

Published in final edited form as:

. 2013 January 1; 144(1): 59–65. doi:10.1007/s00706-012-0839-9.

Entropy-enthalpy Compensation of Biomolecular Systems in Aqueous Phase: a Dry Perspective

Liviu Movileanu and Eric A. Schiff

Department of Physics, Syracuse University, Syracuse, New York USA

Abstract

We survey thermodynamic measurements on processes involving biological macromolecules in aqueous solution, which illustrate well the ubiquitous phenomenon of entropy-enthalpy compensation. The processes include protein folding/unfolding and ligand binding/unbinding, with compensation temperatures varying by about 50 K around an average near 293 K. We show that incorporating both near-exact entropy-enthalpy compensation (due to solvent relaxation) and multi-excitation entropy (from vibrational quanta) leads to a compensation temperature in water of about 230 K. We illustrate a general procedure for subtracting solvent and environment-related terms to determine the bare Gibbs free energy changes of chemical processes.

Keywords

Macromolecules; Biological macromolecules; Entropy-enthalpy compensation; Hydrophobic interactions

Introduction

In the last couple of decades, significant progress has been made in understanding temperature-dependence of the specific interactions among various functional biological macromolecules or between biomacromolecules and an array of small ligands of different sizes, charges and interaction affinities. These efforts contributed to accumulation of a substantial amount of thermodynamic parameters that feature these weak interactions in biological systems. In general, the thermodynamic parameters of these molecular biosystems in aqueous phase are derived from equilibrium constant recordings at different temperatures (e.g., either association, K_a , or dissociation constant, K_d) and semi-log Van 't Hoff plots (1). In many examples, the temperature dependence of biomolecular interactions reveals a phenomenon called enthalpy-entropy compensation (EEC) (2–7).

The phenomena of EEC are illustrated in Fig. 1. In the upper panel, the solid squares indicate measurements of the standard entropy and enthalpy changes associated with imino proton exchange in the particular basepairs in RNA (8). As can be seen, there is a linear trend of these data with a positive slope, which is the compensation effect. Each individual basepair's entropy and enthalpy change can be denoted ΔS_i and ΔH_i . The series of measurements is evidently fitted well by a linear relationship:

$$\Delta S = (\Delta H - \Delta G_c) / T_c, \quad (1)$$

where we denote the intercept of the fitting line on the enthalpy axis as the compensation free-energy ΔG_C , and the reciprocal slope of the plot as the compensation temperature T_C . The lower panel of Fig. 1 illustrates the Gibbs free energy measurements of the same series. The magnitudes of the free energies are significantly smaller than the enthalpies, which indicates that there is significant compensation of the terms ΔH and $T\Delta S$ in the expression $\Delta G \equiv \Delta H - T\Delta S$. This is anticipated when the compensation temperature T_C is reasonably close to the experimental temperature T , as was true for these measurements.

There are two very general models that have been proposed to explain the ubiquity of EEC for macromolecules in water. First, the hydrophobic and hydrogen bonding interactions between the water and the macromolecules lead to large associated entropy and enthalpy terms. Ben-Naim suggested the colorful distinction between the entropy and enthalpy changes when the structure of the water is “frozen”, and the changes incorporating the “relaxation” of the water. Several papers in the last decades have concluded that these relaxation enthalpy and entropy terms compensate perfectly each other; the line labeled $\Delta H/T$ illustrates this relationship; this perspective has been reviewed by some of its originators in Ref. (5;9;10). This “near-exact” compensation effect applies in all solvents, but is particularly significant for water.

The second general effect is the entropy associated with the quantization of vibrations in the solvent, which can be inferred from vibrational spectroscopy of the solvent. This “multi-excitation entropy” effect leads to an entropy compensation temperature T_V that applies to all the enthalpy of a macromolecular process, and not just to the solvent relaxation enthalpy. This effect has been reviewed by some of its proponents in Ref. (6;11).

In addition to these general effects, each individual macromolecular system may have additional internal compensation terms. However, it is not known whether or how these various compensation effects should add together to determine the final entropy-enthalpy relationship of a system of macromolecules, nor is a procedure established for parsing an individual set of measurements such as that in Fig. 1 to determine the relative contributions of the several effects.

Results and Discussion

Survey of EEC compensation for biological macromolecules in water

We have collected experimental estimates for the compensation temperature T_C and the compensation free energy change ΔG_C in Table 1. The compensation temperatures, T_C , are clustered around 293 K, which is the typical measurement temperature. There are many deviations from this temperature point that will be discussed below. The Gibbs compensation free energy ΔG_C has values in the range -44 to 58 kJ/mol.

Before commenting on some of the individual entries in the table, we note a general issue regarding the parameter estimates. Pioneering work of Krug and collaborators (12;13) showed that a linear relationship between enthalpy and entropy differences is sensitive to the correlation of the statistical errors when the differences are derived from van 't Hoff analyses (enthalpy obtained from a graph of the equilibrium constant vs. reciprocal temperature). Recently, Starikov and Norden (14) has emphasized that entropy-enthalpy relations based on calorimetric measurements are relatively insensitive to this problem. We have identified the measurement techniques in Table I. We have not otherwise discriminated against estimates for T_C and ΔG_C based on van 't Hoff analyses, which can be valid when errors are sufficiently small (3;15).

