as empirical parameters, not to be used for other purposes, because of the continuum solvent approximation.

Two different electrostatic approaches for computing solvation free energies have been found to give similar results [30] when the same partial charges are used in both methods. The Langevin dipoles method of Warshel and coworkers [31] and the FDPB method of Honig and coworkers [22] both give free energies of polar solvation which agree within 4–8 kJ/mol for a large set of tripeptides of varying sequences and backbone conformations [30]. The PARSE parameter set used with DelPhi reproduces closely the free energies of polar solvation of the 4 amides in Table 1, as expected: the partial charges of the PARSE parameter set have been optimized for a set of model compounds that includes these amides [22].

## 7. Amide H-bonds represented by partial charges

The interaction of water with polar groups in small molecules can be represented successfully by electrostatic models based on partial charges assigned to the polar atoms [22,31,32]. Can the peptide hydrogen bond itself be described by an electrostatic model based on partial charges assigned to the N, H, O and C atoms of the peptide group? (The NH and CO groups are customarily assumed neutral, so that placing a − charge on O or a + charge on N is equivalent to placing dipoles on the CO and NH groups.). This long-standing question (see Pauling [33]) was analyzed by Lifson, Hagler and coworkers [34], who in the early 1970s undertook the development of a consistent force field for representing interactions between biological molecules. They analyzed the crystal structures of H-bonded amides and carboxylic acids, in conjunction with data such as heats of sublimation, and found that a simple potential function of the Lennard–Jones–Coulomb type successfully describes the non-bonded interaction energy between two atoms, $v(r)$, as a function of $r$, the distance separating their centers.

$$v(r) = A/r^9 - C/r^6 + q_i q_j/r \quad (3)$$

$A$ and $C$ are coefficients of the repulsive and attractive terms of the van der Waals interaction and $q_i$, $q_j$ are the partial charges on the two atoms describing their coulombic interaction.

These authors found that different bond energies and bond lengths of NH•••O and OH•••O H-bonds are a natural consequence of the different van der Waals radii of N and O. Moreover, they found that the simple potential function in Eq. (3) reproduces closely the torsion angles of the H-bonds in crystal structures, which include H-bonded dimers as well as chains of H-bonded monomers. Modern work [35,36] continues to hold open, however, the relative sizes of the contributions to the peptide hydrogen bond from covalency and from simple electrostatics, of the type shown in Eq. (3).

## 8. Electrostatic solvation approach to the enthalpy of alanine helix formation

The electrostatic solvation approach can be used to estimate the enthalpy of alanine helix formation, as indicated schematically in this cycle.

$$\begin{array}{ccc} \text{helix (g)} & \Leftrightarrow & \text{coil (g)} \\ \Updownarrow & & \Updownarrow \\ \text{helix (aq)} & \Leftrightarrow & \text{coil (aq)} \end{array} \quad (4)$$

The hydration/dehydration steps indicated by ⇕ are given by calculated values for $\Delta G_{ESF}$ of the peptide group in the helix and coil states. The approximation $\Delta G_{ESF} \sim \Delta H$ gives the enthalpy change from the calculated $\Delta G_{ESF}$. The net enthalpy change for helix(aq) → coil(aq) is known experimentally ($3.8 \pm 0.1$ kJ/mol [13]) and the enthalpy change for helix (g) ⇔ coil (g) can in principle be found by quantum mechanics calcu-

lations for the gas phase. When the enthalpy changes for all four steps of the cycle have been found, the results provide a test of the self-consistency of this approach.

