IV. SOLVATION - CLASSICAL THERMODYNAMICS - STABILITY

1. Driving forces in protein folding

a. Hydrophobicity is the dominant force driving protein folding.

Protein folding was initially thought to be driven by *intramolecular hydrogen bonding* following the original proposition of Alfred Mirsky and Linus Pauling in 1936 [Mirsky, 1936 #52]. This idea was useful in leading Pauling to conceive the role of hydrogen bonds in stabilizing secondary structures such as α-helices and β-sheets [Pauling, 1951 #51; Pauling, 1951 #50] and DNA. Yet, the concept of hydrogen bonding tendency as the major force driving protein folding was shaken in 1959 by the so-called *Kauzmann hypothesis* [Kauzmann, 1959 #27]. Kauzmann drew attention to the *equal stability* or strength of hydrogen bonds formed either between pairs of polar groups on a given protein, or between the protein and water molecules. The *protein-water* hydrogen bonds might then compete efficiently with the *intramolecular* hydrogen bonds and easily break the folded state. In fact, approximately half of all polar groups in native structures are solvent-exposed and half buried in the interior, and the polar groups are almost invariably hydrogen bonded, either to other backbone or side chain groups in the interior or to water molecules on the surface [Chothia, 1975 #48; Chothia, 1976 #47; Richards, 1977 #49]. They exhibit no net preference between intramolecular or intermolecular hydrogen bonds. There must be another factor responsible for the coherent organization of amino acids into compact globular forms. And this was envisioned by Kauzmann to be *hydrophobicity*.

b. All potential hydrogen bonding groups are satisfied in folded structures.

The incentive for hydrogen bond formation between polar groups in the protein interior is quite strong. For buried polar groups, formation of intramolecular hydrogen bonds seems to be the only way of sustaining the hydrophobic environment of the core, and in the absence of such interactions, these groups would tend to denature the protein, in the course of seeking a hydrogen bonding partner. Analysis of protein structures indicates, for example, that more than 80% of the polar groups are involved in regular, hydrogen-bonded secondary structure, one-third of which occur between backbone polar groups [Stickle, 1992 #1].

c. What is hydrophobicity?

The simplest definition would be the *low solubility* of *nonpolar* substances in water. Low solubility of a given compound means that its mixture with water, i.e. its *hydration*, is accompanied by an increase in free energy. A thermodynamic measure of solubility is the *Gibbs free energy change of hydration*, ΔG^{hyd}, involved in the transfer of this substance from pure liquid state into aqueous media. ΔG^{hyd} may be expressed as

$$\Delta G^{hyd} = - RT \ln K = \Delta H^{hyd} - T \Delta S^{hyd} \qquad \text{(IV.1.1)}$$

Here ΔH^{hyd} and ΔS^{hyd} are the enthalpy and entropy changes accompanying the transfer of the species from pure liquid to water, K is the equilibrium constant for the transfer reaction, also referred to as the *solubility constant*.

In a broader sense, hydrophobicity can also be viewed as the tendency of nonpolar groups on a given molecule in aqueous media to aggregate so as to minimize their contact with water, and maximize the intramolecular *van der Waals interactions*. In this latter definition, which has currently found widespread use, the hydrophobicity entails two effects: (i) the low solubility of nonpolar species, which drives the formation of a *hydrophobic core*, and (ii) the non-specific (homogeneous) attractive van der Waals interactions existing between the *nonpolar* species in the core, which further enhance or stabilize compact globular forms.

d. Nonpolar molecules show an aversion, entropic in origin, to water.

Data for the transfer of nonpolar species from pure liquid into water indicates that (i) the low solubility or the large positive free energy change, ΔG^{hyd}, which indicates the aversion of the nonpolar molecules to water, and (ii) the large negative entropies of transfer. The latter emerges as the major component responsible for the observed free energy change. The enthalpic contribution is relatively small. We also note that the transfer of nonpolar solutes into water causes a large positive increase in the heat capacity of the system, suggesting that enthalpic effects opposing solute insertion become stronger at higher temperature.

The change in entropy upon hydration is attributed to the ordered packing of water molecules around nonpolar compounds. This leads to a substantial loss in entropy for the part of water coming into contact with these compounds. Thus, the major origin of the hydrophobic behavior of the nonpolar solvents is their adverse entropic effect on water, and not an unfavorable enthalpic interaction between the solute and solvent . The net effect is, however, the aggregation of nonpolar species into dense, insoluble blocks. This phenomenon is referred to as *hydrophobic collapse*.

e. There is a competition between solvent entropy gain and protein entropy loss upon folding.

