Long-Range Interactions Dominate the Inverse-Temperature Dependence of Polypeptide Hydration Free Energies

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Direct, all-atom calculations of the free energy of hydration of aqueous deca-alanine structures — holistically including backbone and side-chain interactions together — show that attractive interactions and the thermal expansion of the solvent explain the inverse temperature signatures that have been interpreted traditionally in favor of hydrophobic mechanisms for stabilizing the structure and function of soluble proteins.

Keywords: polypeptide hydration free energies — long-range interactions — inverse temperature dependence

I. SIGNIFICANCE STATEMENT

Solution environments of soluble proteins are intrinsic to the thermodynamic stability of those aqueous macromolecular species. Only recently has molecular theory and simulation progressed to the stage that hydration free energies of soluble proteins can be evaluated holistically, including peptide backbone moieties at the same level as side-chain groups. The new results provide surprising insight into inverse temperature dependences that have been implicated in cold denaturation of these structures. Thus, these results should change our qualitative appreciation of the solution influence on the stability and function of biomolecular structures.

II. INTRODUCTION

Solution environments of soluble proteins are intrinsic to the thermodynamic stability of those macromolecular structures. Typically, a protein is divided into hydrophilic and hydrophobic moieties, then characteristic hydration free energy contributions are assigned, and assembled additively, to rationalize their solution structure, stability, and function. The hydrophilic/hydrophobic assignment is often somewhat arbitrary. But distinctive temperature dependences — so-called inverse temperature dependences [1] — that are characteristic of hydrophobic free energies support the hydrophilic/hydrophobic dichotomy. Strengthening of hydrophobic stabilization with increasing temperature in a physiological range is a simple example of inverse temperature behavior, and viewed in the decreasing temperature direction rationalizes cold denaturation [2]. Nevertheless, Klotz pointed-out some time ago [3] that inverse temperature behavior can be observed in aqueous chemical equilibria such as simple carboxylic acid dissociation involving highly hydrophobic species. Knowledge of the hydration thermodynamics of small molecule analogues of groups comprising the protein indeed do frame views of the dominant forces in protein folding [4, 5]. Though molecular simulations have played a decisive role in filling-out this knowledge, calculating the hydration thermodynamics of a protein holistically at the level that is possible for, say, CH₄ has remained unaddressed. With developments in the molecular quasi-chemical theory [6–10] of solutions and the associated simulation implementation [11, 12], this situation has changed [13]. Refinements in the simulation methodology [14, 15] have made it possible to interrogate the thermodynamics of macromolecules at a level that has only been undertaken for small solutes [16–18] Here we bring those tools to bear on the temperature dependence of the hydration thermodynamics of a polypeptide, thus characterizing the thermodynamic forces driving protein folding.

We study the hydration thermodynamics of the deca-alanine polypeptide in helical and an extended coil conformation. The coil conformation, labeled C₀, has the least negative hydration free energy of the coil states studied earlier [16], bounding the free energy of the un-
folded ensemble from above [17]. We also study the thermodynamics of a pair of helices in contact. The helix pair serves as a model of protein tertiary structure. The long axis of the helices are parallel, and the helix macrodipoles are either anti-parallel, as might occur in a helix-turn-helix motif, or parallel, as might occur in a helix bundle. (In nature helices are not perfectly aligned, but this issue is secondary to the questions studied here.) We also contrast the studies on the deca-alanine with the hydration of CH₄, the small-molecule analogue of the alanine side-chain.

Our results for hydration of the poly-alanine peptides show that the sign of the partial molar excess entropy, $s^{\text{(ex)}}$, and the partial molar heat capacity, $c_p^{\text{(ex)}}$, are just as found for CH₄. In the disassembly of the helix-pair, for example, although we recover the well-known $\Delta e_p^{\text{(ex)}} > 0$ observed in protein unfolding, this signature reflects the weakening of the effective protein-solvent attractive interactions. In a curious twist, the thermal expansion of the water matrix, implicated in hydrophobic interactions [19–21], also plays an important role in the temperature signatures in the protein models.

III. THEORY

The calculation of $\mu^{\text{(ex)}}$ and its entropic $T_s^{\text{(ex)}}$ and enthalpic $h^{\text{(ex)}}$ contributions follows earlier work [14–18]. Briefly [8, 9, 22], the excess chemical potential is given by $\beta\mu^{\text{(ex)}} = \ln\langle e^{\beta\epsilon} \rangle$, averaging $\epsilon$ over the binding energy distribution $P(\epsilon)$. As usual, $\beta = 1/k_B T$, with $T$ the temperature and $k_B$ the Boltzmann constant. We regularize [10, 23] the calculation of $\mu^{\text{(ex)}}$ by introducing an auxiliary field $\phi(r; \lambda)$ that moves the solvent away from the solute, thereby tempering the solute-solvent binding energy. The conditional distribution $P(\epsilon|\phi)$ is better characterized than $P(\epsilon)$, and in calculations we adjust $\lambda$, the range of the field, to control approximation of $P(\epsilon|\phi)$ as a Gaussian distribution.

With the introduction of the field [13–16]

$$\beta\mu^{\text{(ex)}} = -\ln p_0[\phi] + \beta\mu^{\text{(ex)}}[P(\epsilon|\phi)] + \ln x_0[\phi],$$

the quasi-chemical [24] organization of the potential distribution theorem [7–9]. The individual contributions are functionals of the auxiliary field, as indicated. The packing and chemistry contributions are the proximal solvent contributions that are added back to the long-range, regularized problem to complete the calculation. Fig. 1 provides a schematic description of Eq. 1.

