Molecular-Level Thermodynamic Switch Controls Chemical Equilibrium in Sequence-Specific Hydrophobic Interaction of 35 Dipeptide Pairs

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ABSTRACT Applying the Planck-Benzinger methodology, the sequence-specific hydrophobic interactions of 35 dipeptide pairs were examined over a temperature range of 273–333 K, based on data reported by Nemethy and Scheraga in 1962. The hydrophobic interaction in these sequence-specific dipeptide pairs is highly similar in its thermodynamic behavior to that of other biological systems. The results imply that the negative Gibbs free energy change minimum at a well-defined stable temperature, $\langle T_s \rangle$, where the bound unavailable energy, $T\Delta S^o = 0$, has its origin in the sequence-specific hydrophobic interactions, are highly dependent on details of molecular structure. Each case confirms the existence of a thermodynamic molecular switch wherein a change of sign in $\Delta Cp^o(T)_{reaction}$ (change in specific heat capacity of reaction at constant pressure) leads to true negative minimum in the Gibbs free energy change of reaction, $\Delta G^o(T)_{reaction}$, and hence a maximum in the related equilibrium constant, K_{eq}. Indeed, all interacting biological systems examined to date by Chun using the Planck-Benzinger methodology have shown such a thermodynamic switch at the molecular level, suggesting its existence may be universal.

INTRODUCTION

Shortly after T. H. Benzinger published an article in 1971 entitled ''Thermodynamics, Chemical Reactions and Molecular Biology,'' the question arose whether thermochemical descriptions of biochemical systems that existed in the literature were inadequate or wrong or both.

Benzinger (1971) proposed a thermal work function to take into account both Boltzmann statistical energy effects and energies of quantum-mechanical bonds. Inasmuch as the latter are usually not altered significantly in macromolecular reactions, it was Benzinger's conjecture that the large-scale and long-range changes of conformation which accompany protein folding or assembly might generate significant energy differences due to the cumulative alteration of their numerous covalent bond structures.

After years of detailed study on interacting protein systems and their thermodynamic parameters, it is now possible to formulate a different viewpoint, one that is both adequate and consistent when applied to biological systems, and that is the necessity of considering innate thermodynamic quantities in structural biology.

Analyses by Chun of available and highly precise data on protein folding in several ribonuclease systems as well as six self-associating protein systems over the temperature range 220–360 Kelvin show that the ''chemical bond'' component of the overall enthalpy was of significant magnitude, and that it was independent of temperature (Chun, 1988, 1994). This analysis demonstrated the validity of the central concept. Subsequent studies (Chun, 1995–1999; 2000a,b,c; 2001a,b) have shown unequivocally that each macromolecular process has a temperature-independent component of the enthalpy in addition to the traditionally recognized heat capacity of thermodynamics. Specifically, the temperature-independent component of the chemical bond enthalpy is the inherent strength of the bond as measured at zero degrees Kelvin.

It has been well established in pure and applied chemistry of simple molecules that reaction energies at room temperature can be understood in terms of two contributions, one related to energy differences at 0 K (the innate inherent chemical bond energy) and the other associated with integrals of heat capacity data over a range of temperatures (the thermal agitation energy). The necessity of a comparable separation of energy terms for biological reactions, however, has not been obvious to most workers in structural and molecular biology. The innate thermodynamic quantities, in particular the innate temperature-invariant enthalpy, represent differences in chemical bonding energy of products minus reactants, and thus control the heat of reaction at 0 K. The net difference between the temperature-dependent heat capacities of reaction ($\Delta Cp^o(T)$, taken as products minus reactants, dictates how $\Delta H^o_{reaction}$ behaves at high temperatures.

We believe that we have uncovered a type of physical behavior characteristic of living systems which may prove to be remarkably general. It is, of course, known that living systems can live and operate optimally only at a sharply defined temperature, or over a limited temperature range at best. We assert that this implies that basic biochemical macromolecular interactions exhibit a well-defined negative free energy minimum (that is to say, favorable) as a function of temperature. Such a situation is not common in simple chemical systems, where a monotonic change of ΔG^o, ΔH^o, and K_{eq} over an experimental temperature range is typical.

In all biological interactions, $\Delta H^o(T)$ and $\Delta S^o(T)$ are positive at low temperature. As reaction temperature increases, both $\Delta H^o(T)$ and $\Delta S^o(T)$ become negative, creating a negative Gibbs free energy minimum: that is

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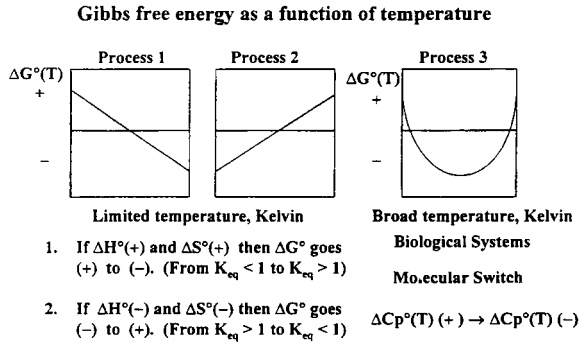

FIGURE 1 In all biological interactions, $\Delta H^o(T)$ and $\Delta S^o(T)$ are positive at low temperature. As reaction temperature increases, both $\Delta H^o(T)$ and $\Delta S^o(T)$ become negative, creating a negative Gibbs free energy minimum: process 1 of the chart goes to process 2, creating process 3, as shown in Table 1. We have effectively demonstrated that a temperature-dependent heat capacity of reaction, $\Delta Cp^o{}_{reaction}$, which is positive at low temperature, switches to a negative value at a temperature in the ambient range. We refer to it as a ''thermodynamic molecular switch.'' It determines the behavior patterns of the Gibbs free energy change, and hence a change in the equilibrium constant, K_{eq}, and/or spontaneity.

process 1 of the chart as shown in Fig. 1, which goes to process 2, creating process 3 (see Table 1). Fig. 1 and Table 1 provide the physiochemical background information which is necessary to understand the situation. The change of sign in $\Delta Cp^o(T)_{reaction}$ leads to a true negative minimum in the Gibbs free energy of reaction, that is $\Delta Cp^o(+) \rightarrow \Delta Cp^o(-)$, designated as a thermodynamic molecular switch (Chun, 2000a,b,c; 2001a,b; 2002; 1995–1999).

In the examination of the pair-wise hydrophobic interaction of 35 sequence-specific dipeptides over the temperature range of 273–333 K reported by Nemethy and Scheraga in 1962, the critical factor driving the process is a temperature-dependent heat capacity change of reaction, $\Delta Cp^o(T)_{reaction}$, which is positive at low temperature but switches to a negative value at a temperature well below the ambient range. This change of sign of the critically important $\Delta Cp^o(T)_{reaction}$ (product minus reactants) has such significant consequences that we refer to it as a ''thermodynamic molecular switch,'' and it has been observed not only in these dipeptide pairs but also in a wide variety of biological systems. It determines the behavior patterns of the Gibbs free energy change, and hence a change in the equilibrium constant, K_{eq}, and/or spontaneity. Note that $\Delta G^o(T)_{reaction} = -RTlnK_{eq}$, so that the roles of ΔG^o and K_{eq} are rigidly coupled. The subsequent, mathematically predictable changes in ΔH^o, ΔS^o, ΔW^o, and ΔG^o which arise as a result of this thermodynamic molecular switch are demonstrated in these pair-wise, sequence-specific hydrophobic interactions.

The Giauque function and Planck-Benzinger thermal work function

One form of the free energy function, the Giauque function (Giauque, 1930a,b; Giauque and Blue, 1930; Giauque and Kemp, 1938; Giauque and Meads, 1941), $(G^o_T - H^o_0)/T = -\psi^o/T$, where $-\psi^o = (G^o_T - - H^o_0)$, has been extensively used in chemistry and physics (Moelwyn-Hughes, 1957; Lewis and Randle, rev. by Pitzer and Brewer, 1961).

TABLE 1 Enthalpy-entropy compensation in chemical reactions where $\Delta G^o = \Delta H^o - T\Delta S^o$

	ΔG^o	ΔH^o	ΔS^o	$T\Delta S^o$	Observations
1	–	+	+	–	Entropy-driven process
2	–	–	–	+	Enthalpy-driven process
3	+	+	+	–	Entropy-driven at high temperature
4	–	–	+	–	Reaction always proceeds at all temperatures

As experimentally observed in interacting biological systems, at low temperature, ΔH^o and ΔS^o are both positive, becoming negative as temperature increases, whereas ΔG^o changes from positive to negative, then reaches a negative value of maximum magnitude at $\langle T_s \rangle$, and finally becomes positive as temperature increases. That is, process 1 goes to process 2, creating cooperative enthalpy-entropy compensation between $\langle T_h \rangle$ and $\langle T_m \rangle$. This process is illustrated schematically in Fig. 1.

An equivalent formulation has recently found application in biochemical literature as the Planck-Benzinger thermal work function (Chun, 1988–1999; 2000a,b,c; 2001a,b; 2002), where the application to a given situation is quite different. It is probably true that the Giauque function could have been used and would have been appropriate—it simply was not. Rather, Benzinger pursued a separate path, although one that can be related to the work of Giauque and other chemists.

Planck's characteristic function may be expressed as $\psi = (H/T) - S$. A proper rearrangement yields the Gibbs free energy function, $[\psi T = H - TS]$. Substitution of Kirchhoff's expression, $\Delta H(T) = \Delta H(T_0) + \int_0^T \Delta CpdT$ and $\Delta S = \int_0^T (\Delta Cp/T)dT$ into the above Gibbs free energy function, yields $\Delta G = \Delta H(T_0) - [T\int_0^T (\Delta Cp/T)dT - \int_0^T \Delta CpdT]$. By Benzinger's definition,

$$\Delta W = \left[T\int_0^T (\Delta Cp/T)dT - \int_0^T \Delta CpdT \right].$$

The Planck-Benzinger thermal work function is therefore expressed as

$$\Delta W(T) = \Delta H(T_0) - \Delta G(T).$$

Here the Planck-Benzinger thermal work function (Benzinger, 1971; Planck via Ogg, 1927), represents the strictly thermal components of any intra- or intermolecular bonding term, that is, energy other than the inherent difference of the 0 K portion of the interaction energy, $\Delta H(T_0)$.

The latter is the only energy term in constant pressure processes at absolute zero Kelvin. Thus, $\Delta W(T)$ expresses completely the thermal energy difference of the process involved. Application of the Planck-Benzinger thermal work function permits the separation of 0-K energy differences

and energy differences associated with heat capacity integrals for a fuller understanding of reaction energies.

Measuring enthalpy values

Enthalpies of reaction are frequently measured at or near room temperature (298 K) for a variety of theoretical and practical reasons; for instance, the relationship between $\Delta H^{o}_{reaction}$ and the temperature coefficient of the equilibrium constant, K_{eq}, $dlnK_{eq}/d(1/T) = -\Delta H^{o}(T)/R$. Kirchhoff (Gibbs, 1878; Moelwyn-Hughes, 1957; Lewis and Randall, 1961) stated $\Delta H^{o}_{298} = \Delta H^{o}(T_0) + \int_0^T \Delta Cp^{o} dT$, where this last term represents the thermal agitation energy (heat capacity integrals), inasmuch as the constant term $\Delta H^{o}(T_0)$ represents the enthalpy of reaction at 0 K. For small molecules, reaction enthalpies are often obtained around room temperature, and the heat of reaction is estimated in terms of the innate temperature-invariant enthalpy, $\Delta H^{o}(T_0)$.

T. L. Cottrell (1958) pointed out 43 years ago that ΔH^{o}_{298} and $\Delta H^{o}(T_0)$ differ only by ~1% in small molecules, but in 1971 T. H. Benzinger made the crucial observation that this difference is large in biological macromolecules due to the large magnitude of the heat capacity integrals (thermal agitation energy). In other words, for small molecules, $[\Delta H^{o}_{298} - \Delta H^{o}(T_0)]$ is a correction of only a few percent, whereas for biological macromolecules, the heat capacity integrals can be large, from 10% up to 50% of the total heat of reaction. At present the scientific literature provides no ''silver bullet''; that is, no highly accurate method for evaluating $[\Delta H^{o}_{298} - \Delta H^{o}(T_0)]$ in large biological macromolecules; however, Chun's work (Chun, 1988–1999; 2000a,b,c; 2001a,b; 2002) has extensively addressed the problem.

The Planck-Benzinger approach

To analyze the thermodynamic processes operating in a sequence-specific hydrophobic interaction of the 35 dipeptide pairs, it is necessary to extrapolate the thermodynamic parameters over a much broader temperature range. The enthalpy, entropy, and heat capacity terms are evaluated as partial derivatives of the Gibbs free energy function defined by Helmholtz-Kelvin's expression (Moelwyn-Hughes, 1957; Lewis and Randall, 1961):

$$\partial\Delta G(T)/\partial T = -\Delta S(T), \quad \{\partial\Delta G(T)/T\}/\partial T = -\Delta H(T)/T^2$$

$$\partial\Delta H(T)/\partial T = \Delta Cp(T), \quad \partial\Delta S(T)/\partial T = \Delta Cp(T)/T.$$

In continuing studies on dozens of interacting protein systems, it has been shown in our laboratory that the third-order polynomial function provides a good fit in the temperature range accessible in biochemical systems (Chun, 1988–1999; 2000a,b,c; 2001a,b; 2002). In fact, it is shown to be correct in the low-temperature limit. The rationale for selecting the linear and nonlinear third-order (T^3 model) polynomial functions for $\Delta G^{o}(T) = \alpha + \beta T^2 + \gamma T^3$ (macromolecular interaction) and $\Delta H(T) = \alpha + \beta T^3 e^{\gamma T}$ (protein unfolding) are found in the fundamentals of relevant quantum theory (Moelwyn-Hughes, 1957).