Restricting the overall volume occupied by a macromolecule reduces its conformational degrees of freedom, and consequently its entropy because the configurational entropy scales directly with the number of accessible configurations. Let us consider, for instance, the passage from an expanded or coil state, which enjoys Ω_1 possible conformations, into a compact form, in which the number of accessible conformations is reduced to Ω_2 ($\Omega_2 < \Omega_1$). The accompanying change in the *configurational entropy* of the macromolecule is

$$\Delta S^{conf} = S_2 - S_1 = k_B \ln [\Omega_2 / \Omega_1] \qquad \text{(IV.1.2)}$$

which follows from application of the Boltzmann law $S = k_B \ln \Omega$. k_B is the Boltzmann constant and the superscript appended to the ΔS indicates the type (configurational) of entropy change. In the extreme case of a passage to a highly compact form, in which all degrees of freedom are practically suppressed and the final state consists of an unique native microstate, we have $\Omega_2 = 1$. The change in entropy is therefore directly equal to $-k_B \ln \Omega_1$.

Thus, two opposite effects both entropic in nature are involved in hydrophobic collapse:

(i) an effect favoring the collapse, associated with the *dehydration* of nonpolar residues. This is due to the entropy increase of water upon removal of polar residues from solvent-exposed regions.

(ii) an unfavorable *configurational* effect, contributed by the protein itself, due to its transition from an expanded, highly flexible coil configuration into a compact form with significant internal order.

These two effects are additive, so that the total entropy change of unfolding $\Delta S_N{}^U$ becomes

$$\Delta S_N{}^U = \Delta S^{hyd} + \Delta S^{conf} \qquad \text{(IV.1.3)}$$

Although each term on the right-hand side of eq IV.1.3 is significantly large, the resulting $\Delta S_N{}^U$ is relatively small. In fact, $\Delta S_N{}^U$ competes with the enthalpy of unfolding, $\Delta H_N{}^U$, to determine the effective free energy of unfolding $\Delta G_N{}^U$.

As a final remark we note that $\Delta G_N{}^U$ is positive under physiological conditions, so that the native state is stable. An entropy difference $\Delta S_N{}^U$ in favor of the transformation U —>N should have an even more pronounced effect on $\Delta G_N{}^U$ at high temperatures. This is directly implied by the identity $\Delta G_N{}^U = \Delta H_N{}^U - T\,\Delta S_N$. However, most proteins denature upon heating, spoiling this prospect. The explanation lies in the fact that the enthalpic contribution, and more importantly the temperature dependence of both the entropic and enthalpic effects, play a dominant role in determining the thermodynamic balance between folded and unfolded states, as will be illustrated with results from calorimetric studies in § IV.3.

2. Solvation free energies can be estimated by group contribution methods

a. Component Modeling is a useful approach for estimating physicochemical properties of multicomponent systems.

Group contribution methods have been widely used for estimating mixture properties in multicomponent systems. For example, the thermodynamic properties of mixtures, such as their critical temperature, pressure, etc. for phase transition are commonly estimated for engineering purposes by group contribution techniques combining the properties of individual components. At the molecular scale, the *principle of additivity* holds for a number of properties, i.e. the property of the overall molecule is estimated by simply adding up the contribution from individual functional groups or atoms. An example is the total dipole moment of a molecule, found from the vectorial sum of the dipole moments of the polar groups comprising the molecule. Or, bond dissociation/formation energies are added for estimating the total energy required to activate chemical synthesis reactions.

Additivity assumptions play a central role in thermodynamics. Additivity has been called the '4th Law of Thermodynamics" Benson, S. W. 1976 *"Thermochemical Kinetics: Methods of Estimation of Thermochemical Data and Rate Parameters"*, 2nd ed, Wiley & Sons, NY; Schellman, J.1996 "*Thermodynamics*...). Without additivity, chemistry would have limited power. For example, if the heat of formation of covalent compounds were not equal to the sum of the bond enthalpies, - if the heat of formation of carbon dioxide were not equal to twice the heat of a C-O bond- then chemical equilibria and kinetics would not be predictable from

simpler reactions. We could not look up bond energies in tables, compute the energetics of ATP cycles, the breakdown of glucose, or other equilibria.