The packing contribution measures the free energy to create a cavity to accommodate the solute and describes primitive hydrophobic effects [25, 26], i.e., hydration of an ideal hydrophobe. The chemistry contribution captures the role of solute attractive interactions with solvent in the hydration layer within a distance $\lambda$ from the center of the nearest heavy atom. The long-range contribution is the free energy of interaction between the solute and the solvent when solvent is excluded from the hydration layer. The chemistry plus long-range contribution describes the total hydrophilic contribution to hydration.

The packing and chemistry contributions in these calculations are based on a soft-cavity [12, 27]. We find that $\lambda = 5$ Å ensures that the conditional binding energy distribution is Gaussian to a good approximation. We denote this range as $\lambda_G$. The largest value of $\lambda$, labeled $\lambda_{SE}$, for which the chemistry contribution is negligible has a special meaning. It bounds the domain excluded to the solvent. We find $\lambda_{SE} \approx 3$ Å, and emphasize that for the given forcefield and solute geometry, this surface is substantially unambiguous. With this choice, Eq. 1 can be rearranged as,

$$\beta\mu^{\text{(ex)}} = -\ln p_0(\lambda_{SE}) \left[ \begin{array}{c} \text{solvent exclusion} \\ \text{packing} \end{array} \right] + \beta\mu^{\text{(ex)}}[P(\epsilon|\lambda_G)] \left[ \begin{array}{c} \text{long-range} \\ \text{chemistry} \end{array} \right] + \ln \left[ x_0(\lambda_G) \left( \frac{p_0(\lambda_{SE})}{p_0(\lambda_G)} \right) \right].$$

In Eq. 2 the various contributions are identified by the range parameter. Thus, for example, $x_0(\lambda_G) \equiv x_0[\phi(\lambda_G)]$. The revised chemistry term has the following physical meaning. It is the work done to move the solvent interface a distance $\lambda_G$ away from the solute relative to the case when the only role played by the solute is to exclude solvent up to $\lambda_{SE}$. This term highlights the role of short-range solute-solvent attractive interactions on hydration. Interestingly, the range between $\lambda_{SE} = 3$ Å and $\lambda_G = 5$ Å corresponds to the first hydration shell for a methane carbon [23] and is an approximate descriptor of the first hydration shell of groups containing nitrogen and oxygen heavy atoms.

The excess entropy of hydration is given by [15]

$$T_s^{\text{(ex)}} = E^{\text{(ex)}} - kT^2 \alpha_p + p \left( \frac{V^{\text{(ex)}}}{kT} + kT\kappa_T \right) - \mu^{\text{(ex)}} \approx E_{sw} + E_{\text{reorg}} - \mu^{\text{(ex)}}$$

FIG. 1. Quasi-chemical organization of the excess chemical potential. The inner-shell of width $\lambda_G = 5$ Å is the smallest region enclosing the solute for which the solute-solvent binding energy distribution $P(\epsilon|\phi)$ is accurately Gaussian. It approximately corresponds to the traditional first solvation shell of the solute. The shell of width $\lambda_{SE} \leq 3.0$ Å is the envelope for which the chemistry contribution is zero, and thus encloses the volume excluded to the solvent.
where \( \kappa_T \) is the isothermal compressibility of the solvent, \( \alpha_p \) is the thermal expansivity of the solvent, and \( (V^{(ex)}) \) is the excess volume of hydration, and in writing the second line of the equation, we have ignored the small contribution from all these terms. The average excess energy of hydration, \( E^{(ex)} \), is the sum the average solute-water interaction energy \( E_{sw} \) and \( E_{reorg} \), the reorganization energy. Ignoring pressure-volume effects, the excess enthalpy of hydration \( h^{(ex)} = E^{(ex)} \).

RESULTS

Hydration of Methane

For CH\(_4\) (Fig. 2), \( \mu^{(ex)} \) and the individual contributions [(2)] increase with increasing temperature. The solvent exclusion contribution makes the largest numerical contribution to the net free energy followed by the revised chemistry contribution. These contributions are balanced by the long-range attractive contribution, which is favorable and thus an attractive contribution.

Allowing water to flood the previously empty quasi-chemical inner-shell (Fig. 1), should decrease the free energy due to favorable solute-solvent interactions. The (positive) sign of the revised chemistry contribution for methane suggests that in this case, however, the solute is being pushed into unfavorable contact with the solute. In another words, the solvent matrix squeezes the hydrophobe, as suggested earlier [28–30].

The excess enthalpy of hydration \( h^{(ex)} \) has two contributions: (a) \( h_{sw}^{(ex)} \), arising from solute-water interactions and (b) \( h_{reorg}^{(ex)} \), arising from changes in the potential energy of the solvent matrix upon insertion of the solute in the solvent. The reorganization contribution is obtained using a hydration-shell-wise summation process [16, 23, 31–33]. For methane, this contribution converges within the first shell (Supporting Information, SI).