At low temperature, the specific heat capacity at constant volume of a simple solid becomes $C_v = (12\pi^4/5)(N_0K)(kT/h\nu_m)^3$. With a proper substitution of $\theta = h\nu_m/k$ and $R = N_0K$, $C_v = (12\pi^4R/5)(T/\theta)^3$ (Planck via Ogg, 1927; Moelwyn-Hughes, 1957). Clearly, the energy and specific heat are universal functions of (kT/ν_m) or (T/θ). Here h is the Planck constant, k, the Boltzmann constant, C_v is the specific heat at constant volume, and ν_m is the maximum frequency of vibration of an atom in a solid state as in Planck's theory.

The constraints for this fitted model of the Gibbs free energy change as a function of temperature are these: the enthalpy and Gibbs free energy must intersect at zero Kelvin at a positive value and the slope must be zero where $\Delta G^{o}(T_0) = \Delta H^{o}(T_0)$ at 0 K as $T\Delta S^{o}$ approaches zero. The cubic term is necessitated by quantum mechanical considerations, and the presence of the quadratic term is dictated by the data rather than theory (Chun, 2000a,b,c; 1998).

Determination of the Gibbs free energy change as a function of temperature

Nemethy and Scheraga's theoretical treatment of hydrophobic interaction (Nemethy and Scheraga, 1962a, Scheraga, 1994) based on a statistical mechanical theory of the thermodynamic properties of liquid water (Nemethy and Scheraga, 1962b) and of aqueous solutions of hydrocarbon (Nemethy and Scheraga, 1962c) led to determination of the maximum strength between isolated hydrophobic side chains. $\Delta G^{o}(T)$ data for a pair-wise hydrophobic interaction of maximum strength were computed from Nemethy and Scheraga's Table 2 (1962a) based on T^2 model of $\Delta G^{o} = a + bT + cT^2$ over a temperature range of 273–343 K, knowing the polynomial coefficients a, b, and c (Nemethy and Scheraga, 1962a, Scheraga, 1994). Values for the Gibbs free energy change as a function of temperature were computed for the sequence-specific hydrophobic interactions of dipeptide pairs using the T^3 model of $\Delta G^{o}(T) = \alpha + \beta T^2 + \gamma T^3$ (Chun, 2001b).

Computational procedure for sequence-specific hydrophobic interaction of dipeptide pairs

To extrapolate down to 0 K, it is necessary to consider the normal solution states of molecules. Here the 0 K limit would presumably refer to the glassy condition (Chun, 1988–1999; 2000a,b,c; 2001a,b; 2002)—that is, a condition with all thermal agitation frozen out, but retaining the general physical chemical properties of solution—inasmuch as a pure crystalline form of macromolecules is rarely encountered in practice.

This approach requires exact determination of K_{eq} for the relevant biochemical processes as a function of absolute temperature. Of critical importance, however, is the use of precise, correctly formulated expressions for $\Delta Cp^{o}_{reaction}$ as a function of temperature as shown in Eq. (3). In this treatment, data reported by Nemethy and Scheraga using the T^2 model of $\Delta G^{o} = a + bT + cT^2$ for 35 dipeptide pairs were fitted to the T^3 model for a three-term linear polynomial function in the 273–343 K temperature range. To illustrate, plots for ΔG^{o} as a function of temperature for eight identical dipeptide pairs—*Met-Met*, *Ile-Ile*, *Phe-Phe*, *Val-Val*, *Pro-Pro*, *Leu-Leu*, *Cys-Cys*, and *Ala-Ala*—are shown in Figs. 2 *A*–9 *A* and compared with eight other pairs in which a single amino acid is substituted. Values for the inherent chemical bond energy at 0 K and thermal agitation energy of each pair are shown in Table 3. The Gibbs free energy data for all 35 dipeptide pairs were then fitted to the three-term linear polynomial function,

$$\Delta G^{o}(T) = \alpha + \beta T^2 + \gamma T^3. \quad (1)$$

The resulting values for the α, β, and γ coefficients are shown in Table 2. Once evaluated as shown in Figs. 2 *A*–9

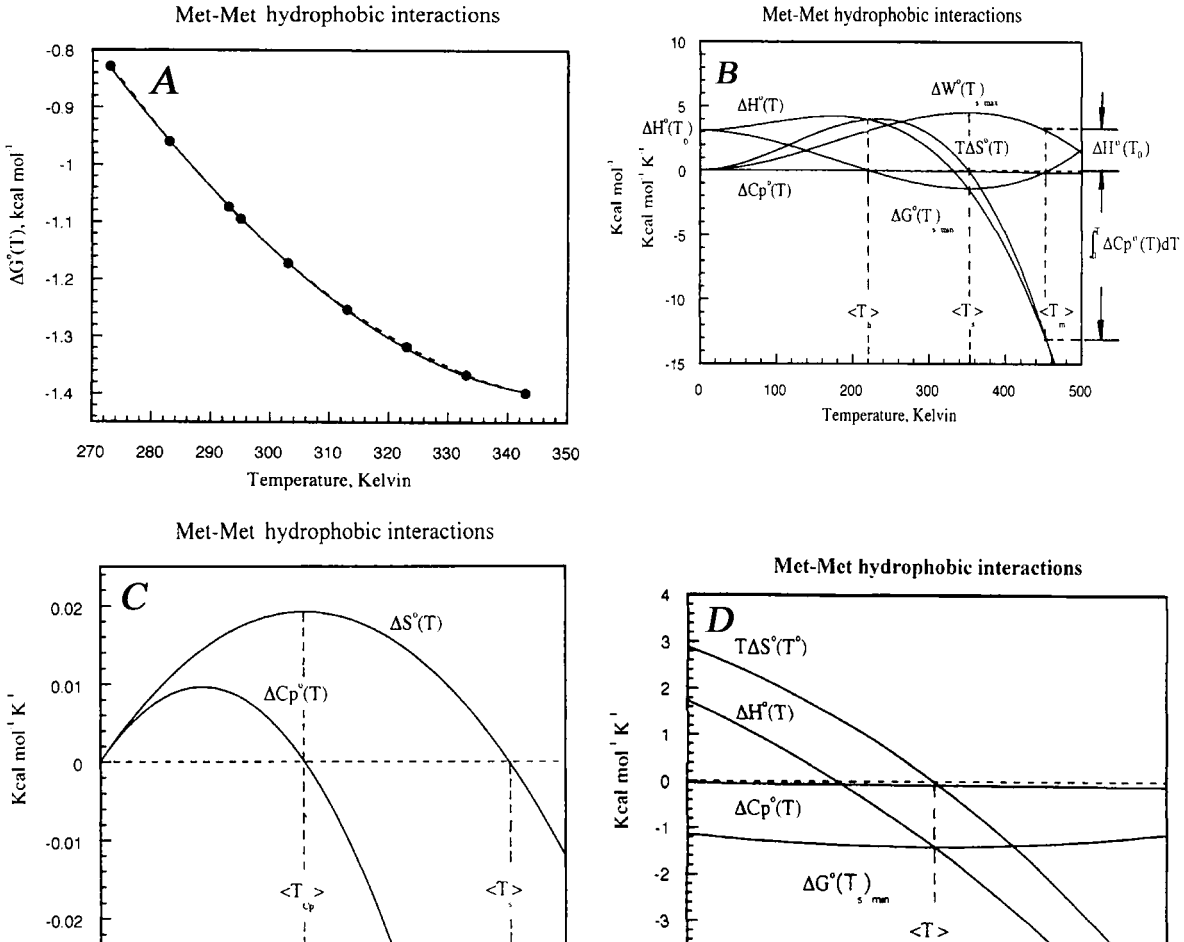

FIGURE 2 (*A*) Thermodynamic plot of the standard Gibbs free energy change of *Met–Met* hydrophobic interaction. Values for $\Delta G^{o}(T)$ as a function of temperature were computed from Nemethy and Scheraga's Table 2 (1962a) in the temperature range of 273–345 K, using the general linear model (T^3 model) procedure of statistical analysis of IMSL subroutine. $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. (*B*) Thermodynamic plot of the Planck-Benzinger thermal work function for *Met–Met* hydrophobic interaction. Each data point between 0 and 500 K was evaluated with extrapolation of *F*-statistics (Chun, 1991; Barr et al., 1985). The solid line represents fitted data, $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. At $\langle T_m \rangle = 445$ K, $\Delta H^{o}(T_0) + \int_{T_0}^{T} \Delta Cp^{o}(T)dT = 3.10 - 14.26 = 11.26$ kcal mol^{-1}. (*C*) A closeup view of a portion of *Met–Met* hydrophobic interaction as shown in Fig. 2 *B*, over the temperature range of 0–400 K, with the magnitude of the *y*-axis reduced to 0.025 to –0.025 kcal mol^{-1}. The thermodynamic molecular switch occurs when $\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle = 175$ K, $\Delta Cp^{o}(T)$ changes sign from positive to negative, whereas $\Delta S^{o}(T) = 0$ or or $T\Delta S^{o}(T)$ changes from positive to negative at $\langle T_s \rangle = 350$ K. (*D*) A closeup view of a portion of *Met–Met* hydrophobic interaction as shown in Fig. 2 *B*, over the temperature range of 300–400 K, with the magnitude of the *y*-axis reduced to 4 to –4 kcal mol^{-1} K^{-1}.

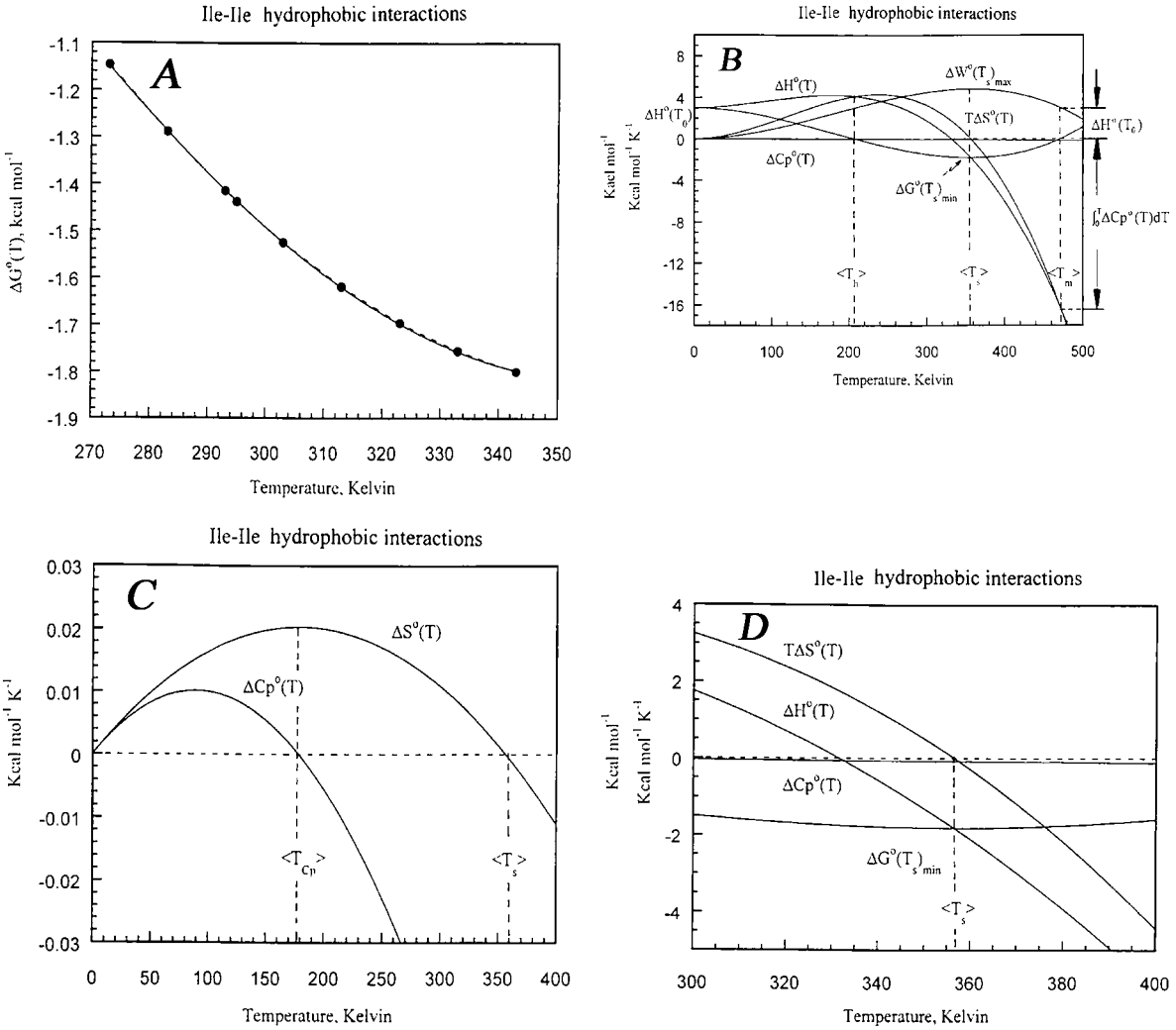

FIGURE 3 (*A*) Thermodynamic plot of the standard Gibbs free energy change of *Ile–Ile* hydrophobic interaction. Values for $\Delta G^{o}(T)$ as a function of temperature were computed from Nemethy and Scheraga's Table 2 (1962) in the temperature range of 273–345 K, using the general linear model (T^3 model) procedure of statistical analysis of IMSL subroutine. $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. (*B*) Thermodynamic plot of the Planck-Benzinger thermal work function for *Ile–Ile* hydrophobic interaction. Each data point between 0 and 500 K was evaluated with extrapolation of *F*-statistics (Chun, 1991; Barr et al., 1985). The solid line represents fitted data, $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. At $\langle T_m \rangle = 475$ K, $\Delta H^{o}(T_0) + \int_{T_0}^{T} \Delta Cp^{o}(T)dT = 2.99 - 17.10 = -14.11$ kcal mol^{-1}. (*C*) A closeup view of a portion of *Ile–Ile* hydrophobic interaction as shown in Fig. 3 *B*, over temperature range of 0–400 K, with the magnitude of the *y*-axis reduced to 0.03 to –0.03 kcal mol^{-1}. The thermodynamic molecular switch occurs when $\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle = 180$ K, $\Delta Cp^{o}(T)$ changes sign from positive to negative, whereas $\Delta S^{o}(T) = 0$ or $T\Delta S^{o}(T)$ changes from positive to negative at $\langle T_s \rangle = 355$ K. (*D*) A closeup view of a portion of *Ile–Ile* hydrophobic interaction as shown in Fig. 3 *B*, over the temperature range of 300–400 K, with the magnitude of the *y*-axis reduced to 4 to –4 kcal mol^{-1} K^{-1}.