The application of group contribution methods, also termed *Component Modeling*, to biomolecular systems has given insights into the folding of proteins or RNA molecules. Folding or binding in a macromolecule may involve the formation or disruption of many interactions, so an appropriate model fragment process might be the desolvation of two monomers, or two atoms, and the formation of a contact between them.

Probably the first component model for macromolecules was the Flory-Huggins theory for polymer conformational change (1943, 1953). The first component model for proteins was the work of Jacobsen and Linderstrøm-Lang (1949) showing that protein volume changes were much smaller than were to be expected from sums of volume changes of the titrations of all the amino acid COOH groups.

The desolvation free energy involved in protein folding has been modeled as the sum of free energies of water-to-oil transfers of amino acids in the hydrophobic core. Similarly, the free energy of partitioning a drug into a membrane is modeled as a sum of the free energies of water-to-oil partitioning of the chemical substituents -the methylene groups, hydroxyl groups, aromatic rings, etc. This approach is also known as QSAR (quantitative structure-activity relationships) or QSPR (quantitative structure-property relationships).

b. Thermodynamic data on transferring solutes between different phases and/or solvents give information on the solvation behavior of biomolecules

Table IV.2.1 lists the group contributions derived from experiments, that have been utilized for estimating the hydration energies of proteins [Makhatadze, 1993 #4; Privalov, 1993 #5; Makhatadze, 1990 #52; Makhatadze, 1995 #188]. The hydration energies listed in this table refer to the transfer *from the gas phase to aqueous environment*. The group contributions are generally negative, indicating that there are more favorable intermolecular interactions in the densely packed liquid phase than in the gas phase. Alternatively, it is possible to compare the solvation energies associated with the transfer *from organic solvent into water*. These are on the contrary accompanied by an increase in free energy reflecting the hydrophobic character of nonpolar groups.

At low temperatures, near room temperature, solvation is favored by enthalpy, but is strongly opposed by entropy. At higher temperatures, the roles of enthalpy and entropy are reversed: solute insertion is opposed by enthalpy and favored by entropy. This particular temperature dependence of solvation preferences for nonpolar solutes will be shown in § IV.3 to be reflected in the unfolding thermodynamics of proteins.

TABLE IV.2.1

Group Contributions to Hydration Enthalpies and Gibbs Free Energies (from Gas to Water)[(a)]

Group	ΔH^{hyd} [(a)] (kJ/mol)	ΔG^{hyd} [Cabani, 1981 #56] (kJ/mol)
$-CH_2-$	-3.39	0.72
$-CH_3$	-8.28	3.72
-OH	-39.60	-25.62
$-NH_2$	-38.61	-23.64
> C=O	-25.05	-23.13
-COOH	-43.94	-31.71
-NH-	-38.74	-25.74
-CH <	2.83	-1.62
> C <	8.45	-4.86
- S -	-19.56	-14.41
-CH =	-4.91	-0.60
-C=[(b)]	2.63	-4.58
-N=	-20.31	-16.81
-CONH-	**-59.57**	**-48.87**

[(a)] at 25°C, from the work of Makhatadze and Privalov [Makhatadze, 1993 #4; Privalov, 1993 #5] using data on linear and cyclic organic compounds [Cabani, 1981 #56; DellaGatta, 1986 #60; Dec, 1984 #57; Hallen, 1986 #58] [(b)]group in aromatic ring.

c. Solvation free energies of proteins can be viewed as the result of the contributions from individual residues

The calculation of the solvation free energies of proteins by adding the contributions from residues, proposed by Eisenberg and McLachlan for example, is an example of Component Modelling based on the additivity principle [Eisenberg, 1986 #55]. The solvation contribution ΔG^{sol} to the free energy change of folding ($-\Delta G_N^U$) is calculated in this approach from a sum over the free energies of transfer of individual amino acids from the protein interior to an aqueous environment, i.e.

$$\Delta G^{sol} = \sum_R \Delta G_R^{sol} = \sum_R \left\{ \Delta G_R^{sol}(A_N) - \Delta G_R^{sol}(A_U) \right\} \qquad \text{(IV.2.1)}$$

Here $\Delta G_R^{sol}(A)$ is the free energy change accompanying the transfer of residue R from the protein interior into an aqueous environment. The argument designates the solvent-exposed nonpolar area of residue R in the native (A_N) and unfolded (A_U) conformations (see § 1.x).