These calculations are internally consistent. The heat capacity at a given temperature can be obtained as either \( c_p^{(ex)} = (\partial h^{(ex)}/\partial T)_{N,p} \) or \( c_p^{(ex)} = T(\partial s^{(ex)}/\partial T)_{N,p} \). The first is \( c_p^{(ex)}[h^{(ex)}] \) and the second is \( c_p^{(ex)}[s^{(ex)}] \) (Fig. 2). These analyses assume that these quantities are constant over the temperature range considered herein. Within statistical uncertainties these values are the same from both paths. \( c_p^{(ex)} \approx 31 \text{ cal/mol-K}, h^{(ex)} = -1.2 \text{ kcal/mol}, \) and \( s^{(ex)} = -12.1 \text{ cal/mol-K} \) are in fair agreement with the experimental values of about 49 cal/mol-K, -2.70 kcal/mol, and -16 cal/mol-K [34], respectively. Deficiencies of both the solute model and the water model impact the numerical agreement, of course; being consistent in these choices should be lead to reliable physical conclusions. \( s^{(ex)}[T = 298.15 \text{ K}] \) obtained from a temperature derivative of \( \mu^{(ex)} \) agrees with the value from (3), emphasizing the internal consistency of these calculations.

Hydration of the helix-pair

Fig. 4 collects the results on the hydration of the helix-pair in which the helices are aligned with the macrodipoles anti-parallel, as might be found in a helix-turn-helix motif. As before, the thermodynamic components emphasize the internal consistency of the data.

The trends in the data are similar to what one finds for the helix or coil conformers (Fig. 3). The decrease with increasing temperature of the solvent exclusion contribution is even more clearly displayed for the larger helix-pair complex. We can consider the heat capacity change in the disassembly of the helix-pair to a pair of isolated helices, a simple model of disassembly of protein complexes. The heat capacity change, \( \Delta c_p^{(ex)} \), in this process is 32 \pm 128 cal/mol-K — we use the \( c_p^{(ex)}[h^{(ex)}] \) values throughout. The uncertainty is necessarily high for reasons noted above, but focusing on the mean value, the trends suggest a positive contribution, just as was found

Hydration of helix and coil conformers

Moving to consider hydration of the helix and coil conformers (Fig. 3), note the internal consistency of those calculations. The comparatively large uncertainty in \( c_p^{(ex)} \) arises from \( h_{reorg}^{(ex)} \). The shell-wise calculation of the reorganization contribution considerably reduces the uncertainty relative to naive direct differencing of potential energies of the entire solvent bath with and without the solute. Still, it is difficult to reduce that uncertainty to what is possible for \( \mu^{(ex)} \). Comparisons based upon preliminary trials support the numerical accuracy of the evaluations of \( h^{(ex)} \), and hence \( c_p^{(ex)} \).

The free energy \( \mu^{(ex)} \) increases with temperature as do the contributions from revised chemistry and long-range interactions. \( \mu^{(ex)} < 0 \) (SI), in contrast with what is observed for CH\(_4\). The revised chemistry contribution is also negative (in contrast to CH\(_4\)), indicating that the flooding of the inner shell with solvent is accompanied by a lowering of the free energy of the solute. The long-range contribution is negative and increases with temperature, just as is found for CH\(_4\). The solvent exclusion contribution for the peptides decreases with increasing temperature, whereas it increases with increasing temperature for CH\(_4\). That is, the solvent exclusion contribution for a collection of repulsive cavities (with \( \lambda = 3 \text{ Å} \)) of the shape of the peptide does not conform to the behavior expected for a single repulsive \( \lambda = 3 \text{ Å} \) cavity.

Examining the heat capacity data, we find that for the peptides used in this study, the backbone solvent contribution makes the largest contribution, whereas the reorganization contribution is smaller and similar in magnitude to the contribution from side-chain solvent interactions. This is in contrast to the behavior of CH\(_4\), where reorganization dominates solute-solvent interactions in \( c_p^{(ex)} \).
FIG. 2. Left panel: The hydration free energy of methane, following (2). With the expected opposition of long-range/attractive interactions to the other contributions, the unfavorable net hydration free energy (filled-pentagons) increases with increasing \( T \), the classic hydrophobic inverse temperature behavior. Note that the difference between the unfavorable solvent exclusion contribution and the net hydration free energy decreases with increasing \( T \), showing that long-range/attractive interactions dominate the inverse temperature behavior in this hydrophobic hydration phenomenon. Right panel: The entropic [crosses: \( T \cdot s^{(ex)} \), (3)] and enthalpic [triangles: \( h^{(ex)} \)] contributions. The filled pentagon is \( T \cdot s^{(ex)} \) from the temperature derivative of \( \mu^{(ex)} \). Contributions from solute-solvent interaction \( h^{(ex)}_{sw} \) (red circles) and solvent reorganization \( h^{(ex)}_{reorg} \) combine to give \( h^{(ex)} \). \( -h^{(ex)}_{reorg} \) is plotted to show all the data on the same plot. The several result-sets are identified by the heat capacity (in cal/mol-K) from the corresponding contribution. Error bars are ±\( \sigma \) standard error of the mean.