There are some problems, however. The coil(aq) conformation must be known accurately before its $\Delta G_{ESF}$ can be calculated and likewise the coil(g) conformation must be known accurately before quantum calculations can be performed on it. A solution to these problems is provided, in principle, by writing out the coil conformation explicitly as an ensemble of species without peptide H-bonds whose backbone conformations are known.

$$\begin{array}{ccccccccc} \text{helix (g)} & \Longleftrightarrow & \alpha\text{(g)} & \Longleftrightarrow & \beta\text{(g)} & \Longleftrightarrow & P_{II}\text{ (g)} & \Longleftrightarrow & \bullet\bullet\bullet \\ \Updownarrow & & \Updownarrow & & \Updownarrow & & \Updownarrow & & \\ \text{helix(aq)} & \Longleftrightarrow & \alpha\text{(aq)} & \Longleftrightarrow & \beta\text{(aq)} & \Longleftrightarrow & P_{II}\text{(aq)} & \Longleftrightarrow & \bullet\bullet\bullet \end{array} \quad (5)$$

In scheme (5), α is the α-helix backbone conformation in a species without peptide H-bonds, α is the extended, i.e. β-strand, conformation, and $P_{II}$ is the backbone conformation of the polyproline II helix. The problem of determining the actual distribution of backbone conformations of alanine peptides without H-bonds is currently being studied by NMR methods (N. Kallenbach, personal communication). There is a problem in determining $\Delta G_{ESF}$ in the α-backbone conformation if the value for an interior residue in a long peptide without H-bonds is desired, because peptide H-bonds are formed when 4 or more consecutive residues are in the α-backbone conformation.

ESF calculations have been reported for some alanine peptides with either the α or β-backbone conformation in peptides without H-bonds [24][40] and for two helices with either 5 or 15 alanine residues [24]. The results provide information about steps in scheme (5). (1) The interaction with water of the peptide group in an alanine helix has $\Delta G_{ESF} = -10.5$ kJ/mol for the central residue (number 8) in a 15-residue helix [24]. Consequently, water still interacts rather strongly with the peptide group in a helix even though the peptide H-bond is formed. This finding contradicts the common assumption that there is no interaction with water once the peptide H–bond is made. Most of the interaction energy is with the peptide CO group: $\Delta G_{ESF} = -8.4$ kJ/mol [24]. (2) *N*-methylacetamide is a poor model for the interaction between water and the peptide group in the coil state, because $\Delta G_{ESF}$ is $-51.0$ kJ/mol for *N*-methylacetamide (Table 1) but is only $-33.1$ kJ/mol for an interior peptide group in a β-strand peptide [24]. (3). The energy of a helical peptide H-bond, in a solvent-exposed helix, should depend substantially on the number of neighboring helical H-bonds because of the long-range nature of the dipole–dipole interactions among the peptide NH and CO dipoles. This effect is evident from the change in the value of $\Delta G_{ESF}$ with residue number near either end of the 15–residue alanine helix [24], when considering only residues both of whose peptide NH and CO groups are H-bonded. A recent report states that the amide H-bond energies in chains of H-bonded formamide molecules depend strongly on both the length of the chain and H-bond position in the chain [37].

Known information about steps in scheme (5) can be combined to give a very approximate estimate of ΔH for alanine helix formation, by connecting the upper and lower rows of the cycle through the steps β(aq) → β(g) and helix(g) → helix(aq), and then omitting the other steps in (5), notably the steps involving α(aq) and α(g). This shortened cycle gives $+33.1 - 27.6 - 10.5 + \Delta H = 0$, or $\Delta H = +5.0$ kJ/mol for helix(aq) → β(aq), in satisfactory agreement with the measured value for helix (aq) → coil(aq) of $+3.8$ kJ/mol [13].

## 9. Concluding comments

Taking a H-bond inventory has been a standard approach to understanding the role of peptide H-bonds in protein folding [18]. Evaluating backbone solvation via an electrostatic approach provides an alternative approach to understanding peptide H-bonds. The use of an electrostatic approach to represent the polar solvation free energies of small molecules is now well established [22,31,32], although discussion continues about how to improve the calculations. Experimental solvation data for small molecules suggest that backbone solvation should be a major factor in the energetics of protein folding [23–25]. The electrostatic

approach shows by calculation how backbone solvation should affect the energetics of protein folding [16,24,30,38]. Two major predictions are: (1) the H-bonded peptide group still interacts strongly with water, in both solvent-exposed helices [24] and β-hairpins [38], and (2) non-polar side chains reduce the interaction between water and the backbone substantially, and this effect is likely to be the major cause of differences in both helix [24] and β-structure [38] propensities (see also [30,39]).