Gilli and colleagues (1994) have compiled ligand (drug) binding experiments for thirteen macromolecule systems including ten biological receptors (16). A linear regression of the scatter plot between the standard enthalpies and entropies provided a compensation temperature of 278 ± 4 K and a free energy of interaction at the compensation temperature ΔG_C^0 of -39.9 ± 0.9 kJ/mol (16). Values of ΔH° and ΔS° were obtained from Van 't Hoff plots of the drug-receptor binding equilibrium curves, which were linear in the temperature range 273 – 310 K.

The data analysis of thermodynamic parameters ΔH° and ΔS° was applied to the system DNA binding site TGACGTCA – bZIP domain in Jun transcription factor (17). Methodical modifications of the sequence of the DNA binding revealed EEC with $\Delta G_C = -3.15$ kJ/mol (18). In contrast, systematic alterations in the length of the binding protein (bZIP domain) resulted in an EEC signature with a compensation temperature of 291 K and $\Delta G_C = -37.4$ kJ/mol.

We also show an example related to the thermostability of double-stranded DNA (dsDNA) and double-stranded RNA (dsRNA) oligonucleotides. Individual base-pair stability of dsDNA and dsRNA was pursued using optimized NMR methodology (8). Steinert and colleagues (2012) (8) found that the compensation temperature, T_c , for short dsDNA and dsRNA oligonucleotides is 322 and 333 K, respectively. Remarkably, this coincides with the melting temperature of the two double-stranded oligonucleotides (Table 1).

Differential Raman spectroscopy (19;20) and Van 't Hoff plots (1) were employed to examine the structural alterations and thermodynamics of the premelting and melting transitions in long dsDNA polymers poly(dA-dT)-poly(dA-dT) and poly(dA)-poly(dT). Similar to Steinert and colleagues' study (8), the compensation temperature of these polynucleotides was 345 and 348 K (Table 1), respectively, which was very close to the melting temperature, T_m , of these biopolymers of 344 and 349 K, respectively.

EEC was found in the energetics of protein folding and stability (2;4;21;22). EEC is one of the most puzzling process in molecular recognition employing a folded protein and a ligand (23–25). Dunitz (1995) has hypothesized that enthalpically more favorable binding interactions between a ligand and a protein would result in a greater restriction of the moieties, so more entropy adverse (26). However, we think that more than a single mechanism is involved in the EEC process in a biomolecular system in aqueous phase, such as the role of cosolutes and osmotic stress, the solvation and water orientation as well as the dynamics of water binding to the polypeptide side chains, hydrophobic hindrance, and so on (24;27–29). Therefore, a defragmentation of the EEC quantitative data in components reflecting each contributing mechanism is an intimidating and persistent challenge. In addition, the protein systems in aqueous phase experience a variety of conformational transitions leading even to negative activation enthalpies (30–32), which certainly requires compensatory negative activation entropies.

General mechanisms for EEC and near-exact EEC

We briefly describe two rather general mechanisms for EEC involving a macromolecule in water (3;6), and then we'll discuss the particular subset of compensation temperatures shown in Fig. 2. We shall call the first mechanism “near-exact entropy enthalpy compensation”, which we'll denote eEEC. Near-exact compensation implies that $\Delta S \approx \Delta H/T$, and thus implicitly that the Gibbs free energy $\Delta G \equiv \Delta H - T\Delta S$ is much smaller than the enthalpy ($\Delta G \ll \Delta H$). The second mechanism has been called “multi-excitation entropy”, which we'll denote MEE. MEE roughly implies that $\Delta S = \Delta H/T_V$. T_V is a temperature largely determined by the vibrational frequency spectrum of the solvent or matrix; in the case of a

narrow spectrum centered at optical frequency ω_0 , $T_V \approx \hbar\omega_0/K_B$, where \hbar and K_B are Planck's and Boltzmann's constants, respectively. In this paper, we only summarize these results; there have been several comprehensive reviews that should be consulted for a discussion of the underlying theories (3;6;9;11).

Several authors have recognized the possibility of near-exact entropy-enthalpy compensation (eEEC) in solvents such as water with structure that “relaxes” around a solute molecule, and we would anticipate that these effects would be especially significant for macromolecules in aqueous solution. The effect can be strong, and can be thought of as a change in the entropy ΔS^R associated with the water surrounding the solute molecules. This relaxation entropy is *exactly* compensated by a corresponding relaxation enthalpy: $\Delta H^R = T\Delta S^R$ (5;9;10).