It has generally been assumed in studies of partitioning of nonpolar solutes between an organic phase and water that the free energy is a function of solute surface area alone. Dividing the transfer free energy by solute surface area then yields a *surface tension coefficient*, γ, which provides a measure of hydrophobicity when measured for alkane/water systems. A value of about 25 cal/(mol.Å^2) has been ascribed to γ [Chothia, 1974 #189] based on experimental data. This value multiplied with the total nonpolar surface area of individual residues has been utilized for estimating the residue-specific solvation free energies $\Delta G_R^{sol}(A_U)$ in the denatured state, assuming that (i) amino acid nonpolar groups are fully

exposed to solvent in the denatured state of the protein, and (ii) transfer free energies depend on the surface area of solute, alone. For the folded state, on the other hand, no unique set of $\Delta G_R^{sol}(A_N)$ values can be calculated. $\Delta G_R^{sol}(A_N)$ depends on the solvent-accessible surface area (ASA) of residue R in the particular folded conformation, and should be calculated separately for each investigated tertiary structure. The methods of Lee and Richards [Lee, 1971 #71; Richmond, 1978 #70] and of Connolly [Connolly, 1983 #271; Connolly, 1983 #270] are widely utilized for calculating surface areas.

The proportionality between transfer free energy and exposed surface area is an approximation. Flory-Huggins theory demonstrates for example, that the molar volumes of the different species play an important role in determining solubility, because the chemical potentials depend on molecular size (volume). The consideration of volume-dependent contribution to ΔG^{sol} has the effect of increasing the apparent surface tension to about 45 cal/(mol.Å^2) [Sharp, 1991 #79; Sharp, 1991 #83]. Table IV.2.2 lists the $\Delta G_R^{sol}(A_U)$ values corresponding to the two approximations.

TABLE IV.2.2

Amino Acid Solvation Free Energies (kJ/mol)

Residue	ΔG_R^{sol}[(a)]	$\Delta G_R^{sol}(\Phi)$[(b)]	$\Delta G_R^{sol}(\Phi)$[(c)]
Gly	0.00	0.00	0.00
Ala	2.80	1.75	4.26
Val	6.27	6.94	14.63
Leu	7.94	9.70	20.40
Ile	7.94	10.28	20.31
Pro	5.01	4.09	10.45
Cys	1.59	5.60	13.29
Met	10.03	7.02	15.84
Thr	2.17	1.46	6.36
Ser	0.04	- 0.21	-4.72
Phe	9.61	10.20	21.40
Trp	10.87	12.80	25.62
Tyr	6.69	5.47	16.26
Asn	- 2.51	- 3.43	1.63
Gln	- 0.92	-1.25	5.85
Asp	- 5.01	-4.39	-5.56
Glu	- 3.17	-3.63	3.22
His	2.67	0.75	8.10
Lys	- 2.38	-5.64	4.18
Arg	- 8.78	-5.72	3.22

(a) See eq IV.2.1 ; (b) free energy of transfer from protein interior into aqueous environment based on the hydrophobicity (Φ) measurements of Fauchère and Pliska [Fauchère, 1983 #72]; (c) free energy of solvation from the hydrophobicity (Φ) scale of Honig and collaborators [Sharp, 1991 #79] using 45 cal/(mol.Å^2) [Sharp, 1991 #83] for the energy of solvation of unit nonpolar surface area instead of 25 cal/(mol.Å^2).

d. Solvation contribution to unfolding free energies of residues can be approximated as the sum of atomic contributions.

An estimation of the solvation free energy of residues can be made by expressing $\Delta G_R^{sol}(A)$ in terms of the contributions of all atomic groups in R as

$$\Delta G_R^{sol}(A) = \Delta\sigma(C) \sum_i A_R(C_i) + \Delta\sigma(N/O) \sum_i A_R(N_i/O_i) +$$
$$\Delta\sigma(O^-) \sum_i A_R(O^-_i) + \Delta\sigma(N^+) \sum_i A_R(N^+_i) + \Delta\sigma(S) \sum_i A_R(S_i) \quad \text{(IV.2.2)}$$

In the above equation, $\Delta\sigma(C)$, $\Delta\sigma(N/O)$, $\Delta\sigma(O^-)$, $\Delta\sigma(N^+)$ and $\Delta\sigma(S)$ are the *atomic solvation parameters* of the five classes of atoms: carbon (C), uncharged oxygen or nitrogen (N/O), charged oxygen (O^-), charged nitrogen (N^+) and sulphur (S). The summations are performed over atoms of each class belonging to residue R, by considering their solvent-exposed areas $A_R(C_i)$, $A_R(N_i/O)$, *etc*. in the conformation of interest. In Table IV.2.3 the atomic solvation parameters proposed by Eisenberg and McLachlan are presented. In the same table are listed the set of parameters [Pickett, 1993 #8] based on the transfer free energies that include volume effects [Sharp, 1991 #79].