A, the α, β, and γ coefficients were fitted to other thermodynamic parameters. $\Delta H^{o}(T)$, $\Delta Cp^{o}(T)$, $T\Delta S^{o}(T)$, and $\Delta W^{o}(T)$ are defined by Helmholtz-Kelvin expression as follows:

$$\Delta H^{o}(T) = \alpha - \beta T^2 - 2\gamma T^3, \quad (2)$$

$$\Delta Cp^{o}(T) = -2\beta T - 6\gamma T^2, \quad (3)$$

$$T\Delta S^{o}(T) = -2\beta T^2 - 3\gamma T^3, \quad (4)$$

$$\Delta W^{o}(T) = -\beta T^2 - \gamma T^3. \quad (5)$$

To extrapolate down to 0 K, it is necessary to consider normal solution states of macromolecules. Here the 0 K limit would presumably refer to the glassy condition (Chun, 1996), as described earlier.

A built-in restriction in the extrapolation procedure is that the values for $\Delta H^{o}(T)$ and $\Delta G^{o}(T)$ determined from the polynomial functions intersect at zero Kelvin with zero slope on a thermodynamic plot, thus obeying Planck's definition of the Nernst heat theorem (Planck via Ogg, 1927; Moelwyn-Hughes, 1957). By definition, the value of $\Delta H^{o}(T_0)$ will be positive. Other polynomial functions failed to meet all three restrictions of $\Delta H^{o}(T)$ and $\Delta G^{o}(T)$, intersecting at zero

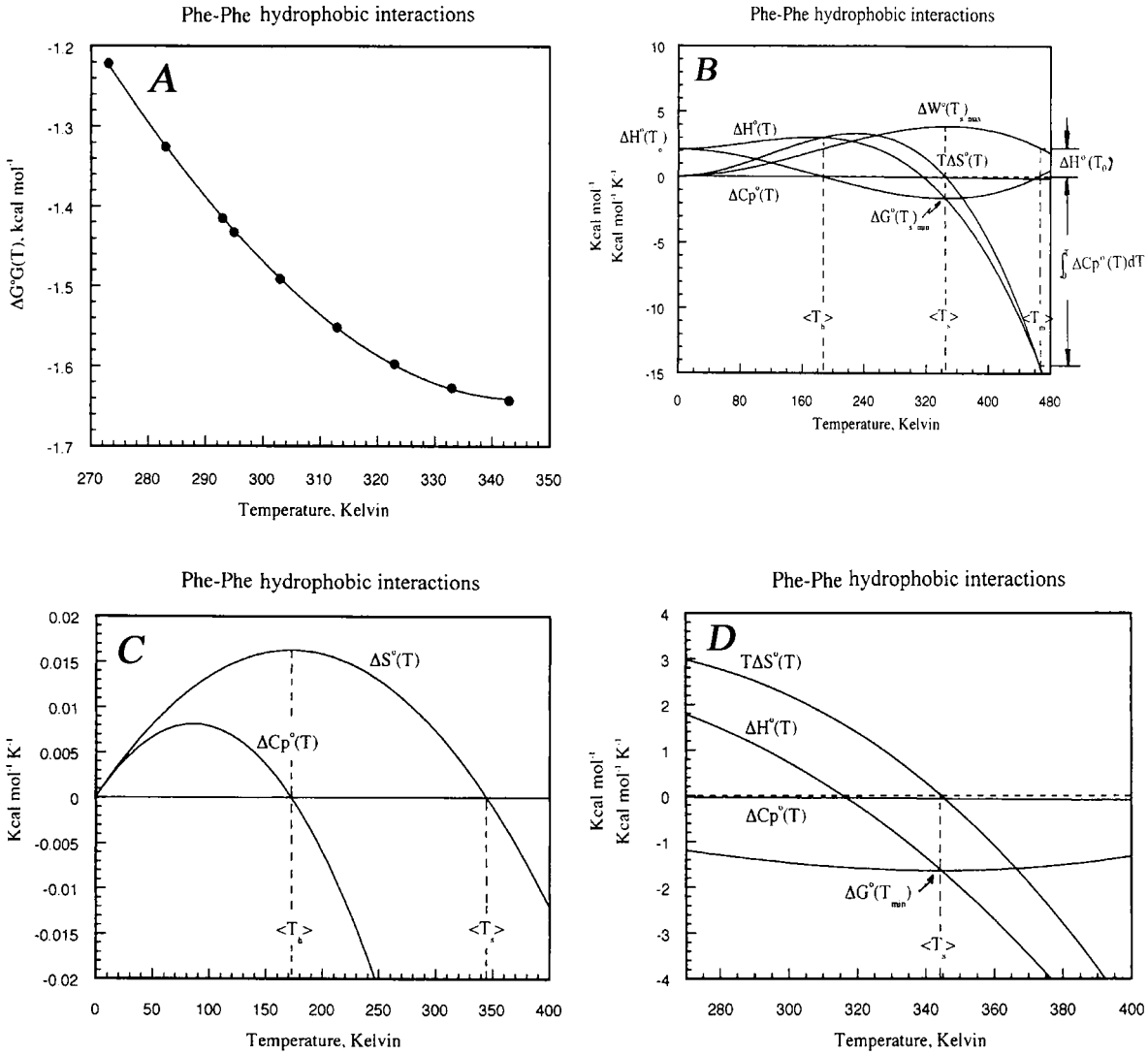

FIGURE 4 (*A*) Thermodynamic plot of the standard Gibbs free energy change of *Phe–Phe* hydrophobic interaction. Values for $\Delta G^{o}(T)$ as a function of temperature were computed from Nemethy and Scheraga's Table 2 (1962) in the temperature range of 273–345 K, using the general linear model (T^3 model) procedure of statistical analysis of IMSL subroutine. $F = 0.0001$, thus the goodness of fit the experimental data was 99.9% or better in each case. (*B*) Thermodynamic plot of the Planck-Benzinger thermal work function for *Phe–Phe* hydrophobic interaction. Each data point between 0 and 480 K was evaluated with extrapolation of *F*-statistics (Chun, 1991; Barr et al., 1985). The solid line represents fitted data, $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. At $\langle T_m \rangle = 465$ K, $\Delta H^{o}(T_0) + \int_{T_0}^{T} \Delta Cp^{o}(T)dT = 2.20 - 14.20 = -12.00$ kcal mol^{-1}. (*C*) A closeup view of a portion of *Phe–Phe* hydrophobic interaction as shown in Fig. 4 *B*, over temperature range of 0–400 K, with the magnitude of the *y*-axis reduced to 0.02 to –0.02 kcal mol^{-1}. The thermodynamic molecular switch occurs when $\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle = 175$ K, $\Delta Cp^{o}(T)$ changes sign from positive to negative, whereas $\Delta S^{o}(T) = 0$ or $T\Delta S^{o}(T)$ changes from positive to negative at $\langle T_s \rangle = 355$ K. (*D*) A closeup view of a portion of *Phe–Phe* hydrophobic interaction as shown in Fig. 4 *B*, over the temperature range of 280–400 K, with the magnitude of the *y*-axis reduced to 4 to –2 kcal mol^{-1} K^{-1}.

Kelvin with zero slope and $\Delta H^{o}(T_0)$ being positive, and thus were discarded.

It is clear that a temperature-dependent model simpler than the third-order Gibbs polynomial model (T^3 model) cannot be used at low temperature, and has been found to be unacceptable at room temperature; therefore it would be reasonable to apply a more complex model in the intervening temperature region only if the facts demand a more complex fit. The facts do not require a different function; the model as described has been found to have both strong correlative power and very good predictive power.

The fitted thermal data [$\Delta G^{o}(T)$, $\Delta H^{o}(T)$, $\Delta W^{o}(T)$, $T\Delta S^{o}(T)$, and $\Delta Cp^{o}(T)$] are reasonable not only over the measured experimental range (near room temperature) but also in the low temperature limit. The fitting curves do nothing strange in the experimentally inaccessible region, but rather smoothly approach the low temperature limit. The thermal dependency presented here is the best (and essentially the only) approach known.

In the thermodynamic methods based on the development of Planck-Benzinger methodology (Chun, 1988–1999; 2000a,b,c; 2001a,b; 2002), computed values must be in

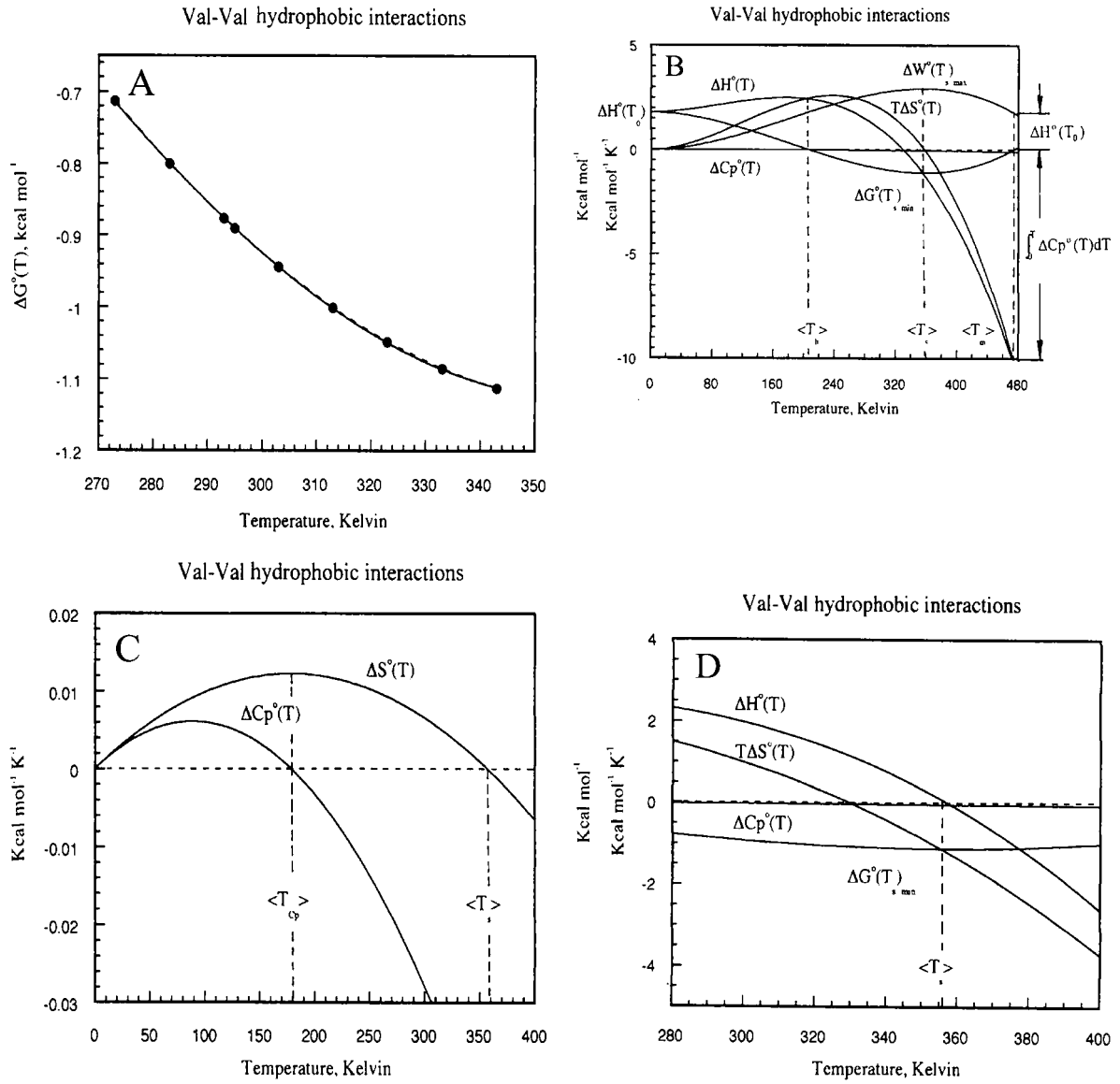

FIGURE 5 (*A*) Thermodynamic plot of the standard Gibbs free energy change of *Val–Val* hydrophobic interaction. Values for $\Delta G^{o}(T)$ as a function of temperature were computed from Nemethy and Scheraga's Table 2 (1962) in the temperature range of 273–345 K, using the general linear model (T^3 model) procedure of statistical analysis of IMSL subroutine. $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. (*B*) Thermodynamic plot of the Planck-Benzinger thermal work function for *Val–Val* hydrophobic interaction. Each data point between 0 and 480 K was evaluated with extrapolation of *F*-statistics (Chun, 1991; Barr et al., 1985). The solid line represents fitted data, $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. At $\langle T_m \rangle = 475$ K, $\Delta H^{o}(T_0) + \int_{T_0}^{T} \Delta Cp^{o}(T)dT = 1.80 - 10.30 = -8.50$ kcal mol^{-1}. (*C*) A closeup view of a portion of *Val–Val* hydrophobic interaction as shown in Fig. 5 *B*, over temperature range of 0–400 K, with the magnitude of the *y*-axis reduced to 0.02 to –0.02 kcal mol^{-1}. The thermodynamic molecular switch occurs when $\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle = 180$ K, $\Delta Cp^{o}(T)$ changes sign from positive to negative, whereas $\Delta S^{o}(T) = 0$ or $T\Delta S^{o}(T)$ changes from positive to negative at $\langle T_s \rangle = 355$ K. (*D*) A closeup view of a portion of *Val–Val* hydrophobic interaction as shown in Fig. 5 *B*, over the temperature range of 280–400 K, with the magnitude of the *y*-axis reduced to 2 to –2 kcal mol^{-1} K^{-1}.

agreement with experimentally obtained K_{eq} values (and therefore with values of $\Delta G^{o}(T)$ of reaction) of 35 pair-wise dipeptide interactions over a temperature range of 273–343 K.