Because of this exact compensation, these large relaxation effects do not affect the chemical potential for processes such as solvation. Ben-Naim (10), Grunwald (9), Yu and Karplus (33), and Qian and Hopfield (5) have all given related arguments. They are rather general, and apply to localized processes (solvation, ligand binding, protein folding/unfolding, etc.) embedded in any matrix with relaxation enthalpies significantly larger than the Gibbs free energy of the process. After Ben-Naim, we write:

$$\Delta H_i^0 = \Delta G_i^0 + T\Delta S_i^0, \quad (2)$$

ΔH_i^0 and ΔS_i^0 are the measured enthalpy and entropy change for some process i , and incorporate the relaxation enthalpy and entropy as well as “frozen-state” terms neglecting relaxation:

$$\Delta S_i^0 = \Delta S_i^R + \Delta S_i^F, \quad (3a)$$

$$\Delta H_i^0 = \Delta H_i^R + \Delta H_i^F, \quad (3b)$$

where ΔS_i^F and ΔH_i^F are the frozen state entropy and enthalpy. ΔG_i^0 is the Gibbs free energy of the process, which is unaffected by relaxation because of the exact compensation effect. Now consider a collection of related processes i such as a particular ligand that binds and unbinds to a series of related macromolecules. The varying macromolecules will each have different, but compensating relaxation entropy and enthalpy changes ΔS_i^R and ΔH_i^R .

Presuming that ΔG_i^0 is small compared to ΔH_i^0 , the usual expression:

$$T\Delta S_i^0 = \Delta H_i^0 - \Delta G_i^0 \quad (4)$$

implies that a scatter plot of ΔS_i^0 and ΔH_i^0 will cluster around the line $T\Delta S = \Delta H$, with small deviations ΔG_i^0 . The key feature of eEEC is that the compensation temperature should be the same as the temperature of the measurement.

The multi-excitation entropy mechanism for EEC

The second EEC mechanism that we'll consider is “multi-excitation entropy” (MEE). Several authors (Peacock-Lopez, Suhl, Linert, Khait, Yelon, and Movaghar) proposed related ideas in the 1980s (11;34–37). MEE amounts to an additional entropy change from annihilation of the several vibrational quanta needed to excite a process i over its enthalpy barrier. These can be associated with an additional entropy term $\Delta S_i^V = \Delta H_i^0/T_V$, where $T_V \approx$

$\hbar\omega_0/k_B$ is determined by a characteristic vibrational frequency ω_0 of the matrix or solvent. Theoretically, for a system with a well-defined Einstein mode, we anticipate proportionality as long as (i) T_V is significantly greater than T , and (ii) the enthalpy barrier ΔH_i^0 is significantly greater than \hbar the latter is the “multi-excitation” criterion. (6)

When both are present, the relaxation and MEE entropies add. We write:

$$\Delta S_i = \Delta S_i^F + \Delta S_i^R + \Delta S_i^V = \Delta S_i^F + \Delta H_i^R/T + \Delta H_i^0/T_V$$

$$\Delta S_i = \left(\Delta S_i^F - \Delta H_i^F/T \right) + \Delta H_i^0 \left(\frac{1}{T} + \frac{1}{T_V} \right) = - \frac{\Delta G_i^F}{T} + \Delta H_i^0 \left(\frac{1}{T} + \frac{1}{T_V} \right). \quad (5)$$

This equation is the main tool we use to parse the relative effects of the internal and solvent-related entropies and enthalpies for a series of macromolecule processes. Note that, although the entropy is changed by the MEE term, the enthalpy is not affected. This expression yields the “frozen” free energy ΔG_i^F from the enthalpy and entropy measurements, presuming that T_V can be estimated.

If we presume the first term on the right of eq. (5) is small compared to the second, then the compensation temperature T_S associated solely with solvent effects is *reduced* somewhat from its near-exact value according to:

$$\frac{1}{T_S} = \frac{1}{T} + \frac{1}{T_V}. \quad (6)$$

This equation incorporates eEEC as the limit with large T_V , and it also indicates that systems with substantial relaxation (such as aqueous solutions) are not ideal for the observation of MEE. A clear demonstration of MEE requires compensation temperatures exceeding T significantly, but near-exact compensation generally keeps compensation temperatures below T .

Application to biological macromolecules in water

We can adapt the discussion of the previous section and offer the following perspective on the compensation temperatures summarized in Fig. 2. We think that the near-exact EEC model is likely the best starting point for explaining the otherwise remarkable coincidence that a wide range of experiments on macromolecules in aqueous solution yield a compensation temperature near the measurement temperature. In addition to the near congruence of the measurement and compensation temperatures, for each of the experiments summarized in Fig. 2, the Gibbs free energies were much smaller than the range of enthalpies, which is the second criterion for eEEC as the primary origin of compensation.

The eEEC model is plainly incomplete. As Fig. 2 shows, there is a significant range of compensation temperatures around the value of 295 K, and the eEEC model is silent on this. The MEE model also fails to account for this variability; the MEE model's strength is that it accounts for variation in the compensation temperatures for solvents and matrices with varying vibrational spectra. All of these experiments were done in aqueous solution. We argue that the joint effect of eEEC and MEE is to predict a single compensation temperature for aqueous systems that is significantly lower the measurement temperature.