FIG. 3. As in Fig. 2 but relative to the \( T = 282.15 \) K value. \( h^{(ex)}_w = h^{(ex)}_{BB} + h^{(ex)}_{SC} \), again relative to the value at 282.15 K, is presented separately in the rightmost panel. \( h^{(ex)}_{BB} \) is the contribution from backbone-solvent interaction and \( h^{(ex)}_{SC} \) is the contribution from side-chain solvent interaction. Top row: Helix. Bottom row: Coil.
in early studies on protein unfolding [35]. For the helix-pair→helix+helix reaction, −7 cal/mol-K is contributed from backbone solvent contributions, 7 cal/mol-K from side-chain solvent contributions, and the remainder from water reorganization contributions. Note that the small net \( \Delta c_p^{(ex)} \) comes from large compensating physical contributions, and the agreement of the net \( \Delta c_p^{(ex)} \) with the solvent reorganization contribution is fortuitous. Importantly, one cannot make general claims that the sign of \( \Delta c_p^{(ex)} \) is determined by the reorganization contribution (and hence with models relating to water structuring around hydrophobic groups).

It is well appreciated by now that attractive solute forces are exhibited differently in the context of hydrophobic interactions in contrast to hydrophobic hydration [1, 21, 23, 36].

The hydration of the peptide models clearly shows that the observed \( c_p^{(ex)} > 0 \) and \( s^{(ex)} < 0 \) arise from the attractive protein-solvent contributions to hydration and not from primitive hydrophobic contributions. What explains the weakening of the effective protein-solvent attraction with increasing temperature? We turn to this question next.

Expansion of the solvent matrix as a basis for understanding the temperature signatures

In the temperature range considered here, water expands upon heating. Consequently, the inner shell population decreases. The mean binding energy of the solute is well correlated with the number of water molecules in the inner-shell (Fig. 5). This effect is small for a small solute, but is amplified at the scale of the peptide. The mean binding energy of the solute with the solvent in the inner shell is weaker (or less favorable) at the higher temperature. The distribution of the binding energy for a given coordination (SI) supports this suggestion.

DISCUSSION

In the hydration of CH\(_4\), we find that \( s^{(ex)} < 0 \) and \( c_p^{(ex)} > 0 \). The negative entropy of hydration is often interpreted in terms of specific solvent iceberg structures. The positive \( c_p^{(ex)} \) is then interpreted as arising from the heat required to “melt” the “iceberg.”

The simple explanation for these signatures suggested by the present results follows from the gradual decrease in solvent population around the solute as the temperature is increased. Not only does the population decrease, but for a given population of solvent around the solute, the attractive binding energy of the solvent with the solute becomes less favorable (Fig. 5) as does the interaction between solvent molecules (SI). These changes contribute, respectively, to the solute-solvent interaction part of the enthalpy and the reorganization term. This then leads to \( c_p^{(ex)} > 0 \). Then the increasing \( \mu^{(ex)} \) with increasing temperature implies \( s^{(ex)} < 0 \).

For the protein, in contrast to what is observed for CH\(_4\), the \( s^{(ex)} < 0 \) and \( c_p^{(ex)} > 0 \) signatures do not arise from the temperature dependence of the hydrophobic contribution. Thus, the present work shows that attractive protein solvent interactions play an important, even dominant, role in protein solution thermodynamics. Our work suggests the need to characterize backbone-solvent interactions, which contribute to the heat capacity of hydration. This extends to an explanation for cold denaturation: with decreasing temperature, the hydration of the unfolded state is preferred over the folded state because of the favorable hydration of the peptide backbone. Exploring this hypothesis further is left for future studies.

A. Methods: Molecular models

The simulation approach closely follows previous work [16]. The relevant simulation details are summarized in the Supporting Information. The deca-alanine peptide was modeled with an acetylated (ACE) N-terminus and n-methyl-amide (NME) capped C-terminus. The extended \( \beta \)-conformation (\( \phi, \psi = -154 \pm 12, 149 \pm 9 \)) was aligned with the end-to-end vector along the diagonal of the simulation cell. The single helix and the helix-pair are aligned with the long axis along the x-axis of the cell. These structures were taken from our earlier work [16]. In the hydration calculation the molecules have a fixed conformation. The CH\(_4\) group is constructed from the CH\(_3\) side-chain of the alanine residue. Version C31 of the CHARMM [37] forcefield with correction(cmap) terms for dihedral angles [38] was used for the protein models and for CH\(_4\). Water molecules were described by the TIP3P [39, 40] designed for the CHARMM forcefield.

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FIG. 4. As in Fig. 3, results for the hydration of the helix-pair with anti-parallel macro-dipoles.

FIG. 5. The conditional mean binding energy of the solute with the solvent in the inner-shell versus the occupation number n of the inner-shell. Inset: the probability distribution of coordination states \{x_n\} versus n. Note that the \(x_0\) member of this distribution gives the chemistry contribution \([1]\). Left panel: Data for CH\(_4\). Right panel: Data for the coil conformer.

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Supplemental text

Long-Range Interactions Dominate the
Inverse-Temperature Dependence of Polypeptide
Hydration Free Energies

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S.1 Simulation Details

The calculation of the hydration free energy using the regularization procedure closely follows earlier studies.\textsuperscript{1,2} We refer the reader to the original articles for complete details. We briefly note the following.

The chemistry and packing contributions are calculated using thermodynamic integration. To build the field to its eventual range of $\lambda_G = 5$ Å, we progressively apply the field, and for every unit Å increment in the range, we compute the work done in applying the field using a five-point Gauss-Legendre quadrature.\textsuperscript{3} At each Gauss-point, the system was simulated for $t_{total}$ (time units) and the last $t_{prod}$ (time units) used for accumulating data. Error analysis and error propagation was performed as before: the standard error of the mean force was obtained using the Friedberg-Cameron algorithm\textsuperscript{5,6} and in adding multiple quantities, the errors were propagated using standard variance-addition rules.