Why is Helmholtz-Kelvin's expression considered to be a continuous function?

It is true that a change of phase for a specific chemical substance causes an infinite discontinuity in *Cp*, an infinite discontinuity in *S* and *H*, and an abrupt change in the slope of *G* versus *T*. Similar relevant data for phase changes in biological reactions are generally lacking; the reference condition at 0 K is taken as a glassy solid. In this case, all thermodynamic functions are continuous down to 0 K.

Evaluation of the innate temperature-invariant enthalpy for hydrogen-bonded water, in equilibrium with nonhydrogen-bonded water molecules, is based on Helmholtz free energy data reported by Nemethy and Scheraga (1962b). The

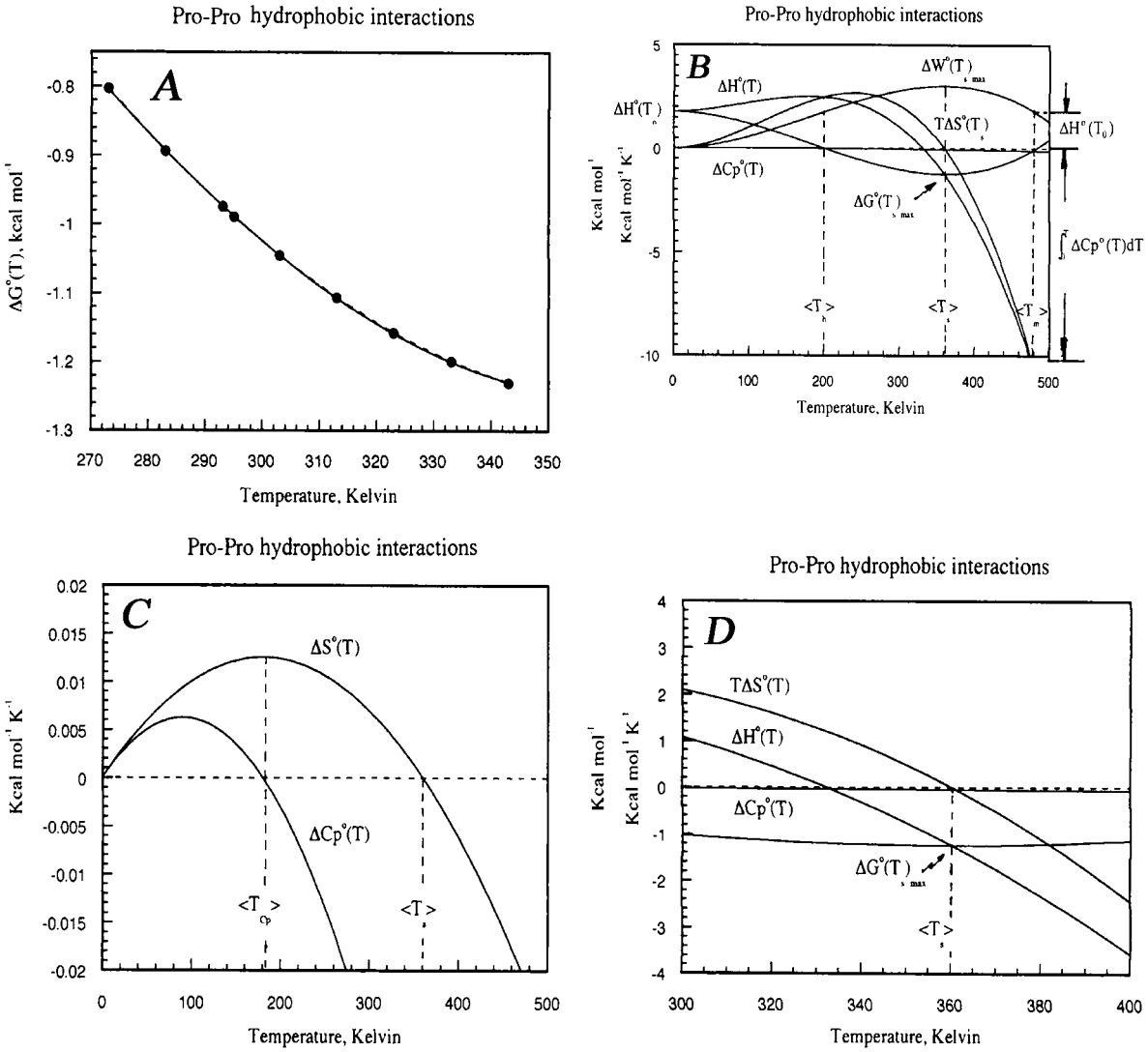

FIGURE 6 *A*. Thermodynamic plot of the standard Gibbs free energy change of *Pro–Pro* hydrophobic interaction. Values for $\Delta G^{o}(T)$ as a function of temperature were computed from Nemethy and Scheraga's Table 2 (1962) in the temperature range of 273–345 K, using the general linear model (T^3 model) procedure of statistical analysis of IMSL subroutine. $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. (*B*) Thermodynamic plot of the Planck-Benzinger thermal work function for *Pro–Pro* hydrophobic interaction. Each data point between 0 and 500 K was evaluated with extrapolation of *F*-statistics (Chun, 1991; Barr et al., 1985). The solid line represents fitted data, $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. At $\langle T_m \rangle = 480$ K, $\Delta H^{o}(T_0) + \int_{T_0}^{T} \Delta Cp^{o}(T)dT = 1.78 - 10.65 = -8.87\,\text{kcal mol}^{-1}$. (*C*) A closeup view of a portion of *Pro–Pro* hydrophobic interaction as shown in Fig. 6 *B*, over temperature range of 0–500 K, with the magnitude of the *y*-axis reduced to 0.02 to –0.02 kcal mol^{-1}. The thermodynamic molecular switch occurs when $\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle = 185$ K, $\Delta Cp^{o}(T)$ changes sign from positive to negative, whereas $\Delta S^{o}(T) = 0$ or $T\Delta S^{o}(T)$ changes from positive to negative at $\langle T_s \rangle = 355$ K. (*D*) A closeup view of a portion of *Pro–Pro* hydrophobic interaction as shown in Fig. 6 *B*, over the temperature range of 280–400 K, with the magnitude of the *y*-axis reduced to 2 to –2 kcal mol^{-1} K^{-1}.

entropy of the system appears to remain independent of temperature, suggesting that there is no significant temperature-dependent difference in the degree of orientation between unbound and hydrogen bound water molecules in equilibrium in the system. A similar conclusion could be reached based on the dielectric relaxation of water as a function of temperature, as reported by Collie, Hasted, and Ritson (1948). This implies that there is a nonzero entropy difference for the transformation between water monomer and *n*-mer at 0 K (Chun, 1996b). As with most small molecules, the thermal agitation energy is minimal. $\Delta W(T) = T\Delta S(T)$, $\Delta H(T) = \Delta H(T_0)$, over the entire temperature range from 0 K to the temperature of interest. $\Delta S(T)$ obtained from Helmholtz free energy data (Nemethy and Scheraga 1962b) and $\Delta S^{o}(T)$ from dielectric relaxation data remain constant and have values of 17.44 cal mol^{-1} deg^{-1} and 4.98 cal mol^{-1} deg^{-1}, respectively. These values are independent of temperature and can be extrapolated to 0 K (Chun, 1996b). There may be a small, finite $\Delta S^{o}(T)$ or ΔS_0 of reaction. For these dilute systems of macro-

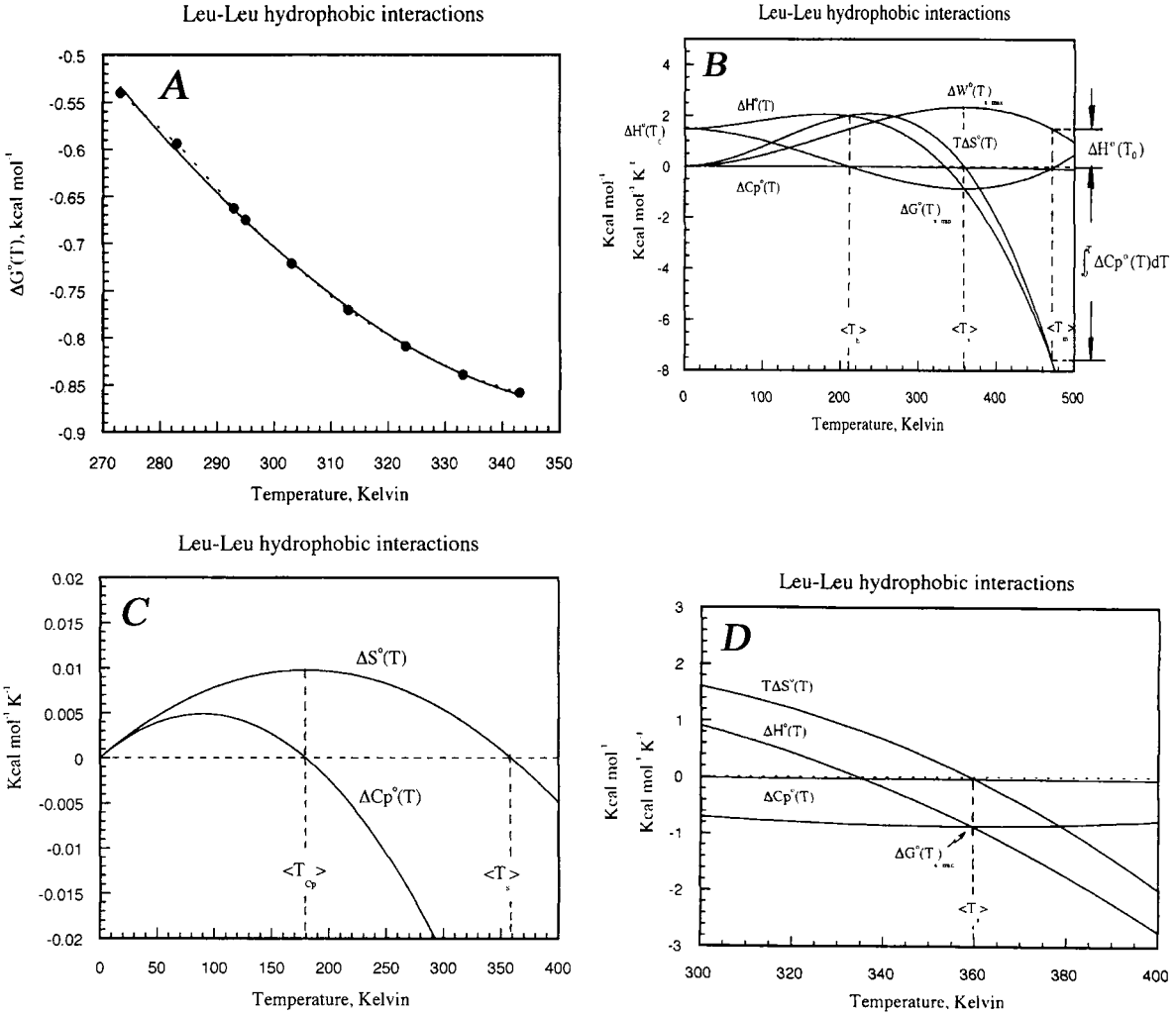

FIGURE 7 (*A*) Thermodynamic plot of the standard Gibbs free energy change of *Leu–Leu* hydrophobic interaction. Values for $\Delta G^{o}(T)$ as a function of temperature were computed from Nemethy and Scheraga's Table 2 (1962) in the temperature range of 273–345 K, using the general linear model (T^3 model) procedure of statistical analysis of IMSL subroutine. $F = 0.0001$, thus the goodness of fit the experimental data was 99.9% or better in each case. (*B*) Thermodynamic plot of the Planck-Benzinger thermal work function for *Leu–Leu* hydrophobic interaction. Each data point between 0 and 500 K was evaluated with extrapolation of *F*-statistics (Chun, 1991; Barr et al., 1985). The solid line represents fitted data, $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. At $\langle T_m \rangle = 575$ K, $\Delta H^{o}(T_0) + \int_{T_o}^{T} \Delta Cp^{o}(T)dT = 1.46 - 7.90 = -6.44$ kcal mol^{-1}. (*C*) A closeup view of a portion of *Leu–Leu* hydrophobic interaction as shown in Fig. 7 *B*, over temperature range of 0–500 K, with the magnitude of the *y*-axis reduced to 0.02 to –0.02 kcal mol^{-1}. The thermodynamic molecular switch occurs when $\Delta Cp^{o}(T) = 0$ at 175 K, $\Delta Cp^{o}(T)$ changes sign from positive to negative, whereas $\Delta S^{o}(T) = 0$ or $T\Delta S^{o}(T)$ changes from positive to negative at $\langle T_s \rangle = 355$ K. (*D*) A closeup view of a portion of *Leu–Leu* hydrophobic interaction as shown in Fig. 7 *B*, over the temperature range of 280–400 K, with the magnitude of the *y*-axis reduced to 3 to –3 kcal mol^{-1} K^{-1}.

molecules the $(\Delta Cp^{o}/T)$ is largely controlled by the solvent water. As already noted, the assumed reference condition is glassy ice at 0 K, which probably does have a small $\Delta S^{o}(T)$. However, $T\Delta S^{o}(T_0) = 0$, in any event. In the case of $\Delta G^{o}(T)$ of formation, phase transition is indeed important. When dealing with $\Delta G^{o}(T)$ of reaction, no phase transition is taking place. In this case, all thermodynamic functions are continuous.