Experimentally (6), there is a fair proportionality between measurements of “isokinetic” temperatures and characteristic vibrational frequencies (determined spectroscopically) for numerous physical systems ranging from electron trapping and defect annealing in semiconductors to chemical reactions in a wide range of solvents or solid surfaces. The isokinetic temperature is analogous to compensation temperature for EEC, but applies to

kinetic measurements instead of equilibrium measurements. The two temperatures need not be identical (38), although one survey that compared their values concludes that they were close (39).

As a provisional measure, we assume that T_V is the isokinetic temperature of about 1.0×10^3 that corresponds to the vibrational band of water at 700 cm^{-1} (11). The predicted compensation temperature for measurements near 293 K is then $T^S = 0.23 \times 10^3 \text{ K}$, which was used in preparing Fig. 1.

This “eEEC+MEE” compensation temperature will not vary for the different macromolecule systems, and thus cannot explain the spread of the measurements in Fig. 2. Beyond these two models, each individual system of molecules will have some relationship of the remaining entropy & enthalpy terms that depend on the details of the macromolecule system. In Fig. 1, we illustrate the “frozen-in” Gibbs free energy change for an RNA/solvent exchange measurement. As can be seen, there is a good linear dependence of this free energy upon the measured enthalpy change of the system. Note that the latter includes the solvent relaxation enthalpy.

Internal entropy-enthalpy compensation in biomacromolecules

Internal entropy and enthalpy changes needn't yield a linear EEC relation. This point has been emphasized by Ford (40), who did calculations of entropies and enthalpies for gas-phase dissociation models, and found compensation, anticompensation, and ill-behaved relationships. However, as summarized in Fig. 2, most biomolecular systems in aqueous phase do exhibit normal compensation. Eq. (5) indicates that this should happen as long as there is a reasonable linear relationship of the internal free energies ΔG_i^F to the total enthalpy ΔH_i^0 ; the fact that compensation temperatures exceed 230 K indicates that the slope of this relationship is positive.

Many authors have suggested entropy-enthalpy models that are specific to the individual macromolecular systems under study. We briefly summarize some of these. At present, none of them enable us to predict the increase of the compensation temperature above 230 K for a particular system.

Liu and Guo (3) published a comprehensive review of EEC that discusses a perspective originally advanced by Larsson (41). For the biomolecules we are discussing, this perspective emphasizes the vibrational properties of the solute macromolecule itself. This seems entirely plausible for macromolecules in solution, and might account broadly for the range of compensation temperatures in Fig. 2.

Another intriguing idea has been advanced in several papers by Starikov and Nordén, who rationalize EEC data in terms of a micro-phase transition (MPT) (14;18). Starikov and Nordén explained that modifications of the nucleotide sequence of the DNA binding site corresponded to an “imaginary artificial heat pump,” whereas the changes of the binding polypeptide chain, which also involved changes in electrostatics, represented a “imaginary artificial refrigerator,” accounting for an equivalent Carnot cycle of the MPT (18). In this respect it is interesting that both the eEEC and MEE models lead to entropy changes that are proportional to the enthalpy change. Such proportionality doesn't account for a non-zero free-energy intercept ΔG_C (see figures). Starikov and Nordén have emphasized that the Carnot interpretation involves energies such as ΔG_C , which are apparently true internal properties of the embedded chemical process.

We note that entropy-enthalpy compensation is involved in measurement of the “temperature factor” of a protein, Q_{10} , which is defined as a ratio between the kinetic rate

constant at the absolute temperature $T + 10$ K and the same kinetic rate constant at T . Values are typically between 2–6 (42–46), which requires an activation enthalpy that is much greater than the thermal energy factor $k_B T$. Large enthalpic contributions (ΔH) to the transition state energies would imply that these transitions would never be observable or would have a very low probability. Therefore, these enthalpic contributions have to be compensated by large entropic contributions (ΔS) to drastically reduce the activation free energies (ΔG) required for such conformational transitions in proteins and other biopolymers (42).

In the most simplistic reasoning, the breaking of inter- and intra-molecular bonds, either covalent or non-covalent, in a biomolecular system in aqueous phase, which includes the molecules under investigation as well as the solvent, will be an endothermic process. This is indicated by an increase in the standard enthalpy, ΔH° (it is a positive parameter). Intuitively, this is accompanied by an enhancement in the molecular mobility and backbone flexibility of the participating molecules, which would result in a greater standard entropy ΔS° (it is a positive parameter) (9;47). The compensatory nature of these thermodynamic parameters means that the absolute values of these parameters cannot be employed as a diagnostic of a particular biomolecular interaction (48;49). Large compensatory values of ΔH° and ΔS° produce a small value of ΔG° , a parameter that characterizes the functionality of a biomolecular system in aqueous phase (50).

Acknowledgments

The authors thank Arthur Yelon (École Polytechnique de Montréal) for Meyer-Neldel discussions over some years and for a critical reading of the manuscript. LM's research has been funded in part by grants from the US National Science Foundation (DMR-1006332) and the National Institutes of Health (R01 GM088403).