TABLE IV.2.3

Atom Type	Δσ, Atomic Solvation Parameter (cal/mol Å^2)	
	Eisenberg & McLachlan[(a)]	Pickett & Sternberg [(b)]
C	16 ± 2	34 ± 3
N/O	- 6 ± 4	7 ± 7
O^-	- 24 ± 10	- 8 ± 16
N^+	- 50 ± 9	- 36 ± 15
S	21 ± 10	53 ± 17

(a) defined in eq IV.2.2. [Eisenberg, 1986 #55] ; (b) from [Pickett, 1993 #8])

3. Experiments on folding thermodynamics

a. Calorimetry shows both heat and cold denaturation

The Gibbs free energy difference between the unfolded and native states of a given protein at a given temperature $\Delta G_N^U(T)$ is accepted as a measure of the stability of that protein at the given temperature. At the transition temperature T_t between native and denatured states this free energy difference vanishes, i.e.

$$\Delta G_N^U(T_t) = 0 \quad \text{(IV.3.1)}$$

or

$$\Delta H_N^U(T_t) = T_t \Delta S_N^U(T_t) \quad \text{(IV.3.2)}$$

In general, the native conformation is preferred at room temperature, i.e. the unfolding free energy $\Delta G_N^U(T) > 0$ at $T \approx 300$ K, which automatically disfavors the unfolded state. Experiments show that an inversion in the sign of ΔG_N^U, and thereby a shift in the equilibrium state from native to unfolded state can be driven either by increasing or by lowering the temperature. These two types of denaturation are called *heat* and *cold* denaturation, respectively. A typical example of a protein that undergoes both heat and cold denaturation is apo-myoglobin (apo-Mb). Figure IV.3.1 illustrates the dependence of the folded fraction of horse apo-Mb on temperature, as detected by circular dichroism.

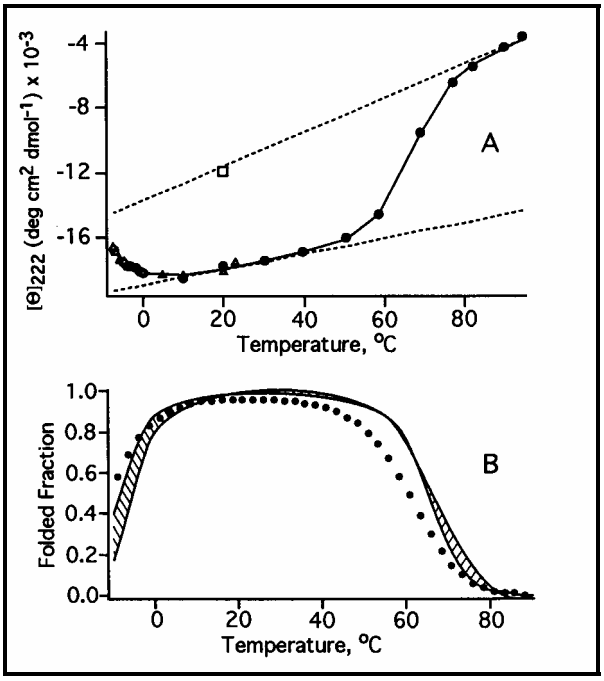

Figure IV.3.1. *Temperature dependence of the folded fraction of horse apo-Mb. Curves are obtained by fitting mean residue ellipticities at 222 nm to a thermodynamic two-state model. The hatched zone indicates the uncertainty in the thermodynamic parameters. The filled circles represent the folded fractions in 3 M glycerol. (Figure 10 of [Ballew, 1996 #170])*