Analysis of Planck-Benzinger thermal work function

A plot of the Gibbs polynomial function, $\Delta G^{o}(T) = \alpha + \beta T^2 + \gamma T^3$, as a function of temperature exhibits an initial value of zero for $\Delta G^{o}(T)$ at $\langle T_h \rangle$, a negative minimum value for $\Delta G^{o}(T)$ at $\langle T_s \rangle$, and the $\Delta G^{o}(T)$ value again reaches zero at $\langle T_m \rangle$ as seen in Figs. 2 *B*–9 *B*. Here $\langle T_s \rangle$ is the stable temperature at which $T\Delta S^{o}(T) = 0$. $\langle T_m \rangle$, as $\Delta G^{o}(T) = 0$, is the temperature at which interaction ceases and $\Delta H^{o}(T_h)(-)$ intersects $T\Delta S^{o}(T_m)(-)$. $\langle T_h \rangle$ is the harmonious temperature at which $\Delta G^{o}(T)$ approaches zero, and $\Delta H^{o}(T_h)(+)$ intersects $T\Delta S^{o}(T_h)(+)$. Values of the innate temperature-invariant enthalpy at $\langle T_h \rangle$, $\langle T_s \rangle$, and $\langle T_m \rangle$ are compared with those at zero K as shown in Figs. 2 *B*–9 *B* (see Tables 3, 4, and 7). The change in inherent chemical bond energy at 0 K, $\Delta H^{o}(T_0)$, ranges from 3.0 kcal mol^{-1} for *Met-Met* or *Ile-Ile* to a low of 0.5 kcal mol^{-1} for *Phe-Ala*, with the values

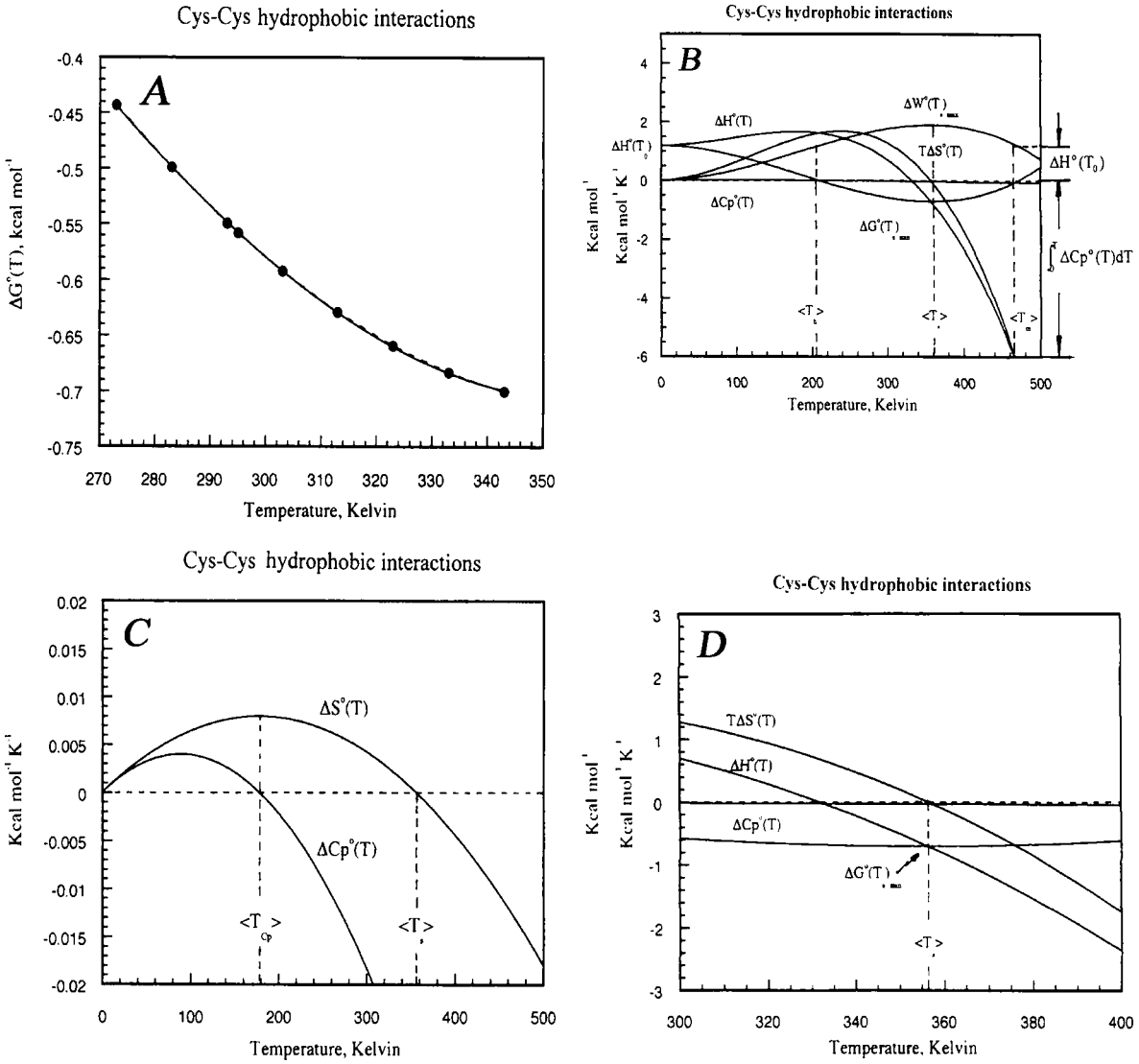

FIGURE 8 (*A*) Thermodynamic plot of the standard Gibbs free energy change of *Cys–Cys* hydrophobic interaction. Values for $\Delta G^{o}(T)$ as a function of temperature were computed from Nemethy and Scheraga's Table 2 (1962) in the temperature range of 273–345 K, using the general linear model (T^3 model) procedure of statistical analysis of IMSL subroutine. $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. (*B*) Thermodynamic plot of the Planck-Benzinger thermal work function for *Cys–Cys* hydrophobic interaction. Each data point between 0 and 500 K was evaluated with extrapolation of *F*-statistics (Chun, 1991; Barr et al., 1985). The solid line represents fitted data, $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. At $\langle T_m \rangle = 475$ K, $\Delta H^{o}(T_0) + \int_{T_o}^{T} \Delta Cp^{o}(T)dT = 1.17 - 6.60 = -5.43$ kcal mol^{-1}. (*C*) A closeup view of a portion of *Cys–Cys* hydrophobic interaction as shown in Fig. 8 *B*, over temperature range of 0–500 K, with the magnitude of the *y*-axis reduced to 0.02 to –0.02 kcal mol^{-1}. The thermodynamic molecular switch occurs when $\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle = 175$ K, $\Delta Cp^{o}(T)$ changes sign from positive to negative, whereas $\Delta S^{o}(T) = 0$ or $T\Delta S^{o}(T)$ changes from positive to negative at $\langle T_s \rangle = 355$ K. (*D*) A closeup view of a portion of *Cys–Cys* hydrophobic interaction as shown in Fig. 8 *B*, over the temperature range of 280 to 400 K, with the magnitude of the *y*-axis reduced to 2 to –2 kcal mol^{-1} K^{-1}.

decreasing as length of the hydrophobic side chains decreases (see Tables 4 and 7). At $\langle T_m \rangle$, the thermal agitation energy for the series of 35 dipeptides is four to five times greater than $\Delta H^{o}(T_0)$. It is clear that the strength and stability of the hydrophobic interaction is determined by the packing density of the side chains, with *Phe-Ala* being the most preferred conformation. Additionally, the thermal agitation energy for the same series of pair-wise sequence-specific hydrophobic interactions, evaluated at $\langle T_m \rangle$, decreases in the same order; that is, as the length of the side chain decreases.

In the eight pairs of identical dipeptides shown in Table 4, $\langle T_h \rangle$, $\langle T_s \rangle$, and $\langle T_m \rangle$ remain relatively constant, with $\langle T_h \rangle$ being close to 210 K, $\langle T_s \rangle$ close to 360 K and $\langle T_m \rangle$ close to 475 K. The exceptions are *Met-Met* and *Pro-Pro*, as shown in the Table. These results suggest that each of these dipeptide molecules may have a slightly different conformational orientation.

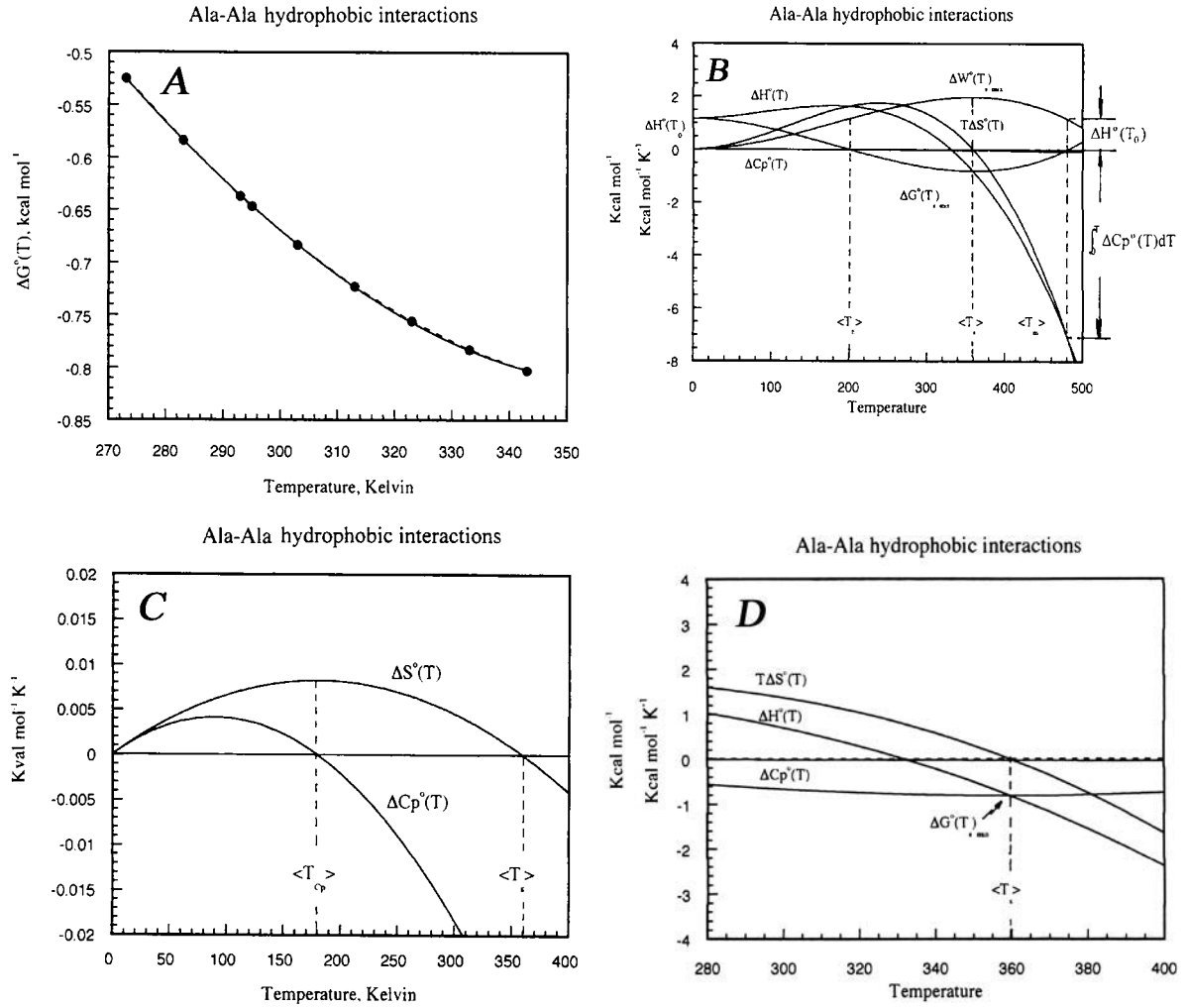

FIGURE 9 (*A*) Thermodynamic plot of the standard Gibbs free energy change of *Ala–Ala* hydrophobic interaction. Values for $\Delta G^{o}(T)$ as a function of temperature were computed from Nemethy and Scheraga's Table 2 (1962) in the temperature range of 273–345 K, using the general linear model (T^3 model) procedure of statistical analysis of IMSL subroutine. $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. (*B*) Thermodynamic plot of the Planck-Benzinger thermal work function for *Ala–Ala* hydrophobic interaction. Each data point between 0 and 500 K was evaluated with extrapolation of *F*-statistics (Chun, 1991; Barr et al., 1985). The solid line represents fitted data, $F = 0.0001$, thus the goodness of fit of the experimental data was 99.9% or better in each case. At $\langle T_m \rangle = 475$ K, $\Delta H^{o}(T_0) = \int_{T_o}^{T} \Delta Cp^{o}(T)dT = 1.16 - 6.70 = -5.54$ kcal mol^{-1}. (*C*) A closeup view of a portion of *Ala–Ala* hydrophobic interaction as shown in Fig. 9 *B*, over temperature range of 0 to 400 K, with the magnitude of the *y*-axis reduced to 0.02 to –0.02 kcal mol^{-1}. The thermodynamic molecular switch occurs when $\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle = 175$ K, $\Delta Cp^{o}(T)$ changes sign from positive to negative, whereas $\Delta S^{o}(T) = 0$ or $T\Delta S^{o}(T)$ changes from positive to negative at $\langle T_s \rangle = 355$ K. (*D*) A closeup view of a portion of *Ala–Ala* hydrophobic interaction as shown in Fig. 9 *B*, over the temperature range of 280–400 K, with the magnitude of the *y*-axis reduced to 4 to –4 kcal mol^{-1} K^{-1}.

In dipeptide pairs with *Phe* at the N-terminus and a single amino acid substituted at the C- terminus, shown in Table 7, $\langle T_h \rangle$ values range from 190 to 230 K, inasmuch as $\langle T_s \rangle$ runs from 345 to 360 K, and $\langle T_m \rangle$ ranges from 443 to 460 K. The values of the inherent chemical bond energy range from 2.2 kcal mol^{-1} in *Phe-Ile* to 0.5 kcal mol^{-1} in *Phe-Ala*, suggesting that each of these dipeptide molecules may have a preferred orientation in sequence-specific interactions.