References

1. Movileanu L, Benevides JM, Thomas GJ. Determination of base and backbone contributions to the thermodynamics of premelting and melting transitions in B DNA. *Nucleic Acids Res.* 2002; 30:3767–3777. [PubMed: 12202762]
2. Lumry R, Rajender S. Enthalpy-Entropy Compensation Phenomena in Water Solutions of Proteins and Small Molecules - A Ubiquitous Property of Water. *Biopolymers.* 1970; 9:1125. [PubMed: 4918636]
3. Liu L, Guo QX. Isokinetic relationship, isoequilibrium relationship, and enthalpy-entropy compensation. *Chem. Rev.* 2001; 101:673–695. [PubMed: 11712500]
4. Lumry R. Uses of enthalpy-entropy compensation in protein research. *Biophys. Chem.* 2003; 105:545–557. [PubMed: 14499917]
5. Qian H, Hopfield JJ. Entropy-enthalpy compensation: perturbation and relaxation in thermodynamic systems. *J. Phys. Chem.* 1996; 105:9292–9298.
6. Yelon A, Movaghar B, Crandall RS. Multi-excitation entropy: its role in thermodynamics and kinetics. *Reports on Progress in Physics.* 2006; 69:1145–1194.
7. Starikov EB, Norden B. Enthalpy-entropy compensation: a phantom or something useful? *J. Phys. Chem. B.* 2007; 111:14431–14435. [PubMed: 18044870]
8. Steinert HS, Rinnenthal J, Schwalbe H. Individual Basepair Stability of DNA and RNA Studied by NMR-Detected Solvent Exchange. *Biophys. J.* 2012; 102:2564–2574. [PubMed: 22713572]
9. Grunwald E, Steel C. Solvent Reorganization and Thermodynamic Enthalpy-Entropy Compensation. *Journal of the American Chemical Society.* 1995; 117:5687–5692.
10. Ben-Naim, A. *Molecular Theory of Water and Aqueous Solutions - Part I: Understanding Water* World Scientific. Singapore: 2009.
11. Linert W, Jameson RF. The Isokinetic Relationship. *Chemical Society Reviews.* 1989; 18:477–505.

12. Krug RR, Hunter WG, Grieger RA. Enthalpy-Entropy Compensation .1. Some Fundamental Statistical Problems Associated with Analysis of Vant Hoff and Arrhenius Data. *Journal of Physical Chemistry*. 1976; 80:2335–2341.
13. Krug RR, Hunter WG, Grieger RA. Statistical Interpretation of Enthalpy-Entropy Compensation. *Nature*. 1976; 261:566–567.
14. Starikov EB, Norden B. Entropy-enthalpy compensation as a fundamental concept and analysis tool for systematical experimental data. *Chemical Physics Letters*. 2012; 538:118–120.
15. Krug RR, Hunter WG, Grieger RA. Enthalpy-Entropy Compensation .2. Separation of Chemical from Statistical Effect. *Journal of Physical Chemistry*. 1976; 80:2341–2351.
16. Gilli P, Ferretti V, Gilli G, Borea PA. Enthalpy-Entropy Compensation in Drug-Receptor Binding. *Journal of Physical Chemistry*. 1994; 98:1515–1518.
17. Seldeen KL, McDonald CB, Deegan BJ, Bhat V, Farooq A. DNA plasticity is a key determinant of the energetics of binding of Jun-Fos heterodimeric transcription factor to genetic variants of TGACGTCA motif. *Biochemistry*. 2009; 48:12213–12222. [PubMed: 19921846]
18. Starikov EB, Norden B. Entropy-enthalpy compensation may be a useful interpretation tool for complex systems like protein-DNA complexes: An appeal to experimentalists. *Applied Physics Letters*. 2012; 100
19. Movileanu L, Benevides JM, Thomas GJ. Temperature dependence of the Raman spectrum of DNA. Part I - Raman signatures of premelting and melting transitions of poly(dA-dT)center dot poly(dA-dT). *J. Raman Spectrosc*. 1999; 30:637–649.
20. Movileanu L, Benevides JM, Thomas GJ. Temperature dependence of the Raman spectrum of DNA. II. Raman signatures of premelting and melting transitions of poly(dA)- poly(dT) and comparison with poly(dA-dT)-poly(dA-dT). *Biopolymers*. 2002; 63:181–194. [PubMed: 11787006]
21. Liu L, Yang C, Guo QX. A study on the enthalpy-entropy compensation in protein unfolding. *Biophys. Chem*. 2000; 84:239–251. [PubMed: 10852311]
22. Blasie CA, Berg JM. Entropy-enthalpy compensation in ionic interactions probed in a zinc finger peptide. *Biochemistry*. 2004; 43:10600–10604. [PubMed: 15301557]
23. Raffa RB. (Extra)thermodynamics of the drug-receptor interaction. *Life Sci*. 1999; 65:967–980. [PubMed: 10499865]
24. Whitesides GM, Krishnamurthy VM. Designing ligands to bind proteins. *Q. Rev. Biophys*. 2005; 38:385–395. [PubMed: 16817982]
25. Marshall GR, Feng JA, Kuster DJ. Back to the future: ribonuclease A. *Biopolymers*. 2008; 90:259–277. [PubMed: 17868092]
26. Dunitz JD. Win some, lose some: enthalpy-entropy compensation in weak intermolecular interactions. *Chem. Biol*. 1995; 2:709–712. [PubMed: 9383477]
27. Qian H, Chan SI. Interactions between a helical residue and tertiary structures: helix propensities in small peptides and in native proteins. *J. Mol. Biol*. 1996; 261:279–288. [PubMed: 8757294]
28. Cooper A, Johnson CM, Lakey JH, Nollmann M. Heat does not come in different colours: entropy-enthalpy compensation, free energy windows, quantum confinement, pressure perturbation calorimetry, solvation and the multiple causes of heat capacity effects in biomolecular interactions. *Biophysical Chemistry*. 2001; 93:215–230. [PubMed: 11804727]
29. Ferrante A, Gorski J. Enthalpy-entropy compensation and cooperativity as thermodynamic epiphenomena of structural flexibility in ligand-receptor interactions. *J. Mol. Biol*. 2012; 417:454–467. [PubMed: 22342886]
30. Oliveberg M, Tan YJ, Fersht AR. Negative activation enthalpies in the kinetics of protein folding. *Proc. Natl. Acad. Sci. U. S. A*. 1995; 92:8926–8929. [PubMed: 7568045]
31. Noronha M, Gerbelova H, Faria TQ, Lund DN, Smith DA, Santos H, Macanita AL. Thermal unfolding kinetics of ubiquitin in the microsecond-to-second time range probed by Tyr-59 fluorescence. *J. Phys. Chem. B*. 2010; 114:9912–9919. [PubMed: 20666531]
32. Cheneke BR, Indic M, van den Berg B, Movileanu L. An Outer Membrane Protein undergoes Enthalpy- and Entropy-driven Transitions. *Biochemistry*. 2012; 51:5348–5358. [PubMed: 22680931]

