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The Molecular Origin of Enthalpy/Entropy Compensation in Biomolecular Recognition

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Abstract

Biomolecular recognition can be stubborn; changes in the structures of associating molecules, or the environments in which they associate, often yield compensating changes in enthalpies and entropies of binding and no net change in affinities. This phenomenon—termed enthalpy/entropy (H/S) compensation—hinders efforts in biomolecular design, and its incidence—often a surprise to experimentalists—makes interactions between biomolecules difficult to predict. Although characterizing H/S compensation requires experimental care, it is unquestionably a real phenomenon that has, from an engineering perspective, useful physical origins. Studying H/S compensation can help illuminate the still-murky roles

of water and dynamics in biomolecular recognition and self-assembly. This review summarizes known sources of H/*S* compensation (real and perceived) and lays out a conceptual framework for understanding and dissecting—and, perhaps, avoiding or exploiting—this phenomenon in biophysical systems.

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INTRODUCTION

Biology—and, thus, life—is the sum of coordinated interactions among biomolecules. The specific association of proteins and ligands—and the self-assembly of proteins into multi-protein complexes—guides cellular organization and signal transduction; enables metabolism, growth, and motility; and directs the synthesis and translation of genetic material. Molecular recognition

by biomolecules is centrally important to the molecular foundations of life yet remains frustratingly difficult to rationalize in molecular detail. Small changes in the structures of ligands and proteins often influence binding in unintuitive ways, and there are still—despite decades of research by very skilled scientists—no generalizable methods to predict that influence (100, 114).

The mysteries of binding between biomolecules in water are, perhaps, best illustrated by a commonly encountered phenomenon: enthalpy/entropy (H/S) compensation—compensating (and, frequently, canceling) changes in the enthalpy and entropy of binding that result from structural modifications to binding partners and/or changes in environmental conditions. H/S compensation is alternatively invoked as a general mechanism of biological homeostasis (23) or a common result of experimental error (24, 106). This review begins by summarizing both explanations and focuses, thereafter, on the molecular origin of H/S compensation in systems for which it is unambiguously present and particularly pronounced. Compensating phenomena often determine the navigability of structure–activity landscapes; an understanding of their molecular origins may, thus, reveal approaches for traversing those landscapes in efforts to engineer the activity of biomolecules [e.g., enzymes (64, 73, 99) or riboswitches (69, 116)] or to control the strength of interactions between them [e.g., between low-molecular-weight drugs and proteins (8, 44), antibodies and receptors (1, 61), and proteins and other proteins (6, 68, 89)].

GENERAL SOURCES OF ENTHALPY/ENTROPY COMPENSATION

Recent surveys of H/S compensation have focused on two questions: (*a*) Is it real? and (*b*) Is it general? Treatments of the first question have pointed to common sources of experimental error in thermodynamic measurements (21, 24, 85, 102). Treatments of the second have invoked statistical mechanical analyses of simplified model systems (26, 98) or highlighted—through tabulation (81, 90)—many examples [most commonly, protein folding (86) or protein–protein (47, 95), protein–ligand (10, 107), or protein–nucleotide association (50, 51, 79)].

Experimental Error

Methods to estimate changes in enthalpy and entropy associated with biomolecular interactions are indirect. Older studies tended to rely on Van't Hoff analyses, where estimates of enthalpy and entropy of binding (ΔH°_b and ΔS°_b, respectively) are derived from measurements of dissociation constants (K_ds) at different temperatures:

$$\ln(K_d) = \frac{\Delta H^\circ_b}{RT} - \frac{\Delta S^\circ_b}{R} = \frac{\Delta G^\circ_b}{RT}. \quad 1.$$

The slope and *y* intercept of linear fits to Equation 1 [i.e., plots of $\ln(K_d)$ versus $1/T$] yield estimates of ΔH°_b and ΔS°_b. Such fits assume that values of ΔH°_b and ΔS°_b are independent of temperature, often a poor assumption given the temperature dependence of many properties of proteins, ligands, and water (3, 38, 75). With this approach, errors in ΔH°_b, which tend to be large relative to the magnitude of ΔG°_b, give rise to large errors in ΔS°_b and can, thus, cause an apparent but physically irrelevant form of H/S compensation (70).