It is also apparent that when either *Ala* or *Val* is in the N-terminus, the interactions are the same independent of the hydrophobic amino acids in the C-terminus position, as seen in Tables 10 and 11. Even the inherent chemical bond energies show little deviation from 1.2 kcal mol^{-1} for the *Ala-X* and 2.2 kcal mol^{-1} in the *Val-X* group, with *X* being the hydrophobic group.

1. $\Delta H^{o}(T_0) = \Delta W^{o}(T_h)$, $\Delta H^{o}(T_h)(+)$ intersects $T\Delta S^{o}(T_h)(+)$, $\Delta G^{o}(T) = 0$ at $\langle T_h \rangle$.
2. $\Delta H^{o}(T_0) = \Delta W^{o}(T_s)_{max} + \Delta G^{o}(T_s)_{min}$, $\Delta H^{o}(T_s) = \Delta G^{o}(T_s)_{min}$ at $\langle T_s \rangle$.
3. $\Delta H^{o}(T_0) = \Delta W^{o}(T_m)$, $\Delta H^{o}(T_m)(-)$ intersects $T\Delta S^{o}(T_m)(-)$, $\Delta G^{o}(T) = 0$ at $\langle T_m \rangle$ and one can define the heat of

TABLE 2 Evaluation of $\Delta H^o(T_0)$ and expansion coefficients of the Planck-Benzinger thermal work function for pair-wise (dipeptide), hydrophobic interactions

Dipeptide pair	α,[$\Delta H^o(T_0)$] [kcal mol^{-1}]	β (kcal mol^{-1} K^{-2})	γ (kcal mol^{-1} K^{-3})
Met-Met	3.0966	-1.0930×10^{-4}	2.0730×10^{-7}
Ile-Ile	3.0005	-1.1368×10^{-4}	2.1255×10^{-7}
Ile-Met	2.9978	-1.1013×10^{-4}	2.0712×10^{-7}
Leu-Met	2.4418	-9.9294×10^{-5}	1.6835×10^{-7}
Met-Pro	2.4344	-8.9712×10^{-5}	1.6891×10^{-7}
Leu-Ile	2.4183	-8.9841×10^{-5}	1.6883×10^{-7}
Val-Met	2.3945	-9.1367×10^{-5}	1.7071×10^{-7}
Ile-Pro	2.3855	-9.1759×10^{-5}	1.7157×10^{-7}
Val-Ile	2.2953	-8.8689×10^{-5}	1.6537×10^{-7}
Phe-Ile	2.1882	-7.4297×10^{-5}	1.4309×10^{-7}
Phe-Met	2.1782	-7.4297×10^{-5}	1.4309×10^{-7}
Phe-Phe	2.0917	-9.4230×10^{-5}	1.8222×10^{-7}
Leu-Pro	1.8418	-6.9315×10^{-5}	1.3038×10^{-7}
Val-Leu	1.8242	-6.7305×10^{-5}	1.2662×10^{-7}
Val-Val	1.7901	-6.8521×10^{-5}	1.2287×10^{-7}
Val-Pro	1.7771	-6.8797×10^{-5}	1.2815×10^{-7}
Pro-Pro	1.7587	-6.9482×10^{-5}	1.2854×10^{-7}
Met-Cys	1.6558	-5.470×10^{-5}	1.0428×10^{-7}
Phe-Leu	1.6290	-5.6113×10^{-5}	1.0777×10^{-7}
Phe-Pro	1.6276	-5.9362×10^{-5}	1.1268×10^{-7}
Leu-Cys	1.6242	-5.6292×10^{-5}	1.0596×10^{-7}
Phe-Val	1.5992	-5.7444×10^{-5}	1.0924×10^{-7}
Val-Cys	1.5810	-5.7215×10^{-5}	1.0692×10^{-7}
Ile-Cys	1.5731	-5.5970×10^{-5}	1.0492×10^{-7}
Leu-Leu	1.4656	-5.4357×10^{-5}	1.0084×10^{-7}
Ala-Leu	1.2126	-4.4216×10^{-5}	8.3235×10^{-8}
Ala-Ile	1.1882	-4.4738×10^{-5}	8.3663×10^{-8}
Cys-Cys	1.1882	-4.4738×10^{-5}	8.3663×10^{-8}
Ala-Val	1.1806	-4.5154×10^{-5}	8.42219×10^{-8}
Ala-Ala	1.1606	-4.5582×10^{-5}	8.4213×10^{-8}
Ala-Pro	1.1601	-4.5794×10^{-5}	8.4883×10^{-8}
Ala-Met	1.1601	-4.5930×10^{-5}	8.4884×10^{-8}
Cys-Pro	1.1243	-4.4223×10^{-5}	8.2640×10^{-8}
Phe-Cys	1.0724	-3.7132×10^{-5}	7.1179×10^{-8}
Phe-Ala	0.5320	-1.8930×10^{-5}	3.6202×10^{-8}

Compiled using the general linear model procedure analysis of IMSL subroutine adapted for use in Kaleidagraph 3.5.1. Values for these $\Delta H^o(T_0)$ vary by less than 0.01%. $F = 0.0001$, thus the goodness of fit of the computed data was 99.9% or better in each case.

Dipeptide pair	Chi-square	R-square	F-statistics $PR > F$	$\Delta H^o(T_0)$ (Kcal mol^{-1})
Met-Met	3.0593×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	3.059
Ile-Ile	2.7401×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	3.000
Ile-Met	1.1750×10^{-4}	$R^2 = 0.9996$	$F = 0.0001$	2.998
Leu-Met	2.0429×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	2.442
Met-Pro	2.2078×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	2.434
Leu-Ile	1.9386×10^{-5}	$R^2 = 0.9996$	$F = 0.0004$	2.418
Val-Met	2.1804×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	2.395
Ile-Pro	1.1750×10^{-4}	$R^2 = 0.9996$	$F = 0.0004$	2.386
Val-Ile	5.0401×10^{-4}	$R^2 = 0.9979$	$F = 0.0020$	2.295
Phe-Ile	1.0642×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	2.188
Phe-Met	1.0642×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	2.178
Phe-Phe	1.6016×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	2.092
Leu-Pro	1.0630×10^{-4}	$R^2 = 0.9996$	$F = 0.0004$	1.842
Val-Leu	1.1755×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	1.824
Val-Val	9.0586×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.790
Val-Pro	1.3311×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.777
Pro-Pro	8.6280×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.759
Met-Cys	9.6108×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.656
Phe-Leu	9.1503×10^{-6}	$R^2 = 0.9993$	$F = 0.0002$	1.629
Phe-Pro	4.1956×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	1.629
Leu-Cys	1.0151×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	1.624
Phe-Val	1.0646×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	1.599
Val-Cys	1.3269×10^{-5}	$R^2 = 0.9999$	$F = 0.0001$	1.581
Ile-Cys	8.6832×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.573
Leu-Leu	1.2842×10^{-5}	$R^2 = 0.9993$	$F = 0.0007$	1.467
Ala-Leu	4.4920×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.213
Ala-Ile	4.2501×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.188
Cys-Cys	4.2501×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.188
Ala-Val	4.9610×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.180
Ala-Ala: (Ala-Pro)	4.4350×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.161
Ala-Met	4.4350×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.160
Cys-Pro	4.2501×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.124
Phe-Cys	7.0751×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	1.072
Phe-Ala	1.4849×10^{-6}	$R^2 = 0.9999$	$F = 0.0001$	0.532

Each data point between 0 and 480 K was evaluated with extrapolation of *F*-statistics.

reaction as $\Delta H^o(T) = \Delta H^o(T_0) + \int_{T_0}^{T} \Delta Cp^o(T)dT$.

4. $\Delta H^o(T_0)$ is evaluated at zero K (see Figs. 2 *B*–9 *B*). The values of $\Delta W^o(T)$ and $\Delta G^o(T)$ exhibit a positive maximum and negative minimum, respectively, at $\langle T_s \rangle$; therefore, the innate temperature-invariant enthalpy, $\Delta H^o(T_0) = \Delta W^o(T_s)_{max} + \Delta G^o(T_s)_{min}$ at $\langle T_s \rangle$. The innate temperature-invariant enthalpy at the melting temperature is, by the integrated Kirchhoff expression, $\Delta H^o(T) + \Delta H^o(T_0) + \int_0^T \Delta Cp^o(T)dT$, where $\Delta H^o(T)$ and $T\Delta S^o(T)$ are of the same magnitude, $\Delta W^o(T) + \Delta H^o(T_0)$ and $\Delta G^o(T)$ approaches zero. The nature of the biochemical thermodynamic compensation which takes place between $\langle T_h \rangle$ and $\langle T_m \rangle$ may be characterized by evaluating $\Delta H^o(T_0)$ and the heat capacity integrals (see Figs. 2 *B*–9 *B* and Tables 4, 5, 7, and 8).
5. As shown in Figs. 2 *D*–9 *D* and Table 1, in this hydrophobic interaction, at low temperature, $\Delta H^o(T)$ and $\Delta S^o(T)$ are both positive (entropy-driven process), becoming negative as temperature increases (enthalpy-driven process), whereas $\Delta G^o(T)$ changes from positive to negative then reaches a negative value of maximum magnitude at $\langle T_s \rangle$, and finally becomes positive as temperature increases (see Figs. 2 *D*–9 *D*). That is, process 1 goes to process 2, creating cooperative enthalpy-entropy compensation between $\langle T_h \rangle$ and $\langle T_m \rangle$, as shown in Table 1.

The Planck-Benzinger methodology provides a means of determining the innate temperature-invariant enthalpy, $\Delta H^o(T_0)$, thermal agitation energy, or the heat capacity integrals, $\int_0^T \Delta Cp^o(T)dT$, and allows precise determination

TABLE 3 Comparison of values of $\Delta H^o(T_0)$ at $\langle T_h \rangle$, $\langle T_s \rangle$, $\langle T_m \rangle$, and 0 K for pair-wise dipeptide hydrophobic interactions

Dipeptide pair	$\Delta H^o(T_0)$ at 0 K (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_h \rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_s \rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_m \rangle$ (kcal mol^{-1})	$\int \Delta Cp^o\,(T)dT$ (kcal mol^{-1})	$\langle T_h \rangle$ (K)	$\langle T_s \rangle$ (K)	$\langle T_m \langle$ (K)
Met-Met	3.10	3.10	3.10	3.10	−14.26	220	350	460
Ile-Ile	2.99	2.97	2.99	2.99	−17.1	210	355	475
Ile-Met	2.99	2.96	2.99	2.99	−14.8	210	360	465
Leu-Met	2.44	2.45	2.44	2.43	−12.13	215	355	465
Met-Pro	2.43	2.47	2.43	2.42	−12.15	215	355	460
Leu-Ile	2.42	2.43	2.42	2.42	−12.82	215	355	470
Val-Met	2.39	2.40	2.39	2.39	−13.58	210	355	475
Ile-Pro	2.39	2.38	2.39	2.32	−7.05	215	355	475
Val-Ile	2.29	2.30	2.29	2.29	−13.15	205	355	475
Phe-Ile	2.19	2.19	2.19	2.19	−8.41	230	345	443
Phe-Met	2.18	2.19	2.18	2.19	−7.80	230	345	443
Phe-Phe	2.10	2.10	2.20	2.20	−14.2	200	345	465
Leu-Pro	1.84	1.84	1.84	1.84	−9.95	210	355	470
Val-Leu	1.82	1.85	1.82	1.82	−9.08	215	355	465
Val-Val	1.79	1.83	1.80	1.76	−10.3	210	355	475
Val-Pro	1.78	1.79	1.78	1.79	−10.2	205	360	475
Pro-Pro	1.76	1.75	1.76	1.79	−10.65	200	360	480
Met-Cys	1.66	1.64	1.66	1.64	−5.93	230	350	445
Phe-Leu	1.63	1.63	1.63	1.63	−6.25	230	345	443
Phe-Pro	1.63	1.63	1.62	1.63	−7.75	220	350	460
Leu-Cys	1.62	1.64	1.62	1.60	−7.13	220	355	460
Phe-Val	1.60	1.61	1.60	1.60	−7.53	220	350	460
Val-Cys	1.58	1.58	1.58	1.54	−7.98	210	355	470
Ile-Cys	1.57	1.55	1.60	1.63	−7.05	215	355	460
Leu-Leu	1.42	1.46	1.47	1.45	−7.90	210	360	475
Ala-Leu	1.21	1.21	1.21	1.20	−5.98	215	355	465
Ala-Ile	1.19	1.20	1.19	1.19	−7.48	210	355	465
Cys-Cys	1.19	1.19	1.19	1.15	−6.60	210	355	475
Ala-Val	1.18	1.16	1.17	1.18	−6.70	205	355	475
Ala-Ala	1.16	1.17	1.16	1.16	−6.71	205	360	475
Ala-Pro	1.16	1.15	1.16	1.16	−7.06	200	360	480
Ala-Met	1.16	1.16	1.17	1.16	−7.01	205	360	475
Cys-Pro	1.12	1.13	1.12	1.12	−6.61	205	355	475
Phe-Cys	1.10	1.10	1.07	1.08	−4.12	227	350	450
Phe-Ala	0.53	0.53	0.53	0.53	−2.23	220	350	450

Compiled using the general linear model procedure of statistical analysis of IMSL subroutine adapted for use in Kaleidagraph 3.5.1.

of $\langle T_{Cp} \rangle$, $\langle T_h \rangle$, $\langle T_s \rangle$, and $\langle T_m \rangle$. It is a method for evaluating $[\Delta H^o_{298} - \Delta H^o(T_0)]$, the heat of reaction for biological molecules at room temperature, and provides for a better understanding of cooperative thermodynamic compensation.