33. Yu HA, Karplus M. A Thermodynamic Analysis of Solvation. *Journal of Chemical Physics*. 1988; 89:2366–2379.
34. Peacock-Lopez E, Suhl H. Compensation Effect in Thermally Activated Processes. *Physical Review B*. 1982; 26:3774–3782.
35. Khait YL. Kinetic Many-Body Theory of Short-Lived Large Energy Fluctuations of Small Numbers of Particles in Solids and Its Applications. *Physics Reports-Review Section of Physics Letters*. 1983; 99:239–340.
36. Yelon A, Movaghar B. Microscopic Explanation of the Compensation (Meyer-Neldel) Rule. *Physical Review Letters*. 1990; 65:618–620. [PubMed: 10042969]
37. Yelon A, Movaghar B, Branz HM. Origin and Consequences of the Compensation (Meyer-Neldel) Law. *Physical Review B*. 1992; 46:12244–12250.
38. Yelon A, Sacher E, Linert W. Multi-Excitation Entropy, Entropy-Enthalpy Relations, and their Impact on Catalysis. *Catalysis Letters*. 2011; 141:954–957.
39. Linert W. Concerning the Problem of the Isokinetic Relationship .4. the Evaluation of the Isosubstituent Hammett Parameter by Means of Isokinetic and Isoequilibrium Temperatures. *Australian Journal of Chemistry*. 1986; 39:199–208.
40. Ford DM. Enthalpy-entropy compensation is not a general feature of weak association. *J. Am. Chem. Soc.* 2005; 127:16167–16170. [PubMed: 16287305]
41. Larsson R. Zeolite catalysis and molecular vibrations. *Catalysis Today*. 1988; 3:387–394.
42. Liu B, Hui K, Qin F. Thermodynamics of heat activation of single capsaicin ion channels VR1. *Biophys. J.* 2003; 85:2988–3006. [PubMed: 14581201]
43. Yao J, Liu B, Qin F. Rapid temperature jump by infrared diode laser irradiation for patch-clamp studies. *Biophys. J.* 2009; 96:3611–3619. [PubMed: 19413966]
44. Yao J, Liu B, Qin F. Kinetic and energetic analysis of thermally activated TRPV1 channels. *Biophys. J.* 2010; 99:1743–1753. [PubMed: 20858418]
45. Gupta S, Auerbach A. Temperature dependence of acetylcholine receptor channels activated by different agonists. *Biophys. J.* 2011; 100:895–903. [PubMed: 21320433]
46. Gupta S, Auerbach A. Mapping heat exchange in an allosteric protein. *Biophys. J.* 2011; 100:904–911. [PubMed: 21320434]
47. Calderone CT, Williams DH. An enthalpic component in cooperativity: the relationship between enthalpy, entropy, and noncovalent structure in weak associations. *J. Am. Chem. Soc.* 2001; 123:6262–6267. [PubMed: 11427049]
48. Sharp K. Entropy-enthalpy compensation: fact or artifact? *Protein Sci.* 2001; 10:661–667. [PubMed: 11344335]
49. Prabhu NV, Sharp KA. Heat capacity in proteins. *Annual Review of Physical Chemistry*. 2005; 56:521–548.
50. Cooper A. Thermodynamic analysis of biomolecular interactions. *Curr. Opin. Chem. Biol.* 1999; 3:557–563. [PubMed: 10508661]
51. Seldeen KL, McDonald CB, Deegan BJ, Bhat V, Farooq A. Dissecting the role of leucine zippers in the binding of bZIP domains of Jun transcription factor to DNA. *Biochem. Biophys. Res. Commun.* 2010; 394:1030–1035. [PubMed: 20331972]
52. Kuroki R, Nitta K, Yutani K. Thermodynamic changes in the binding of Ca²⁺ to a mutant human lysozyme (D86/92). Enthalpy-entropy compensation observed upon Ca²⁺ binding to proteins. *J. Biol. Chem.* 1992; 267:24297–24301. [PubMed: 1447179]
53. Houk KN, Leach AG, Kim SP, Zhang XY. Binding affinities of host-guest, protein-ligand, and protein-transition-state complexes. *Angewandte Chemie-International Edition*. 2003; 42:4872–4897.
54. Starikov EB, Norden B. DNA duplex length and salt concentration dependence of enthalpy-entropy compensation parameters for DNA melting. *J. Phys. Chem. B*. 2009; 113:11375–11377. [PubMed: 19719257]
55. Liu G, Gu D, Liu H, Ding W, Luan H, Lou Y. Thermodynamic properties of micellization of Sulfobetaine-type Zwitterionic Gemini Surfactants in aqueous solutions—a free energy perturbation study. *J. Colloid Interface Sci.* 2012; 375:148–153. [PubMed: 22424764]