The starting configuration for each $\lambda$ point is obtained from the ending configuration of the previous point in the chain of states. For the packing contribution, a total of 25 Gauss points span $\lambda \in [0, 5]$. For the chemistry contribution, since solvent never enters $\lambda < 2.9$ Å, conservatively we simulate $\lambda \in [2.5, 5]$ for a total of 13 Gauss points.

For CH$_4$, the long-range contribution was obtained using the inverse form of the potential distribution theorem in the presence of the external field with range $\lambda_G = 5$ Å. In this case, from the end-point of the chemistry calculation, we create the system with $\lambda_G = 5$ Å. The system was simulated for a total $t_{lr,total}$ (time units) and solute-solvent interaction accumulated over $t_{lr,prod}$ (time units).

For the peptide models, the long-range contribution was obtained using the forward form of the potential distribution theorem in the presence of the external field with range $\lambda_G = 5$ Å. In this case, from the end-point of the packing calculation, we create the system with $\lambda_G = 5$ Å. The system was simulated for a total $t_{lr,total}$ (time units) and neat solvent frames archived over $t_{lr,prod}$ (time units). We then do test particle insertions in the neat solvent frame to obtain the solute-solvent binding energy distribution.
Specifics for each system is given below.

S.1.1 CH$_4$

The water box comprises 992 TIP3P$^{7,8}$ water molecules. For the packing and chemistry calculations, at each $\lambda$, $t_{total} = 4$ ns and $t_{prod} = 3$ ns. Frames were archived every 100 fs for further analysis. For the long-range calculation, the system with $\lambda_G = 5$ Å was simulated for 5 ns and frames archived every 400 fs for analysis.

For the excess enthalpy and coordination number analysis, the well-equilibrated solute-solvent system (with no external field) was further simulated for 5 ns and frames archived every 200 fs for analysis.

S.1.2 Helix and coil conformers

As before$^1$ we used a box of 3500 TIP3P water molecules. For the packing and chemistry calculations, at each $\lambda$, $t_{total} = 1$ ns and $t_{prod} = 0.75$ ns. (We checked to ensure that neglecting more data does not perceptibly change the results.) Frames were archived every 50 fs for further analysis. (For the 298.15 K case alone, the simulations were repeated 4 times and the results averaged. We emphasize that the results from the four independent repeats were consistent within the statistical uncertainty of each run.) For the long-range calculation, the system with $\lambda_G = 5$ was simulated for $t_{lr,total} = 4$ ns. Data was accumulated over the last $t_{lr,prod} = 2$ ns, with frames saved every 500 fs. For temperatures except 298.15 K, this procedure was repeated twice. The resulting data was pooled together and analyzed as before.$^{1,2}$ For the 298.15 K case, for the outer calculation we simulated for $t_{lr,total} = 11$ ns and accumulated data for $t_{lr,prod} = 10$ ns, with frames saved every 500 fs. This procedure was repeated twice and the results pooled and analyzed. We emphasize that the Gaussian description of shorter segments of the long 10 ns production in the 298.15 K case was in excellent agreement with the Gaussian description using all the data. This confirms the robustness of the Gaussian and shows that our choices for the other temperatures are still
very conservative.

For the excess enthalpy and coordination number analysis, the well-equilibrated solute-solvent system (with no external field) was simulated for 4 ns and frames archived every 200 fs for analysis.

S.1.3 Helix-pairs

For the helix-pairs, the chemistry and packing calculation was as for the isolated helix. For the long-range calculation, for the 298.15 K case, the system was equilibrated for 1 ns. Five independent 2 ns runs were performed and the data analyzed. For the rest of the temperatures, $t_{lr,\text{total}} = 11$ ns of which $t_{lr,\text{prod}} = 10$ ns was used for accumulating data. Frames were saved at the same rate as for the isolated helix.

For the excess enthalpy calculation, the well-equilibrated solute-solvent system (with no external field) was simulated for a total of 3 ns and the last 2.75 ns used for analysis. Frames were archived every 500 fs for a total of 5500 frames. Because of a factor of 3 less data (compared to the isolated protein case), the statistical uncertainties were correspondingly higher.

S.2 Entropy from the temperature derivative of $\mu^{(ex)}$

To check the consistency of $T_s^{(ex)}$ calculated at $T = 298.15$ K using Euler’s relation (Eq. 3, main text) with $T_s^{(ex)} = -T(\partial \mu^{(ex)}/\partial T)_{N,p}$, we can either take the derivative of $\mu^{(ex)}$ using a central difference formula or by differentiating a model fit to the calculated $\mu^{(ex)}$ values. Statistically, the latter approach is to be preferred given the logarithmic dependence of $\mu^{(ex)}$ on $T$. To this end, consistent with the analysis of $c_p^{(ex)}$ (main text), we assume constant heat capacity over the temperature range of interest and set $\mu^{(ex)}_{\text{model}} = a + b \cdot T + c \cdot T \log T$ with
To estimate the model parameters $a, b, c$, we minimize
\[ \chi^2 = \sum_i \left( \frac{\mu_{\text{calc}}(T_i) - \mu_{\text{model}}(T_i)}{\sigma(T_i)} \right)^2, \]  
(S.1)