Nemethy and Scheraga's method of analysis

Fitting the T^2 model of $\Delta G^o(T) = a + bT + cT^2$, for the Gibbs free energy as a function of temperature of Nemethy and Scheraga (1962a, Scheraga, 1994) and the T^3 model of

TABLE 4 Comparison of $\Delta H^o(T_0)$ at $\langle T_h \rangle$, $\langle T_s \rangle$, $\langle T_m \rangle$, and 0 K for pair-wise dipeptide (*X-X*) hydrophobic interactions

Dipeptide pair	$\Delta H^o(T_0)$ at 0 K	$\Delta H^o(T_0)$ at $\langle T_h \rangle$	$\Delta H^o(T_0)$ at $\langle T_s \rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_m \rangle$	$\int_O^T \Delta Cp^o(T)dT$	$\langle T_h \rangle$ (K)	$\langle T_s \rangle$ (K)	$\langle T_m \rangle$ (K)
Met-Met	3.10	3.10	3.10	3.00	−14.26	220	350	460
Ile-Ile	2.99	2.96	2.99	2.99	−17.10	210	355	475
Phe-Phe	2.10	2.10	2.20	2.20	−14.20	190	345	465
Val-Val	1.79	1.83	1.82	1.78	−10.30	210	355	475
Pro-Pro	1.76	1.75	1.76	1.79	−10.65	200	360	480
Leu-Leu	1.42	1.46	1.47	1.45	−7.90	210	360	475
Cys-Cys	1.19	1.19	1.16	1.16	−6.60	205	360	475
Ala-Ala	1.16	1.17	1.16	1.16	−6.70	210	360	475

Compiled using the general linear model procedure of statistical analysis of IMSL subroutine adapted for use in Kaleidagraph 3.5.1.

TABLE 5 Thermodynamic molecular switch in Gibbs free energy change

Dipeptide pair (*X-X*)	Thermodynamic quantities	Molecular switch at temperature (*K*)	Effect on sign	
			$\Delta G^{o}(T)$	$\Delta H^{o}(T)$
Met-Met	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	220	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	350	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	460	(−) → (+)	
	$\Delta H^{o}(T)$	333		(+) → (−)
Ile-Ile	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	210	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	355	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	475	(−) → (+)	
	$\Delta H^{o}(T)$	333		(+) → (−)
Phe-Phe	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	190	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	345	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	465	(−) → (+)	
	$\Delta H^{o}(T)$	317		(+) → (−)
Val-Val	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	210	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	355	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	465	(−) → (+)	
	$\Delta H^{o}(T)$	328		(+) → (−)
Pro-Pro	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	205	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	360	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	483	(−) → (+)	
	$\Delta H^{o}(T)$	333		(+) → (−)
Leu-Leu	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	210	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	360	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	475	(−) → (+)	
	$\Delta H^{o}(T)$	334		(+) → (−)
Cys-Cys	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	210	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	355	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	475	(−) → (+)	
	$\Delta H^{o}(T)$	333		(+) → (−)
Ala-Ala	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	200	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	360	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	480	(−) → (+)	
	$\Delta H^{o}(T)$	333		(+) → (−)

$\Delta G^{o}(T) = \alpha + \beta T^2 + \gamma T^3$, based on the Planck-Benzinger methodology, it is apparent from Figs. 2 *A*–9 *A* that both models fit the theoretically computed $\Delta G^{o}(T)$ data equally well over the temperature range of 273–343 K.

In extrapolating Nemethy and Scheraga's data to 0 K to evaluate the thermodynamic parameters, fit of the data for heat capacity versus temperature is no longer fully adequate, therefore an unacceptable prediction of entropy is to be expected. The values for $\Delta H^{o}(T)$ and $\Delta G^{o}(T)$ determined from a linear T^2 polynomial function do not intersect at 0 K with zero slope and thus are inconsistent with Planck's definition of the Nernst heat theorem (Planck via Ogg, 1927). Furthermore, no thermodynamic molecular switch is observed.

The origin of the Gibbs free energy function in interacting biological systems

$\Delta H^{o}(T_0)$ and $\Delta S^{o}(T)$ are simple fundamental thermodynamic functions. In each case the respective value at a given temperature is determined in a straightforward way using the following expression:

$$[\Delta H^{o}_{T} - \Delta H^{o}(T_0)] = \int_0^T \Delta Cp^{o}(T), \quad \text{whereas}$$

$$\Delta S^{o}_{T} = \int_0^T (\Delta Cp^{o}/T)dT.$$

Note that the value of the enthalpy change at 0 K, $\Delta H^{o}(T_0)$, is important, but distinct and separate from the thermal agitation term. Many authors have entirely ignored this, particularly when dealing with biological systems.

In contrast, the Gibbs free energy function is a composite quantity, defined as a tradeoff of $\Delta H^{o}(T_0)$ and $\Delta S^{o}(T)$ terms:

$$\Delta G^{o}(T) = \Delta H^{o}(T) - T\Delta S^{o}(T)$$

$$\Delta G^{o}(T) = \Delta H^{o}(T_0) + \int_0^T \Delta Cp^{o}dT - T\int_0^T (\Delta Cp^{o}/T)dT$$

TABLE 6 Thermodynamic molecular switch in $\Delta Cp^o(T)$ and $\Delta S^o(T)$

Dipeptide pair (*X-X*)	Thermodynamic quantities	Molecular switch at temperature (*K*)	Effect on sign of $\Delta Cp^o(T)$ or $\Delta S^o(T)$
Met-Met	$\Delta Cp^o(T) = 0$ at $\langle T_{Cp}\rangle$	175	(+) → (−)
	$\Delta S^o(T) = 0$ at $\langle T_s\rangle$	350	(+) → (−)
Ile-Ile	$\Delta Cp^o(T) = 0$ at $\langle T_{Cp}\rangle$	175	(+) → (−)
	$\Delta S^o(T) = 0$ at $\langle T_s\rangle$	355	(+) → (−)
Phe-Phe	$\Delta Cp^o(T) = 0$ at $\langle T_{Cp}\rangle$	175	(+) → (−)
	$\Delta S^o(T) = 0$ at $\langle T_s\rangle$	345	(+) → (−)
Val-Val	$\Delta Cp^o(T) = 0$ at $\langle T_{Cp}\rangle$	175	(+) → (−)
	$\Delta S^o(T) = 0$ at $\langle T_s\rangle$	355	(+) → (−)
Pro-Pro	$\Delta Cp^o(T) = 0$ at $\langle T_{Cp}\rangle$	180	(+) → (−)
	$\Delta S^o(T) = 0$ at $\langle T_s\rangle$	360	(+) → (−)
Leu-Leu	$\Delta Cp^o(T) = 0$ at $\langle T_{Cp}\rangle$	175	(+) → (−)
	$\Delta S^o(T) = 0$ at $\langle T_s\rangle$	360	(+) → (−)
Cys-Cys	$\Delta Cp^o(T) = 0$ at $\langle T_{Cp}\rangle$	175	(+) → (−)
	$\Delta S^o(T) = 0$ at $\langle T_s\rangle$	355	(+) → (−)
Ala-Ala	$\Delta Cp^o(T) = 0$ at $\langle T_{Cp}\rangle$	175	(+) → (−)
	$\Delta S^o(T) = 0$ at $\langle T_s\rangle$	360	(+) → (−)

With proper rearrangement, this equation also yields the Giauque and Planck-Benzinger thermal work functions (Chun, 1988–1999; 2000a,b,c; 2001a,b; 2002). In consequence $\Delta G^o(T)$ displays an interesting variety of behavior patterns as the temperature changes. ΔG^o can change sign (K_{eq} from <1 to >1 or vice versa) only if ΔH^o and ΔS^o remain of the same sign (see Tables 1, 5, and 8, and Figs. 2 *D*–9 *D*). That is to say (*i*), if ΔH^o is (+) and ΔS^o is (+), then ΔG^o goes from (+), unfavorable to (−), which is favorable (from $K_{eq} < 1$ to $K_{eq} > 1$); and (*ii*) if ΔH^o is (−) and ΔS^o is (−), then ΔG^o goes from (−), favorable to (+), which is unfavorable (from $K_{eq} > 1$ to $K_{eq} < 1$).

In all biological interactions, $\Delta H^o(T)$ and $\Delta S^o(T)$ are positive at low temperature. As reaction temperature increases, both $\Delta H^o(T)$ and $\Delta S^o(T)$ become negative; that is process 1 of the chart goes to process 2: $\Delta H^o(+)$ and $\Delta S^o(+) \rightarrow \Delta H^o(-)$ and $\Delta S^o(-)$, creating a negative Gibbs free energy minimum as shown in Fig. 1 (see Table 1). In this thermodynamic switch unique to and characteristic of hydrophobic interaction, $\Delta Cp^o(T)$ changes from positive to negative at $\langle T_{Cp}\rangle = 0$, shown in Figs. 2 *C*–9 *C*. As seen in Tables 6 and 9, the Gibbs free energy change switches sign at $\langle T_h\rangle$ and $\langle T_m\rangle$, where $\Delta G^o(T) = 0$, and reaches a negative minimum at $\langle T_s\rangle$. The $\langle T_s\rangle$ values range from 345 K to 360 K at $T\Delta S^o(T) = 0$ (see Tables 6–9, and Figs. 2 *C*–9 *C*).

$$\Delta G^o(T)(+) \rightarrow \Delta G^o(T_s)_{min}(-) \rightarrow \Delta G^o(T)(+)$$

$$\Delta S^o(T)(+) \rightarrow \Delta S^o(T)(-) \text{ where } \Delta S^o = 0 \text{ at } \langle T_s\rangle.$$

At temperature $\langle T_s\rangle$, a simple algebraic sum of $\Delta W^o(T_s)$ and $\Delta G^o(T_s)_{min}$ yields the value of $\Delta H^o(T_0)$, i.e., $\Delta H^o(T_0) = \Delta W^o(T_s)_{max} + \Delta G^o(T_s)_{min}$. At $\langle T_s\rangle$ there exists an optimal balance of $\Delta H^o(T_s) = \Delta G^o(T_s)_{min}$ and $T\Delta S^o(T) = 0$, so there will be minimum negative Gibbs free energy change and the maximum work can be accomplished.

The results in the 35 dipeptide pairs examined as shown in Table 3 and Figs. 2 *C*–9 *C* suggest that the negative Gibbs free energy minimum at a well-defined $\langle T_s\rangle$ has its origin in the sequence-specific hydrophobic interactions, which are highly dependent on details of molecular structure.

A molecular-level thermodynamic switch controls chemical equilibrium in sequence-specific hydrophobic interactions

For the pair-wise, sequence-specific hydrophobic interactions examined, $\Delta Cp^o(T)$ reaches a maximum at ~90 K, inasmuch as the sign changes from positive to negative where $\langle T_{Cp}\rangle$ is ~180 K, the temperature at which $\Delta Cp^o(T) = 0$. Similarly, $\Delta S^o(T)$ reaches a maximum at 180 K, inasmuch as the sign changes from positive to negative at $\langle T_S\rangle = 345$–360 K, the temperature at which $T\Delta S^o(T) = 0$, as shown in Figs. 2 *C*–9 *C* (see Tables 6 and 9).

The significance of the negative Gibbs free energy minimum in equilibria of sequence-specific dipeptides and on a wider scope, of biochemical systems, is that one is dealing with a true condition of stability, that is, the maximum K_{eq} of the reaction. It is no surprise that the temperature of maximum stability should be found over

TABLE 7 Comparison of values of $\Delta H^o(T_0)$ at $\langle T_h\rangle$, $\langle T_s\rangle$, $\langle T_m\rangle$, and 0 K for pair-wise dipeptide (*Phe-X*) hydrophobic interactions

Dipeptide pair (*Phe-X*)	$\Delta H^o(T_0)$ at 0 K (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_h\rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_s\rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_m\rangle$ (kcal mol^{-1})	$\int \Delta Cp^o\,(T)dT$ (kcal mol^{-1})	$\langle T_h\rangle$ (*K*)	$\langle T_s\rangle$ (*K*)	$\langle T_m\rangle$ (*K*)
Phe-Phe	2.10	2.10	2.20	2.20	−14.2	190	345	465
Phe-Ile	2.19	2.19	2.19	2.19	−8.41	230	345	443
Phe-Met	2.18	2.19	2.18	2.19	−7.80	230	345	443
Phe-Leu	1.63	1.63	1.63	1.63	−6.25	230	345	443
Phe-Pro	1.63	1.63	1.62	1.63	−7.75	220	350	460
Phe-Val	1.60	1.61	1.60	1.60	−7.53	220	350	460
Phe-Cys	1.10	1.10	1.07	1.08	−4.12	227	350	450
Phe-Ala	0.53	0.53	0.53	0.53	−2.23	220	350	450

Compiled using the general linear model procedure of statistical analysis of IMSL subroutine adapted for use in Kaleidagraph 3.5.1.