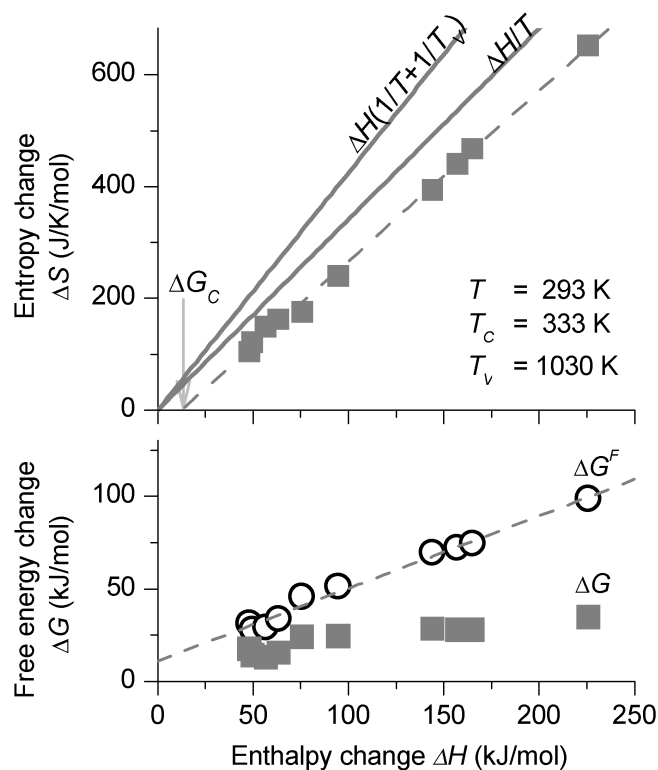


Figure 1. (upper) The symbols are measurements of entropy change and enthalpy change for imino proton exchange for several basepairs in RNA. The dashed fitting line yields the compensation temperature T_C and free energy ΔG_C for the measurements. The line labeled $\Delta H/T$ shows exact compensation; the line labeled $\Delta H/(1/T + 1/T_v)$ shows the effects of water's structural relaxation and vibrational quantization (multi-excitation entropy). (lower) The solid symbols show Gibbs free energy measurements on the base pairs ΔG_i ; The open symbols show the free energies ΔG_i^F after correction for water's structural relaxation and vibrational quantization.