using the interior point method within Mathematica. In Eq. S.1, $\mu_{\text{calc}}(T_i)$ is the calculated value of $\mu^{(ex)}$ at the temperature $T_i$ and $\sigma(T_i)$ is the associated standard error of the mean value. To estimate the uncertainty of $T_s^{(ex)}$ from the derivative path, we repeated the above minimization procedure for synthetic data sets obtained by drawing random variates using $\mu_{\text{calc}}(T_i)$ and $\sigma(T_i)$ as the mean and standard deviation. We repeated this simulation procedure over 100 times and found the numerical estimates to be well-converged. (In practice, as few as 10 data sets suffice to obtain a robust estimate of the mean value.) The standard deviation of the distribution of $T_s^{(ex)}$ values calculated over the synthetic data set is taken as the estimate of the uncertainty of $T_s_{\text{model}}^{(ex)}$ calculated from the derivative of $\mu_{\text{model}}^{(ex)}$.

Table S.I below collects the results from the above analysis. Please note that $c_{p,\text{model}}^{(ex)}$ is obtained from a second derivative of $\mu_{\text{model}}^{(ex)}$ with respect to temperature, and not surprisingly, the variation is rather large. For comparison, we reproduce $T_s^{(ex)}$ from Euler’s relation (Eq. 3 main text) and $c_p^{(ex)}$ from the temperature derivative of $h^{(ex)}$.

| Species | $T_s_{\text{model}}^{(ex)}$ (in kcal/mol) | $T_s^{(ex)}$ | $T_s^{(ex)}_{\text{Euler}}$ | $c_{p,\text{model}}^{(ex)}$ | $c_p^{(ex)}[h^{(ex)}]$ |
|---------|-----------------------------------|----------|-----------------|-----------------|-----------------|
| CH$_4$  | $-2.7 \pm 0.5$                    | $-3.6 \pm 0.4$ | 16 (0, 60)    | 31 ± 4          |
| Helix   | $-39 \pm 5$                      | $-40 \pm 1$   | 1198 (0, 2480) | 265 ± 48       |
| Coil    | $-54 \pm 4$                      | $-54 \pm 2$   | 1278 (0, 2120) | 340 ± 82       |
| Helix pair (antiparallel) | $-64 \pm 9$ | $-65 \pm 3$ | 1585 (0, 3385) | 498 ± 108      |
| Helix pair (parallel)  | $-71 \pm 11$                     | $-71 \pm 3$   | 1866 (0, 4270) | 541 ± 69       |
S.3 Reorganization energy by shells at 298.15 K

The calculation of the reorganization contribution follows the procedure exhaustively documented before.\textsuperscript{1,2} Please note that we define an inner-shell around the peptide as the union of shells of radius $\lambda$ centered on the peptide heavy atoms. $\lambda \leq 5.5 \, \text{Å}$, $5.5 < \lambda \leq 8.5 \, \text{Å}$, and $8.5 < \lambda \leq 11.5 \, \text{Å}$ defined the first, second, and third shells, respectively. For the reorganization calculation, the definition of the inner shell was slightly increased by 0.5 Å, but this change has no bearing on the final thermodynamic quantity $h^{(\text{ex})}$.

![Diagram showing convergence of reorganization energy as a function of the shells of water around the solute. For methane the reorganization contribution converges within the first shell, whereas we require the first two shells for the helix and coil conformers. The same holds true for the helix-pair.](image-url)

Figure S1: Convergence of reorganization energy as a function of the shells of water around the solute. For methane the reorganization contribution converges within the first shell, whereas we require the first two shells for the helix and coil conformers. The same holds true for the helix-pair.
S.4  Hydration of the helix-pair with helix macro-dipoles oriented in the parallel configuration.

Figure S2: As in Figure 4 (main text) for a helix-pair, but with helices in a parallel configuration in the helix pair.

S.5  Effect of expansion of the matrix

Figure S3: Variation of the energy of the water cluster in the inner shell. Please note that the cluster energy with the rest of the bulk solvent or the solute is not taken into consideration.
Figure S4: Figure showing the distribution of solute interaction with solvent in the inner shell for a defined coordination $n$ of the inner shell. Observe that both the low energy tail and the high energy tails are affected by increase in temperature. This suggests that the solvent samples both the repulsive wall and the weaker attractive tail as temperature is increased.

S.6 Hydration of CH$_4$

### Table S.II: Quasicomponents

| Temperature | Solvent exclusion | Revised Chemistry | Long-range | $\mu^{(ex)}$ |
|-------------|------------------|------------------|------------|-------------|
| 282.15      | 2.47 ± 0.01      | 2.00 ± 0.05      | −2.21 ± 0.003 | 2.26 ± 0.06 |
| 290.15      | 2.48 ± 0.01      | 1.98 ± 0.04      | −2.18 ± 0.003 | 2.28 ± 0.04 |
| 298.15      | 2.49 ± 0.01      | 2.01 ± 0.05      | −2.15 ± 0.003 | 2.35 ± 0.05 |
| 306.15      | 2.52 ± 0.01      | 2.06 ± 0.05      | −2.13 ± 0.003 | 2.45 ± 0.05 |
| 314.15      | 2.53 ± 0.01      | 2.08 ± 0.04      | −2.09 ± 0.003 | 2.52 ± 0.04 |
Table S.III: Thermodynamic components