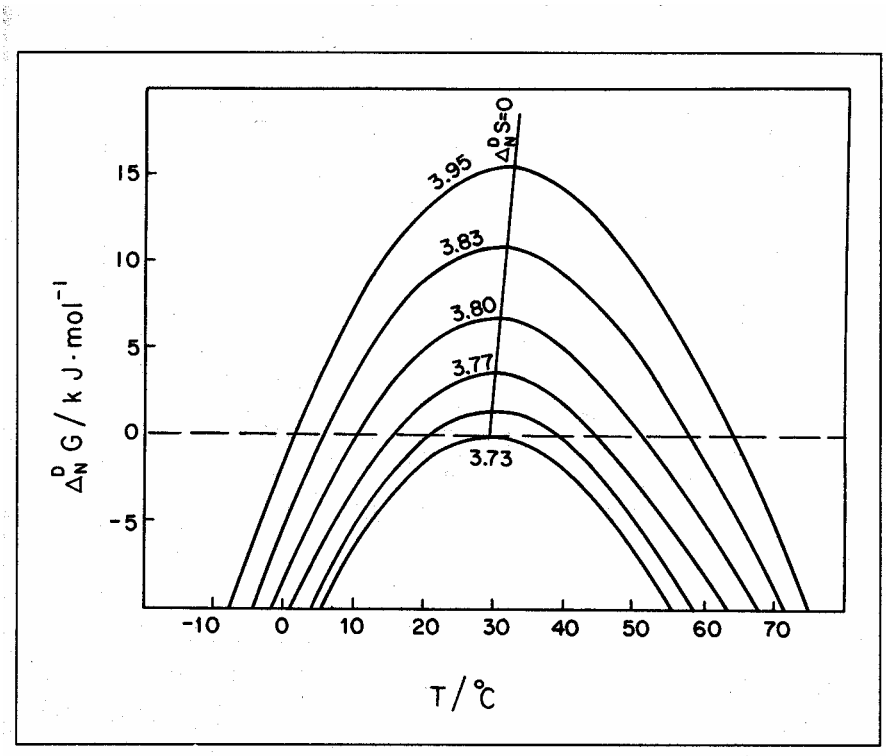

Figure IV.3.2. *Dependence of unfolding Gibbs free energy on temperature, for metmyoglobin in acetate buffer solution. The curves refer to different pH values, as indicated. We note that the unfolding free energy is negative below pH = 3.7, which indicates that the protein is denatured irrespective of temperature. The native state is stabilized at increasingly broader temperature ranges with increasing pH. The maxima in the curves indicate the temperature at which the entropic contribution to unfolding vanishes. (from Fig 3.11 of* [Creighton, 1992 #16])

g. Hydrophobic contribution to denaturation free energy conforms to the solvation behavior of small molecules.

In an attempt to visualize the contribution of the hydrophobic (HΦ) effect to ΔG_N^U Privalov and Makhatadze proposed the thermodynamic cycle displayed in *Figure IV.3.3*. Therein the free energy change contributed by the solvent-exposure of the originally buried nonpolar groups is designated as $\Delta G^{H\Phi}$. G is a state function, i.e. ΔG depends on the initial and final states, irrespective of the path of the transition. Therefore, it is possible to break down the original process into individual steps, whose contributions can be more readily calculated. In *Figure IV.3.3*, the change $\Delta G^{H\Phi}$ is essentially viewed as consisting of three steps: (i) the transfer of buried nonpolar groups into vacuum, (ii) the disruption of their van der Waals (vdW) contacts in vacuum, and (iii) the hydration of nonpolar groups. The energetics involved in steps (ii) and (iii) are ΔG^{vdW} (= ΔH^{vdW}) and ΔG_{npl}^{hyd}, respectively. No energy change occurs in step (i) because the neighborhood remains unchanged. Therefore, the contribution of the hydrophobic effect to stability is

$$\Delta G^{H\Phi} = \Delta H^{vdW} + \Delta G_{npl}^{hyd} \qquad \text{(IV.3.9)}$$

The latter can be divided into enthalpic and entropic contributions, $\Delta G^{H\Phi} = \Delta H^{H\Phi} - T\Delta S^{H\Phi}$, which upon substitution of $\Delta G_{npl}^{hyd} = \Delta H_{npl}^{hyd} - T\Delta S_{npl}^{hyd}$ permits us to identify

$$\begin{aligned} \Delta H^{H\Phi} &= \Delta H^{vdW} + \Delta H_{npl}^{hyd} \\ \Delta S^{H\Phi} &= \Delta S_{npl}^{hyd} \end{aligned} \qquad \text{(IV.3.10)}$$