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## References

[1] J.A. Schellman, The stability of hydrogen-bonded peptide structures in aqueous solution, C.R. Trav. Lab. Carslberg Ser. Chim. 29 (1955) 230–259.
[2] R.L. Baldwin, α-Helix formation by peptides of defined sequence, Biophys. Chem. 55 (1995) 127–135.
[3] R. Aurora, G.D. Rose, Helix capping, Protein Sci. 7 (1998) 21–38.
[4] B.H. Zimm, J.K. Bragg, Theory of the phase transition between helix and random coil in polypeptide chains, J. Chem. Phys. 31 (1959) 526–535.
[5] S. Lifson, A. Roig, On the theory of helix–coil transition in polypeptides, J. Chem. Phys. 34 (1961) 1963–1974.
[6] I.M. Klotz, J.S. Franzen, Hydrogen bonds between model peptide groups in solution, J. Am. Chem. Soc. 84 (1962) 3461–3466.
[7] I.M. Klotz, Non-covalent bonds in protein structure, Brookhaven, Symp. Biol. 13 (1960) 25–48.
[8] B.A. Shirley, P. Stanssens, U. Hahn, C.N. Pace, Contribution of hydrogen bonding to conformational stability of ribonuclease T1, Biochemistry 31 (1992) 725–732.
[9] B. Honig, A.-S. Yang, Free energy balance in protein folding, Adv. Protein Chem. 46 (1995) 27–58.
[10] S. Marqusee, V.H. Robbins, R.L. Baldwin, Unusually stable helix formation in short alanine-based peptides, Proc. Natl. Acad. Sci. USA 86 (1989) 5286–5290.
[11] C.A. Rohl, A. Chakrabartty, R.L. Baldwin, Helix propagation and N-cap propensities of amino acids measured in alanine-based peptides in 40% trifluoroethanol, Protein Sci. 5 (1996) 2623–2637.
[12] E.J. Spek, C.A. Olson, Z. Shi, N.R. Kallenbach, Alanine is an intrinsic α-helix stabilizing amino acid, J. Am. Chem. Soc. 121 (1999) 5571–5572.
[13] M. Lopez, D.-H. Chin, R.L. Baldwin, G.I. Makhatadze, The enthalpy of the alanine peptide helix measured by isothermal titration calorimetry using metal-binding to induce helix formation. Proc. Natl. Acad. Sci. USA 99 (2002) 1298–1302.
[14] J.M. Scholtz, S. Marqusee, R.L. Baldwin, et al., Calorimetric determination of the enthalpy change for the α-helix to coil transition of an alanine peptide in water, Proc. Natl. Acad. Sci. USA 88 (1991) 2854–2858.
[15] J.M. Richardson, K.W. McMahon, C.C. MacDonald, G.I. Makhatadze, MEARA sequence repeat of human CstF–64 polyadenylation factor is helical in solution. A spectroscopic and calorimetric study, Biochemistry 38 (1999) 12869–12875.
[16] N. Ben-Tal, D. Sitkoff, I.A. Topol, A.-S. Yang, S.K. Burt, B. Honig, Free energy of amide hydrogen bond formation in vacuum, in water and in liquid alkane solution, J. Phys. Chem. B 101 (1997) 450–457.
[17] J.B.O. Mitchell, S.L. Price, On the relative strengths of amide...amide and amide...water hydrogen bonds, Chem. Phys. Lett. 180 (1991) 517–523.
[18] A.R. Fersht, The hydrogen bond in molecular recognition, Trends Biochem. Sci. 12 (1987) 301–304.
[19] P.W. Atkins, Physical chemistry, Fifth Ed., Freeman and Co, New York, 1996.
[20] A. Ben-Naim, Y. Marcus, Solvation thermodynamics of non-ionic solutes, J. Chem. Phys. 81 (1984) 2016–2027.
[21] B. Lee, Analyzing solvent reorganization and hydrophobicity, Methods Enzymol. 259 (1995) 555–577.
[22] D. Sitkoff, K.A. Sharp, B. Honig, Accurate calculation of hydration free energies using macroscopic solvent models, J. Phys. Chem. 98 (1994) 1978–1988.
[23] G.I. Makhatadze, P.L. Privalov, Contribution of hydration to protein folding thermodynamics. I. The enthalpy of hydration, J. Mol. Biol. 232 (1993) 639–659.
[24] F. Avbelj, P. Luo, R.L. Baldwin, Energetics of the interaction between water and the helical peptide group and its role in determining helix propensities, Proc Natl. Acad. Sci. USA 97 (2000) 10786–10791.
[25] R. Wolfenden, Interaction of the peptide bond with solvent water: a vapor phase analysis, Biochemistry 17 (1978) 201–204.
[26] G. Della Gatta, G. Barone, V. Elia, Enthalpies of solvation for *N*-alkylamides in water and in carbon tetrachloride at 25 °C, J. Solution Chem. 15 (1986) 157–167.
[27] J.D. Dunitz, The entropic cost of bound water in crystals and biomolecules, Science 264 (1994) 670.
[28] A.A. Rashin, B. Honig, Reevaluation of the Born model of ion hydration, J. Phys. Chem. 89 (1985) 5588–5593.
[29] A.R. Henn, W. Kauzmann, Equation of state of a random network, continuum model of liquid water, J. Phys. Chem. 93 (1989) 3770–3783.
[30] F. Avbelj, Amino acid conformational preferences and solvation of polar backbone atoms in peptides and proteins, J. Mol. Biol. 300 (2000) 1335–1359.
[31] J. Florian, A. Warshel, Langevin dipoles model for ab initio calculations of chemical processes in solution:

parameterization and application to hydration free energies of neutral and ionic solutes and conformational analysis in aqueous solution, J. Phys. Chem. B 101 (1997) 5583–5595.

[32] C.J. Cramer, D.G. Truhlar, An SCF solvation model for the hydrophobic effect and absolute free energies of aqueous solvation, Science 256 (1992) 213–217.

[33] L. Pauling, The Nature of the Chemical Bond, Second edition, Cornell Univ. Press, Ithaca, NY, 1940, pp. 284–327.

[34] S. Lifson, A.T. Hagler, P. Dauber, Consistent force field studies of intermolecular forces in hydrogen-bonded crystals. 1. Carboxylic acids, amides and the C=O••• H hydrogen bonds, J. Am. Chem. Soc. 101 (1979) 5111–5121.

[35] F. Weinhold, Nature of H-bonding in clusters, liquids, and enzymes: an ab initio, natural bond orbital perspective, J. Mol. Struct. (Theochem) 398–399 (1997) 181–197.

[36] J.J. Dannenberg, L. Haskamp, A. Masunov, Are hydrogen bonds covalent or electrostatic? A molecular orbital comparison of molecules in electric fields and H-bonding environments, J. Phys. Chem. A 103 (1999) 7083–7086.

[37] N. Kobko, L. Paraskevas, E. Del Rio, J.J. Dannenberg, Cooperativity in amide hydrogen-bonding chains: implications for protein folding models, J. Am. Chem. Soc. 123 (2001) 4348–4349.

[38] F. Avbelj, R.L. Baldwin, Role of backbone solvation in determining thermodynamic β-propensities of the amino acids. Proc. Natl. Acad. Sci. USA 99 (2002) 1309–1313.

[39] F. Avbelj, L. Fele, Role of main-chain electrostatics, hydrophobic effect and side-chain conformational entropy in determining the secondary structure of proteins, J. Mol. Biol. 279 (1998) 665–684.