| Temperature | \( h_{\text{sw}}^{(\text{ex})} \) | \( h_{\text{reorg}} \) | \( h^{(\text{ex})} \) | \( T_s^{(\text{ex})} \) |
|-------------|-------------------------------|-----------------|-----------------|-----------------|
| 282.15      | -3.39 ± 0.01                  | 1.80 ± 0.30     | -1.60 ± 0.30    | -3.80 ± 0.30    |
| 290.15      | -3.34 ± 0.01                  | 2.00 ± 0.40     | -1.40 ± 0.40    | -3.60 ± 0.40    |
| 298.15      | -3.30 ± 0.01                  | 2.10 ± 0.40     | -1.20 ± 0.40    | -3.60 ± 0.40    |
| 306.15      | -3.25 ± 0.01                  | 2.50 ± 0.40     | -0.80 ± 0.40    | -3.20 ± 0.40    |
| 314.15      | -3.21 ± 0.01                  | 2.60 ± 0.30     | -0.60 ± 0.30    | -3.10 ± 0.30    |

S.7 Hydration of deca-alanine helix

Table S.IV: Quasicomponents

| Temperature | Solvent exclusion | Revised Chemistry | Long-range | \( \mu^{(\text{ex})} \) |
|-------------|-------------------|-------------------|------------|-----------------|
| 282.15      | 44.7 ± 0.5        | -53.2 ± 0.7       | -32.8 ± 0.2 | -41.4 ± 0.6     |
| 290.15      | 45.3 ± 0.3        | -52.9 ± 0.5       | -32.1 ± 0.2 | -39.7 ± 0.4     |
| 298.15      | 44.4 ± 0.4        | -51.6 ± 0.6       | -31.6 ± 0.1 | -38.9 ± 0.5     |
| 306.15      | 44.2 ± 0.3        | -51.1 ± 0.4       | -30.9 ± 0.2 | -37.8 ± 0.4     |
| 314.15      | 43.9 ± 0.3        | -50.2 ± 0.4       | -30.8 ± 0.2 | -37.1 ± 0.4     |
Table S.V: Thermodynamic components

| Temperature | $h_{bb}^{(ex)}$ | $h_{sc}^{(ex)}$ | $h_{reorg}^{(ex)}$ | $h^{(ex)}$ | $T_s^{(ex)}$ |
|-------------|----------------|----------------|------------------|-----------|-------------|
| 282.15      | $-129.0 \pm 0.1$ | $-26.10 \pm 0.02$ | $71.3 \pm 1.8$ | $-83.8 \pm 1.8$ | $-42.5 \pm 1.9$ |
| 290.15      | $-127.5 \pm 0.1$ | $-25.80 \pm 0.02$ | $72.7 \pm 1.8$ | $-80.6 \pm 1.9$ | $-40.9 \pm 1.9$ |
| 298.15      | $-125.9 \pm 0.1$ | $-25.30 \pm 0.02$ | $72.8 \pm 1.3$ | $-78.4 \pm 1.3$ | $-39.6 \pm 1.4$ |
| 306.15      | $-124.6 \pm 0.1$ | $-25.01 \pm 0.03$ | $72.7 \pm 2.2$ | $-76.9 \pm 2.2$ | $-39.1 \pm 2.2$ |
| 314.15      | $-123.2 \pm 0.1$ | $-24.60 \pm 0.02$ | $72.6 \pm 2.0$ | $-75.2 \pm 2.0$ | $-38.1 \pm 2.0$ |

S.8 Hydration of deca-alanine coil $C_0$

Table S.VI: Quasicomponents

| Temperature | Solvent exclusion | Revised Chemistry | Long-range | $\mu^{(ex)}$ |
|-------------|------------------|------------------|------------|-------------|
| 282.15      | $58.4 \pm 0.1$   | $-79.0 \pm 0.3$  | $-28.8 \pm 0.1$ | $-49.4 \pm 0.3$ |
| 290.15      | $58.3 \pm 0.2$   | $-77.8 \pm 0.4$  | $-28.1 \pm 0.1$ | $-47.7 \pm 0.4$ |
| 298.15      | $58.4 \pm 0.2$   | $-77.0 \pm 0.4$  | $-27.5 \pm 0.0$ | $-46.1 \pm 0.3$ |
| 306.15      | $58.2 \pm 0.2$   | $-75.7 \pm 0.4$  | $-27.0 \pm 0.1$ | $-44.5 \pm 0.3$ |
| 314.15      | $57.3 \pm 0.2$   | $-74.8 \pm 0.3$  | $-26.2 \pm 0.1$ | $-43.7 \pm 0.3$ |
Table S.VII: Thermodynamic components

| Temperature | $h_{bb}^{(ex)}$ | $h_{sc}^{(ex)}$ | $h_{reorg}^{(ex)}$ | $h^{(ex)}$ | $T_s^{(ex)}$ |
|-------------|----------------|-----------------|--------------------|-----------|-------------|
| 282.15      | −163.3 ± 0.1   | −28.00 ± 0.02   | 88.5 ± 1.7         | −102.8 ± 1.7 | −53.5 ± 1.7 |
| 290.15      | −161.6 ± 0.1   | −27.60 ± 0.02   | 90.2 ± 1.7         | −99.0 ± 1.8  | −51.3 ± 1.8  |
| 298.15      | −159.5 ± 0.2   | −27.20 ± 0.02   | 87.1 ± 2.0         | −99.6 ± 2.0  | −53.5 ± 2.1  |
| 306.15      | −157.6 ± 0.1   | −26.90 ± 0.03   | 90.0 ± 2.1         | −94.5 ± 2.1  | −50.0 ± 2.1  |
| 314.15      | −155.5 ± 0.2   | −26.40 ± 0.02   | 90.3 ± 1.5         | −91.6 ± 1.5  | −47.9 ± 1.5  |