In contrast with Van't Hoff analyses, isothermal titration calorimetry (ITC) enables independent estimates of ΔH°_b (from heats of binding) and ΔG°_b (from nonlinear fits to plots of heat generated versus the molar ratio of ligand to macromolecule); the difference in these two parameters yields the entropy of binding:

$$-T\Delta S^\circ_b = \Delta G^\circ_b - \Delta H^\circ_b. \quad 2.$$

Entropies of binding determined via ITC are much less susceptible to systematic error than entropies determined from Van't Hoff plots and are, thus, much more accurate. With that said, because estimates of entropy and enthalpy remain coupled to one another, correlated errors can still yield compensation of a trivial origin (21, 106); careful experimental design, execution, and statistical analysis remain critically important.

Experimental errors leading to H/S compensation are common (21, 85), but they can be minimized with appropriate precautions. Examples of compensating—and statistically significant ($p \leq 0.01$)—differences in enthalpies and entropies of binding between similar processes (differences on the order of 1–15 kcal mol^{-1}) have been observed in many systems (81, 90); the high incidence of such examples motivates a discussion of their physical basis.

Perturbation of a Small Number of Energy Levels

A statistical mechanical argument for H/S compensation outlined by Sharp (98) suggests that it arises from correlated changes in internal energy and entropy that result from perturbations of a system with many closely spaced energy levels. (In biological systems, the difference between internal energy and enthalpy is negligible, and Sharp makes no distinction between them. See the sidebar, Thermodynamic Quantities in Biological Systems, for standard definitions of thermodynamic properties). **Figure 1*a*,*b*** illustrates this argument for a model system with a Gaussian distribution of internal energy levels. **Figure 1*a*** plots the occupancy probability, $P(U)$, of energy levels (U) in an unperturbed system (kcal mol^{-1}) (Equation 3); **Figure 1*b*** shows changes in mean internal energy (ΔU, kcal mol^{-1}) and entropy ($T\Delta S$, kcal mol^{-1}) that result from perturbations

THERMODYNAMIC QUANTITIES IN BIOLOGICAL SYSTEMS

Internal energy (U, kcal mol^{-1}) is the energy associated with the motions, interactions, and bonding of the constituent molecules of a system (96).

Potential energy (V, kcal mol^{-1}) is the work required to bring two molecules together from an infinite distance to a specified distance r (77).

Enthalpy (H, kcal mol^{-1}), a quantity defined out of convenience, describes the heat content of a system (27). It is defined by Equation a, where p and V are the system pressure and molar volume:

$$H \equiv U + pV. \qquad \text{a.}$$

In biological processes, where pressures and molar volumes are small, and where changes in these properties are negligible, enthalpy and internal energy—and changes in enthalpy and internal energy—are indistinguishable from one another:

$$H = \Delta(U + pV) \approx \Delta U. \qquad \text{b.}$$

In discussions of intermolecular potentials, which do not account for entropy, potential energy is equivalent to enthalpy.

Entropy (S, kcal mol^{-1} K) is a measure of the number of microscopic states of a system and commonly used as a metric for disorder (27).

Gibbs free energy (G, kcal mol^{-1}) determines the direction of a spontaneous process; it is a thermodynamic potential that is minimized when a system reaches equilibrium at constant temperature and pressure (27, 77). It is formally defined by Equation c:

$$G \equiv H - TS. \qquad \text{c.}$$

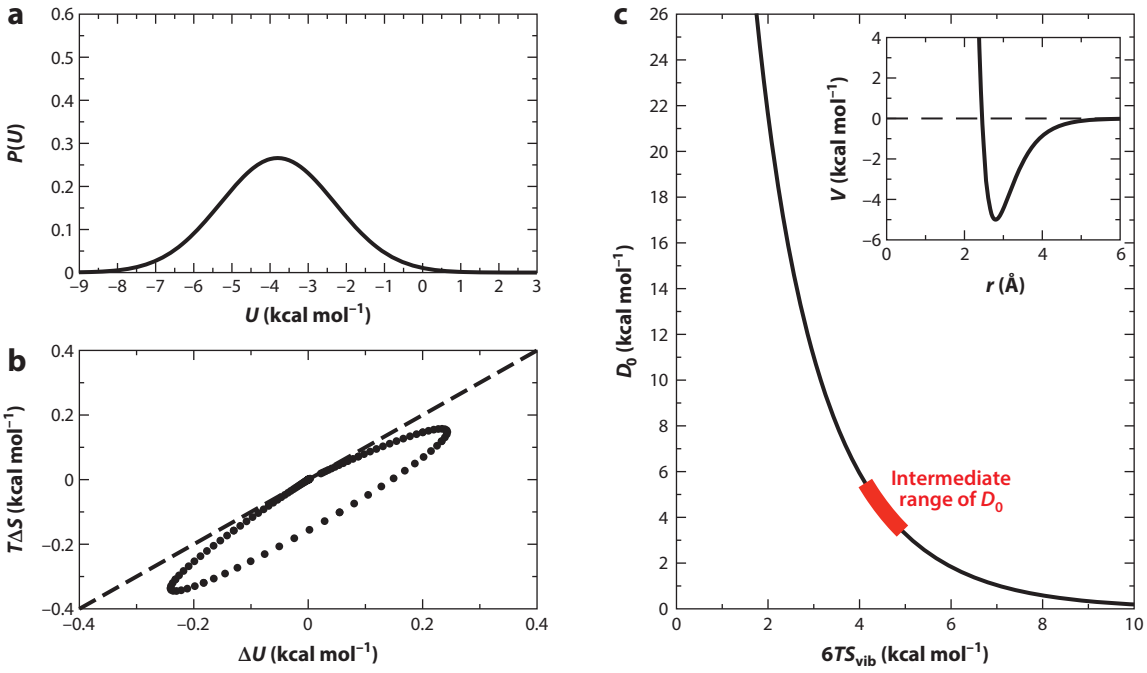


Figure 1

Rationalizations of enthalpy/entropy (H/S) compensation. (*a*) Occupancy probabilities [$P(U)$] for a system with a Gaussian distribution of energy levels ($T = 298$ K; $\sigma = 1.5$ kcal mol^{-1}). (*b*) Correlated changes in internal energy (ΔU) and entropy ($T\Delta S$) for a series of perturbations of different energy levels. Within the limits of experimental precision and/or experimentally accessible perturbations, data from this ellipse can appear linear (98). (*c*) H/S compensation for a hydrogen bond modeled by a Morse potential (*inset*). For these plots, we parameterized a hydrogen bond between a water molecule and a much larger molecule: $D_0 = 5$ kcal mol^{-1}, $r_0 = 2.8$ Å, $\mu = 18$ g mol^{-1}/6.023 $\times$ 10^{23} molecules mol^{-1}. Vibrational entropy (S_{vib}) is calculated from a vibrational partition function as described by Dunitz ($T = 298$ K) (26); estimates of the entropy of bonding ($6TS_{vib}$) assume equal contributions from six vibrational modes (one stretching, two rotational, and three translational modes). Over an intermediate range of dissociation energies [e.g., $D_0 = -\Delta H^\circ_b = 3.5 - 5.5$ kcal mol^{-1} for a typical hydrogen bond (*red highlight*)], enthalpic and entropic terms nearly cancel, leading to weak free energies of binding. Additional abbreviations: D_0, dissociation energy; μ, reduced mass; r_0, equilibrium bond length; S, entropy; T, temperature; U, internal energy; V, potential energy.

of that system (Equations 4 and 5).

$$P(U)\mathrm{d}U = \frac{\mathrm{d}U}{\sqrt{2\pi\sigma^2}} e^{\frac{U}{2\sigma^2} - \frac{U}{k_B T} - \frac{\sigma^2}{2k_B^2 T^2}} \quad 3.$$

$$\Delta U = (U - U')P(U')\mathrm{d}U \quad 4.$$

$$T\Delta S = (U - U' - k_B T)P(U')\mathrm{d}U \quad 5.$$

In Equations 3–5, σ is the standard deviation of the Gaussian distribution of internal energy levels, k_B is the Boltzmann constant, T is temperature (K), $\langle U \rangle$ is the mean internal energy of the unperturbed system, and U' is the perturbed internal energy level. Perturbations of different energy levels yield changes in mean internal energy (ΔU) and entropy ($T\Delta S$) that follow a narrow elliptical profile; data sets sampled from this distribution will, within the limits of experimental precision—or within the constraints of experimentally accessible perturbations—appear linear.