TABLE 8 Thermodynamic molecular switch in Gibbs free energy change

Dipeptide pair (*Phe-X*)	Thermodynamic quantities	Molecular switch at temperature (*K*)	Effect on sign	
			$\Delta G^{o}(T)$	$\Delta H^{o}(T)$
Phe-Phe	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	190	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	345	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	465	(−) → (+)	
	$\Delta H^{o}(T)$	317		(+) → (−)
Phe-Ile	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	230	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	345	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	443	(−) → (+)	
	$\Delta H^{o}(T)$	327		(+) → (−)
Phe-Met	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	230	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	345	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	443	(−) → (+)	
	$\Delta H^{o}(T)$	328		(+) → (−)
Phe-Leu	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	230	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	345	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	443	(−) → (+)	
	$\Delta H^{o}(T)$	328		(+) → (−)
Phe-Pro	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	220	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	350	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	460	(−) → (+)	
	$\Delta H^{o}(T)$	328		(+) → (−)
Phe-Val	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	220	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	350	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	460	(−) → (+)	
	$\Delta H^{o}(T)$	333		(+) → (−)
Phe-Cys	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	227	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	350	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	450	(−) → (+)	
	$\Delta H^{o}(T)$	333		(+) → (−)
Phe-Ala	$\Delta G^{o}(T)$ at $\langle T_h \rangle$	220	(+) → (−)	
	$\Delta G^{o}(T)$ at $\langle T_s \rangle$	350	Negative minimum	
	$\Delta G^{o}(T)$ at $\langle T_m \rangle$	450	(−) → (+)	
	$\Delta H^{o}(T)$	328		(+) → (−)

TABLE 9 Thermodynamic molecular switch in $\Delta Cp^{o}(T)$ and $\Delta S^{o}(T)$

Dipeptide pair (*Phe-X*)	Thermodynamic quantities	Molecular switch at temperature (*K*)	Effect on sign of $\Delta Cp^{o}(T)$ or $\Delta S^{o}(T)$
Phe-Phe	$\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle$	175	(+) → (−)
	$\Delta S^{o}(T) = 0$ at $\langle T_s \rangle$	345	(+) → (−)
Phe-Ile	$\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle$	175	(+) → (−)
	$\Delta S^{o}(T) = 0$ at $\langle T_s \rangle$	345	(+) → (−)
Phe-Met	$\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle$	175	(+) → (−)
	$\Delta S^{o}(T) = 0$ at $\langle T_s \rangle$	345	(+) → (−)
Phe-Leu	$\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle$	175	(+) → (−)
	$\Delta S^{o}(T) = 0$ at $\langle T_s \rangle$	345	(+) → (−)
Phe-Pro	$\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle$	175	(+) → (−)
	$\Delta S^{o}(T) = 0$ at $\langle T_s \rangle$	350	(+) → (−)
Phe-Val	$\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle$	175	(+) → (−)
	$\Delta S^{o}(T) = 0$ at $\langle T_s \rangle$	350	(+) → (−)
Phe-Cys	$\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle$	175	(+) → (−)
	$\Delta S^{o}(T) = 0$ at $\langle T_s \rangle$	350	(+) → (−)
Phe-Ala	$\Delta Cp^{o}(T) = 0$ at $\langle T_{Cp} \rangle$	175	(+) → (−)
	$\Delta S^{o}(T) = 0$ at $\langle T_s \rangle$	350	(+) → (−)

a broad temperature range in biological systems, inasmuch as such systems are known to exist optimally from arctic temperatures to several hundred degrees Centigrade in fumerals of the ocean floor.

These data demonstrate that the critical factor is a temperature-dependent heat of reaction, $\Delta Cp^{o}(T)$, which is positive at low temperature but switches to a negative value at $\langle T_{Cp} \rangle = 180$ K. Note that this thermodynamic switch in the sign of $\Delta Cp^{o}(T)_{reaction}$ causes the change of behavior of $\Delta G^{o}(T)$, hence a change in the equilibrium constant, K_{eq}, and/or spontaneity as shown in Figs. 2 *C*–9 *C*. The subsequent, mathematically predictable changes in $\Delta H^{o}(T)$, $\Delta S^{o}(T)$, $\Delta W^{o}(T)$, and $\Delta G^{o}(T)$ give rise to the observed behavior patterns in pair-wise, sequence-specific hydrophobic interaction.

It is clear that nonlinear temperature dependence of $\Delta Cp^{o}(T)_{reaction}$ is the essence of the thermodynamic molecular switch. In the present instance, the hydrophobic interactions seem to be the best candidates for such behavior.

TABLE 10 Comparison of values of values of $\Delta H^o(T_0)$ at $\langle T_h \rangle$, $\langle T_s \rangle$, $\langle T_m \rangle$, and 0 K for pair-wise dipeptide (*Ala-X*) hydrophobic interactions

Dipeptide pair (*Ala-X*)	$\Delta H^o(T_0)$ at 0 K (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_h \rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_s \rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_m \rangle$ (kcal mol^{-1})	$\int \Delta Cp^o(T)dT$ (kcal mol^{-1})	$\langle T_h \rangle$	$\langle T_s \rangle$	$\langle T_m \rangle$
						(*K*)		
Ala-Leu	1.21	1.21	1.21	1.20	−5.98	215	355	465
Ala-Ile	1.19	1.20	1.19	1.19	−7.48	210	355	465
Ala-Val	1.18	1.16	1.17	1.18	−6.70	205	355	475
Ala-Ala	1.16	1.17	1.16	1.16	−6.71	205	360	475
Ala-Pro	1.16	1.15	1.16	1.16	−7.06	200	360	475
Ala-Met	1.16	1.16	1.17	1.16	−7.01	205	360	475

Compiled using the general linear model procedure of statistical analysis of IMSL subroutine adapted for use in Kaleidagraph 3.5.1.

CONCLUSION

The hydrophobic interaction in these sequence-specific dipeptide pairs—eight of them identical and eight with a single amino acid substituted—is highly similar in its thermodynamic behavior to that of other biological systems, except that the negative Gibbs free energy change minimum at $\langle T_s \rangle$ occurs at a considerably higher temperature, 345–360 K compared to ~300 K. The melting temperature, $\langle T_m \rangle$, is also high, 460–480 K compared to ~343 K in a biological system. The change in innate temperature-invariant enthalpy at 0 K ranges from 3.0 kcal mol^{-1} for *Met-Met* or *Ile-Ile* to a low of 0.5 kcal mol^{-1} for *Phe-Ala*, with the values decreasing as the length of the hydrophobic side chains decreases. At $\langle T_m \rangle$, the thermal agitation energy in these pairs is four to five times greater than $\Delta H^o(T_o)$ in each case, as was true for each of the 35 dipeptide pairs.

The results imply that the negative Gibbs free energy minimum at a well-defined $\langle T_s \rangle$ has its origin in the sequence-specific hydrophobic interactions, which are highly dependent on details of molecular structure.

We believe that we have uncovered a type of physical behavior characteristic of living systems which may prove to be remarkably general. It is, of course, known that living systems can live and operate optimally only at a sharply defined temperature, or over a limited temperature range at best. We assert that this implies that basic biochemical macromolecular interactions exhibit a well-defined negative Gibbs free energy change minimum (that is to say, favorable) at $\langle T_s \rangle$. Such a situation is not common in simple chemical systems, where a monotonic change of ΔG^o, ΔH^o, and K_{eq} over an experimental temperature range is typical.

We have found that the critical factor driving the pair-wise hydrophobic interaction of 35 dipeptides is a temperature-dependent heat capacity change of reaction, $\Delta Cp^o(T)_{reaction}$, which is positive at low temperature but switches to a negative value at a temperature well below the ambient range.

$$\Delta Cp^o(T)(+) \rightarrow \Delta Cp^o(T)(-) \text{ at low temperature}$$

This change of sign of the critically important $\Delta Cp^o(T)_{reaction}$ has such significant consequences that we refer to it as a thermodynamic molecular switch. It determines the behavior patterns of the Gibbs free energy change, and hence a change in the equilibrium constant, and/or spontaneity. All interacting biological systems we have thus far examined using with the Planck-Benzinger approach point to the universality of this thermodynamic switch.

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TABLE 11 Comparison of values of values of $\Delta H^o(T_0)$ at $\langle T_h \rangle$, $\langle T_s \rangle$, $\langle T_m \rangle$, and 0 K for pair-wise dipeptide (*Val-X*) hydrophobic interactions

Dipeptide pair (*Val-X*)	$\Delta H^o(T_0)$ at 0 K (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_h \rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_s \rangle$ (kcal mol^{-1})	$\Delta H^o(T_0)$ at $\langle T_m \rangle$ (kcal mol^{-1})	$\int \Delta Cp^o\ (T)dT$ (kcal mol^{-1})	$\langle T_h \rangle$	$\langle T_s \rangle$	$\langle T_m \rangle$
						(*K*)		
Val-Met	2.39	2.40	2.39	2.39	−13.58	210	355	475
Val-Ile	2.29	2.30	2.29	2.29	−13.15	205	355	475
Val-Leu	1.82	1.85	1.82	1.82	−9.08	215	355	465
Val-Val	1.79	1.82	1.80	1.76	−10.3	210	355	475
Val-Pro	1.78	1.79	1.78	1.79	−10.2	205	360	475
Val-Cys	1.58	1.58	1.58	1.54	−7.98	210	355	470

Compiled using the general linear model procedure of statistical analysis of IMSL subroutine adapted for use in Kaleidagraph 3.5.1.

REFERENCES

Barr, D. J., P. Goodnight, and J. T. Helwig. 1985. SASD GLM 27 and GLM 131 Statistical Analysis. SAS Institute Inc., Cary, NC.

Benzinger, T. H. 1971. Thermodynamics, chemical reactions, and molecular biology. *Nature (Lond.)*. 200:100–103.

Chun, P. W. 2002. Beyond the Planck-Benzinger thermal work function: new insights into the reaction of molecular switches in biology. Per-Olov Lowdin Memorial Symposium. *Intl. J. Quantum Chem.* 87:323–353.

Chun, P. W. 2001a. Thermodynamic molecular switch in micelles. *J. Colloids Surfaces.* 181:183–203.

Chun, P. W. 2001b. Thermodynamic molecular switch in sequence-specific hydrophobic interactions. *Intl. J. Quantum Chem.* 85:697–712.

Chun, P. W. 2000a. Thermodynamic molecular switch in biological systems: ribonuclease S' fragment complementation reactions. *Biophys. J.* 78:416–429.

Chun, P. W. 2000b. Thermodynamic molecular switch in macromolecular interactions. *Cell Biochem. Biophys.* 33:149–169.

Chun, P. W. 2000c. Thermodynamic molecular switch in biological systems. *Intl. J. Quantum Chem.* 80:1181–1198.

Chun, P. W. 1999. Uncovering the innate thermodynamic quantities in protein unfolding. *Intl. J. Quantum Chem.* 75:1027–1042.

Chun, P. W. 1998. Chapter 12. Application of Planck-Benzinger relationships to biology. *In* Methods in Enzymology. Academic Press, New York.

Chun, P. W. 1997. Planck-Benzinger thermal work function: thermodynamic approach to site-specific S-protein and S-peptides interactions in the ribonuclease S' system. *J. Phys. Chem. B.* 101:7835–7843.

Chun, P. W. 1996a. The Planck-Benzinger thermal work function: Determination of the thermodynamic stability of chymotrypsinogen A and ribonuclease A in glycerol. *J. Phys. Chem.* 100:7283–7292.

Chun, P. W. 1996b. New thermodynamic studies on hydrogen bond energy of liquid water, American Chemical Society, Biophysical Chemistry, poster 283, 212nd National American Chemical Society Meeting, Orlando, FL.

Chun, P. W. 1995. New thermodynamic studies on ribonuclease A at low pH. *J. Biol. Chem.* 270:13925–13931.

Chun, P. W. 1994. The Planck-Benzinger thermal work function: definition of temperature-invariant enthalpy in biological systems. *J. Phys. Chem.* 86:6851–6861.

Chun, P. W. 1991. Manual for computer-aided analysis of biochemical processes with Florida 1-2-4. University of Florida, copyright reserved.

Chun, P. W. 1988. Approximation of the Planck-Benzinger thermal work function in protein refolding in ribonuclease systems. *Intl. J. Quantum Chem.* 15:247–258.

Collie, C. H., J. B. Hasted, and D. M. Ritson. 1948. The dielectric properties of water and heavy water. *Proc. Phys. Soc.* 60:145–160.

Cottrell, T. L. 1958. Strength of Chemical Bonds. Academic Press, New York and London. pp21–70.

Giauque, W. F. 1930a. The calculation of free energy from spectroscopic data. *J. Am. Chem. Soc.* 52:4808–4815.

Giauque, W. F. 1930b. The entropy of hydrogen and the third law of thermodynamics, the free energy and dissociation of hydrogen. *J. Am. Chem. Soc.* 52:4816–4831.

Giauque, W. F., and R. W. Blue. 1930. Hydrogen sulfide, the heat capacity and vapor pressure of solid and liquid, the heat of vaporization, a comparison of thermodynamic and spectroscopic values of the entropy. *J. Am. Chem. Soc.* 58:831–837.

Giauque, W. F., and J. D. Kemp. 1938. Entropies of N_2O_4 and NO_2. Heat capacities from 15K to BP. Heat of vaporization and the vapor pressure-equilibrium $N_2O_4 = 2NO_2 = 2NO + O_2$. *J. Chem. Phys.* 6: 40–52.

Giauque, W. F., and P. F. Meads. 1941. The heat capacities and entropies of aluminum and copper from 15 to 300 K. *J. Am. Chem. Soc.* 63:1897–1901.

Gibbs, J. W. 1878. On the equilibrium of heterogeneous substances. Am. J. Sci 3 series 16: 441–458 via Trans. *Conn. Acad. Sci.* 3:228–232.

Lewis, G. N., and M. Randall. 1961. Thermodynamics. K. S. Pitzer, and L. Brewer, Editors. McGraw-Hill, New York. pp164–182; appendix, pp665–668.

Moelwyn-Hughes, E. A. 1957. Physical Chemistry. Pergamon Press, New York. pp90– 103, 264–279, 560–563.

Nemethy, G., and H. A. Scheraga. 1962a. The structure of water and hydrophobic bonding in proteins. III. Thermodynamic properties of hydrophobic bonds in proteins. *J. Phys. Chem.* 66:1773–1789.

Nemethy, G., and H. A. Scheraga. 1962b. Structure of water and hydrophobic bonding in proteins. A model for the thermodynamic properties of liquid water. *J. Chem. Phys.* 36:3382–3400.

Nemethy, G., and H. A. Scheraga. 1962c. Structure of water and hydrophobic bonding in proteins. II. Model for the thermodynamic properties of aqueous solutions of hydrocarbons. *J. Chem. Phys.* 36:3401–3417.

Planck, M. Vorlesungen-uber Thermodynamics, 7th ed. Translated by A. Ogg. 1927. *As* Treatise on Thermodynamics. Longmans, Green and Co., London, England. pp164–182; appendix, 665–668.

Scheraga, H. A. 1994. Chapter 24. *In* Characterization of Chemical and Biological Systems. C. J. Cramer, and D. G. Truhaler, Editors. ACS Symposium Series No. 568.