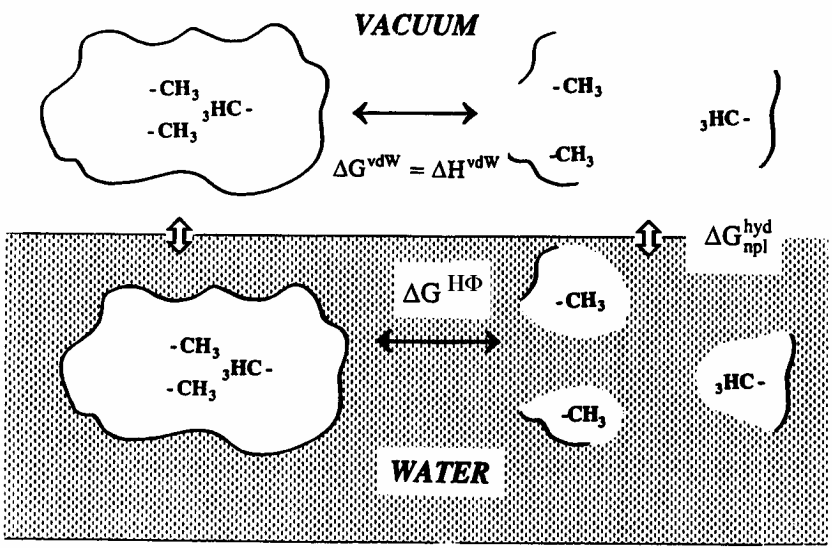

Figure IV.3.3. *Thermodynamic cycle for describing the contribution $\Delta G^{H\Phi}$ of hydrophobic (HΦ) effect on unfolding Gibbs free energy ΔG_N^U. The change $\Delta G^{H\Phi}$ is replaced by three successive steps, the energy costs of which are 0, ΔH^{vdW} and ΔG_{npl}^{hyd}, respectively. (Adapted from Fig 29 of ref [Makhatadze, 1995 #188])*

The temperature dependences of these contributions to unfolding free energies (per unit ASA) are shown in *Figure IV.3.4* [Makhatadze, 1995 #188]. At room temperature, $\Delta H^{H\Phi} = 0$ and $\Delta G^{H\Phi} \approx -T\Delta S^{H\Phi}$, i.e. the hydrophobic effect is purely entropic; whereas at high temperatures the entropic contribution becomes vanishingly small, and $\Delta G^{H\Phi} \approx \Delta H^{H\Phi}$. Another observation of interest is that at low temperatures the hydrophocity is essentially driven by entropy despite the possible adverse enthalpic effect; the latter favors unfolding; whereas at high temperatures the hydrophobic effect is of enthalpic origin. This behavior conforms to that already observed during the transfer of inert gases, or nonpolar solutes, into water.

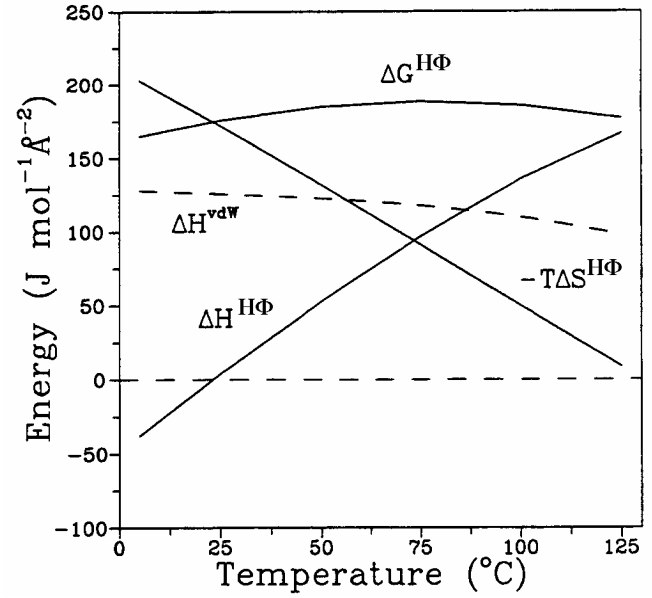

Figure IV.3.4. *Temperature dependence of the hydrophobic contributions to unfolding energetics. Energies are expressed per unit ASA of nonpolar groups. Both the enthalpic and entropic contributions associated with hydrophobic effects are displayed, along with the resulting $\Delta G^{H\Phi}$. Note that at room temperature, $\Delta G^{H\Phi} \approx -T\Delta S^{H\Phi}$, i.e. the hydrophobic effect is purely entropic; whereas at high temperatures $\Delta G^{H\Phi}$ becomes purely enthalpic. Dashed lines represent the contributions of the van der Waals interactions between nonpolar groups to the Gibbs free energy change and enthalpy change driven by hydrophobic effects. (Adapted from Fig 30 of ref [Makhatadze, 1995 #188])*

h. The configurational entropy decrease of the protein accompanying folding can be estimated from statistical analysis of databank structures