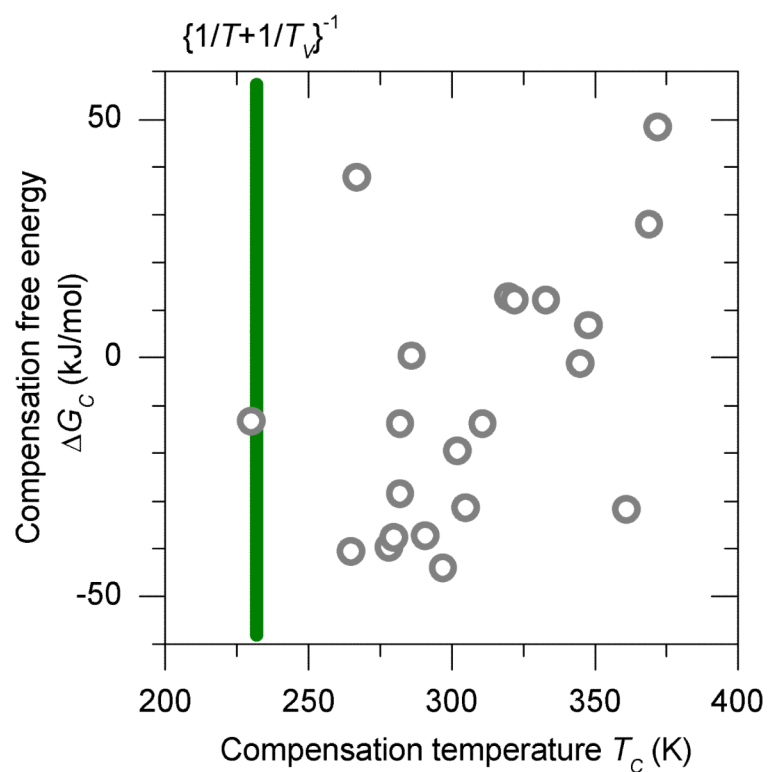


Figure 2. Two-dimensional scatter plot of the compensation temperatures and Gibbs compensation free energies for some biomolecular systems in aqueous phase. See Table I for details. The vertical line at 235 K indicates the compensation temperature that is predicted from the properties of water, and neglecting the internal entropy and enthalpy changes of the macromolecule systems.

Table 1

Compensation temperatures and the Gibbs free energies at the compensation temperature for various chemical and biophysical systems

| T_c (K) | ΔG_C^0 kJ/mol | System | Experimental Approach | Reference |
|-----------|-----------------------|---|--|--|
| 278 | -39.9 | drug-protein receptor binding interactions | Temperature dependence of association constants | Gilli <i>et al.</i> (16) |
| 305 | -31.5 | DNA-transcriptional factor interactions | analytical laser scattering (ALS) in combination with isothermal titration calorimetry (ITC) | Seldeen <i>et al.</i> , 2009 (17) and Starikov and Nordén, 2012 (18) |
| 291 | -37.4 | DNA-transcriptional factor interactions | analytical laser scattering (ALS) in combination with isothermal titration calorimetry (ITC) | Seldeen <i>et al.</i> , 2009 (51) and Starikov and Nordén, 2012 (18) |
| 282 | -28.6 | DNA-drug interactions | Combination of spectroscopic and calorimetric techniques | Starikov and Norden, 2007 (7) |
| 361 | -31.9 | DNA-drug interactions | Combination of spectroscopic and calorimetric techniques | Starikov and Nordén, 2007 (7) |
| 280 | -37.8 | Calcium binding | Calorimetry | Kuroki and colleagues (52) and Sharp, 2001 (48) |
| 286 | 0.4 | Small globular protein unfolding | Calorimetry | Sharp, 2001 (48) |
| 267 | 37.8 | Unfolding of large proteins | Hydrogen exchange protection factors | Sharp, 2001 (48) |
| 282 | -13.9 | Host-guest complexes of cyclodextrins | Calorimetry | Houk <i>et al.</i> , 2003 (53) |
| 311 | -13.9 | Host-guest complexes of non-cyclodextrins | Calorimetry | Houk <i>et al.</i> , 2003 (53) |
| 230 | -13.4 | Host-guest complexes in non-aqueous solution | Calorimetry | Houk <i>et al.</i> , 2003 (53) |
| 297 | -44.1 | Antibody-antigen complexes of proteins and carbohydrates | Calorimetry | Houk <i>et al.</i> , 2003 (53) |
| 320 | 12.6 | Thermally induced unfolding in globular proteins | Two-state analysis of differential scanning calorimetry (DSC) | Cooper <i>et al.</i> , 2001 (28) |
| 322 | 12 | DNA base-pair opening | NMR spectroscopy coupled with temperature dependence of imino proton exchange rates | Steinert <i>et al.</i> , 2012 (8) |
| 333 | 12 | RNA base-pair opening | NMR spectroscopy coupled with temperature dependence of imino proton exchange rates | Steinert <i>et al.</i> , 2012 (8) |
| 372 | 48.3 | Melting of DNA duplex | Differential scanning calorimetry | Steinert <i>et al.</i> , 2012 (54) |
| 369 | 27.9 | Ligand-receptor interactions | Competitive peptide binding assay | Ferrante and Gorski, 2012 (29) |
| 265 | -40.6 | Drug-membrane protein receptor interactions | Calorimetry | Grunwald and Steel, 1995 (9) |
| 302 | -19.7 | Thermodynamic properties of micellization of Sulfobetaine-type Zwitterionic Gemini Surfactants in aqueous solutions | A free energy perturbation study | Liu <i>et al.</i> , 2012 (55) |
| 345 | -1.3 | Melting of nucleic acids Poly(dA-dT)-poly(dA-dT) | Differential Raman spectroscopy | Movileanu, <i>et al.</i> 2002 (1) |
| 348 | 6.7 | Melting of nucleic acids Poly(dA)-poly(dT) | Differential Raman spectroscopy | Movileanu, <i>et al.</i> , 2002 (1) |