S.9 Hydration of helix pair with helix macro-dipoles in the antiparallel configuration

Table S.VIII: Quasicomponents

| Temperature | Solvent exclusion | Revised Chemistry | Long-range | $\mu^{(ex)}$ |
|-------------|------------------|-------------------|------------|--------------|
| 282.15      | 84.1 ± 0.6       | −107.6 ± 0.9      | −42.9 ± 0.2| −66.4 ± 0.7  |
| 290.15      | 83.9 ± 0.5       | −106.8 ± 0.8      | −41.7 ± 0.2| −64.6 ± 0.6  |
| 298.15      | 82.4 ± 0.9       | −103.4 ± 1.3      | −41.2 ± 0.4| −62.2 ± 1.0  |
| 306.15      | 81.3 ± 0.6       | −101.6 ± 0.9      | −40.7 ± 0.2| −61.0 ± 0.7  |
| 314.15      | 80.3 ± 0.6       | −100.5 ± 0.9      | −39.5 ± 0.2| −59.7 ± 0.7  |
Table S.IX: Thermodynamic components

| Temperature | $h_{bb}^{(ex)}$ | $h_{sc}^{(ex)}$ | $h_{reorg}^{(ex)}$ | $h^{(ex)}$ | $T_s^{(ex)}$ |
|-------------|----------------|----------------|-------------------|-----------|-------------|
| 282.15      | $-226.0 \pm 0.4$ | $-45.20 \pm 0.04$ | 134.9 $\pm 2.5$ | $-136.3 \pm 2.5$ | $-69.9 \pm 2.6$ |
| 290.15      | $-222.8 \pm 0.2$ | $-44.40 \pm 0.08$ | 138.8 $\pm 3.3$ | $-128.4 \pm 3.3$ | $-63.8 \pm 3.4$ |
| 298.15      | $-219.8 \pm 0.2$ | $-43.80 \pm 0.07$ | 136.4 $\pm 2.4$ | $-127.2 \pm 2.4$ | $-65.0 \pm 2.7$ |
| 306.15      | $-216.9 \pm 0.2$ | $-43.10 \pm 0.08$ | 136.7 $\pm 3.7$ | $-123.3 \pm 3.7$ | $-62.4 \pm 3.8$ |
| 314.15      | $-214.0 \pm 0.3$ | $-42.30 \pm 0.05$ | 136.6 $\pm 2.8$ | $-119.7 \pm 2.8$ | $-60.0 \pm 2.9$ |

S.10 Hydration of helix pair with helix macro-dipoles in the parallel configuration

Table S.X: Quasicomponents

| Temperature | Solvent exclusion | Revised Chemistry | Long-range | $\mu^{(ex)}$ |
|-------------|------------------|-------------------|------------|-------------|
| 282.15      | 84.3 $\pm 0.9$   | $-109.1 \pm 1.2$ | $-62.7 \pm 0.4$ | $-87.5 \pm 1.0$ |
| 290.15      | 83.2 $\pm 0.6$   | $-107.4 \pm 0.9$ | $-61.1 \pm 0.4$ | $-85.3 \pm 0.8$ |
| 298.15      | 82.0 $\pm 0.8$   | $-105.8 \pm 1.1$ | $-60.1 \pm 0.8$ | $-83.9 \pm 1.1$ |
| 306.15      | 81.5 $\pm 0.9$   | $-104.1 \pm 1.4$ | $-58.4 \pm 0.3$ | $-81.0 \pm 1.0$ |
| 314.15      | 80.8 $\pm 0.6$   | $-102.4 \pm 0.9$ | $-58.5 \pm 0.4$ | $-80.1 \pm 0.8$ |
Table S.XI: Thermodynamic components

| Temperature | $h_{bb}^{(ex)}$ | $h_{sc}^{(ex)}$ | $h_{reorg}$ | $h^{(ex)}$ | $T_s^{(ex)}$ |
|-------------|----------------|----------------|-------------|------------|-------------|
| 282.15      | $-260.0 \pm 0.5$ | $-48.70 \pm 0.08$ | $145.2 \pm 3.4$ | $-163.5 \pm 3.5$ | $-76.0 \pm 3.6$ |
| 290.15      | $-257.3 \pm 0.3$ | $-47.90 \pm 0.05$ | $148.4 \pm 2.2$ | $-156.8 \pm 2.2$ | $-71.5 \pm 2.3$ |
| 298.15      | $-254.6 \pm 0.4$ | $-47.20 \pm 0.09$ | $147.4 \pm 3.1$ | $-154.4 \pm 3.1$ | $-70.5 \pm 3.3$ |
| 306.15      | $-250.9 \pm 0.2$ | $-46.40 \pm 0.05$ | $147.8 \pm 2.0$ | $-149.5 \pm 2.0$ | $-68.5 \pm 2.3$ |
| 314.15      | $-248.5 \pm 0.3$ | $-45.80 \pm 0.04$ | $149.6 \pm 2.0$ | $-144.7 \pm 2.0$ | $-64.6 \pm 2.2$ |

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