The configurational entropy change directly reflects the increase in the number of possible conformations Ω due to the passage from the folded to the unfolded state. Additionally, fluctuations from the local energy minima of microstates, underlying the internal *vibrational* entropy of the macromolecule, should be accounted for in a quantitative assessment of S^{conf}. Thus, the overall configurational entropy of a macrostate composed of Ω configurations is [Karplus, 1981 #76]

$$S^{conf} = - k_B \Sigma_i \, pi \ln p_i + \Sigma_i \, p_i S_i^{vib} \qquad \text{(IV.3.11)}$$

where the summations are performed over all configurations, p_i denotes the equilibrium probability of the ith configuration and S_i^{vib} is its vibrational entropy.

ΔS^{conf} is the difference between the configurational entropies of the macrostates U and N, as $\Delta S^{conf} = S_U^{conf} - S_N^{conf}$. Inasmuch as the native state consists of a unique conformation ($\Omega = 1$),

$$\Delta S^{conf} = - [k_B \sum_i pi \ln p_i + \sum_i p_i S_i^{vib}] - S_N^{vib} \qquad \text{(IV.3.12)}$$

If, furthermore, the vibrational entropies of the conformations are assumed to be the same in both states, the configurational entropy reduces to

$$\Delta S^{conf} = \left[- k_B \sum_{i=1}^{\Omega} p_i \ln p_i \right]_U \qquad \text{(IV.3.14)}$$

The validity of eq IV.3.13 has been demonstrated by comparison of experiments with simulations. The configurational entropy changes determined for each type of amino acid are listed in *Table IV.2.4.* These were extracted [Pickett, 1993 #8] from 50 known protein structures.

TABLE IV.2.4
Side-Chain Conformational Entropies[(a)]

Residue at 300 K[(a)]	TΔS (kcal/mol)
Ala	0.00
Arg	- 2.03
Asn	- 1.57
Asp	- 1.25
Cys	- 0.55
Gln	- 2.11
Glu	- 1.81
Gly	0.00
His	- 0.96
Ile	- 0.89
Leu	- 0.78
Lys	- 1.94
Met	- 1.61
Phe	- 0.58
Pro	0.00
Ser	- 1.71
Thr	- 1.63
Trp	- 0.97
Tyr	- 0.98
Val	- 0.51

[(a)] *from [Pickett, 1993 #8]*

i. A delicate balance between large entropic and large enthalpic effects determines folding equilibrium

The intramolecular contributions ΔG^{conf}, ΔH^{conf} and ΔS^{conf}, to the thermodynamic property changes of unfolding $\Delta G_N{}^U$, $\Delta H_N{}^U$ and $\Delta S_N{}^U$ may be indirectly estimated by subtracting the foregoing hydration contributions ΔG^{hyd}, ΔH^{hyd} and ΔS^{hyd} from the calorimetrically measured denaturation properties. As pointed out above, $\Delta H_N{}^U$ values are relatively small; and the large negative enthalpy of hydration ΔH^{hyd} is counterbalanced by a *large positive* ΔH^{conf} opposing unfolding. Likewise, a marginal entropic driving potential in the unfolding process is inferred from the specific entropies of unfolding $\Delta S_N{}^U$, in spite of the strong unfavorable hydration entropies. This implies the superposition of large, positive internal contributions ΔS^{conf}, in favor of the expanded configurations of the unfolded state.

Thus, the property changes accompanying denaturation may be summarized as: strong hydration enthalpies (of polar groups in particular) favoring unfolded conformations, almost counterbalanced by significantly strong intramolecular attractions taking place in compact folded structures on the one hand and, on the other, a significant decrease in the entropy of the aqueous phase upon contacting polar and nonpolar groups, which is again compensated by the large entropy increase of the protein upon denaturation. It is interesting to note that *the apparent marginal changes in extensive properties result from such a delicate balance between strong entropic and enthalpic effects*.