A second statistical mechanical argument outlined by Williams and colleagues (97) and Dunitz (26) suggests that H/S compensation occurs naturally when the strength of a bond increases (ΔH_b° becomes more negative) and, thus, "tightens" the bonded system ($-T\Delta S_b^\circ$ becomes more positive). **Figure 1*c*** illustrates this argument for a hydrogen bond modeled by a Morse potential (Equations 6–8).

$$V(r) = \frac{D_0}{6}\left[6\left(\frac{r_0}{r}\right)^{12} - 12\left(\frac{r_0}{r}\right)^{6}\right] \quad 6.$$

$$S_{vib} = R\left(\frac{x}{e^x - 1} - \ln\left(e^x - 1\right)\right) \quad 7.$$

$$x = \frac{h\upsilon}{k_B T}, \quad \upsilon = \frac{(f/\mu)^{1/2}}{2\pi}, \quad f = \frac{ml}{(r_0)^2} D_0 \quad 8.$$

In Equations 6–8, $V(r)$ is the potential energy (kcal mol^{-1}) of a hydrogen bond between a water molecule and a much larger molecule separated by a distance r (Å), D_0 is the dissociation energy (kcal mol^{-1}) of the bond, r_0 is the equilibrium bond length (Å), S_{vib} is the vibrational entropy associated with that bond (kcal mol^{-1} K^{-1}), υ is the frequency of stretching (cm^{-1}), h is Planck's constant, μ is the reduced mass of the system (g), and f is the quadratic force constant (kcal mol^{-1} Å^{-1}). [In this section, intermolecular potential energy $V(r)$ is equivalent to enthalpy, and D_0 is equivalent to the enthalpy of bonding (see the sidebar)]. Over an intermediate range of dissociation energies [e.g., $D_0 = -\Delta H_b^\circ = 3.5$–$5.5$ kcal mol^{-1} for a typical hydrogen bond, including hydrogen bonds previously observed to exhibit H/S compensation (62)], enthalpies and entropies nearly cancel.

Statistical mechanical analyses provide qualitative rationalizations of H/S compensation for simple bimolecular interactions [alongside the aforementioned examples, several alternative, but similarly focused explanations have been developed (55, 63, 93)], but they yield few molecular insights into the large, correlated changes in enthalpy and entropy observed in real systems, where proteins, ligands, and/or water engage in complex multi-point interactions. The remainder of this review attempts to glean such insights by examining the molecular determinants of H/S compensation in bimolecular systems.

MODEL SYSTEMS TO STUDY BIOMOLECULAR RECOGNITION

Studies of biomolecular recognition—and H/S compensation in particular—require the use of model systems. Biophysical models, like models in other disciplines (the hydrogen atom in chemistry, or the vibrating string in physics), enable the abstraction of complex processes down to simpler ones that can be studied with empirical observation. Carefully chosen proteins facilitate such abstractions by simplifying the (otherwise highly complex) binding process.

Biomolecular Recognition

The association of a protein and ligand in buffered aqueous solution can be thought of as the sum of nine processes (alternative groupings are also possible) (**Figure 2*a***): (*a*) the formation of protein–ligand contacts, (*b*) the rearrangement of water initially solvating the protein, (*c*) the rearrangement of water initially solvating the ligand, (*d*) the formation of a hydration structure around the protein–ligand complex, (*e*) changes in the conformation of the protein (between bound and unbound states), (*f*) changes in the conformation of the ligand, (*g*) changes in the dynamics

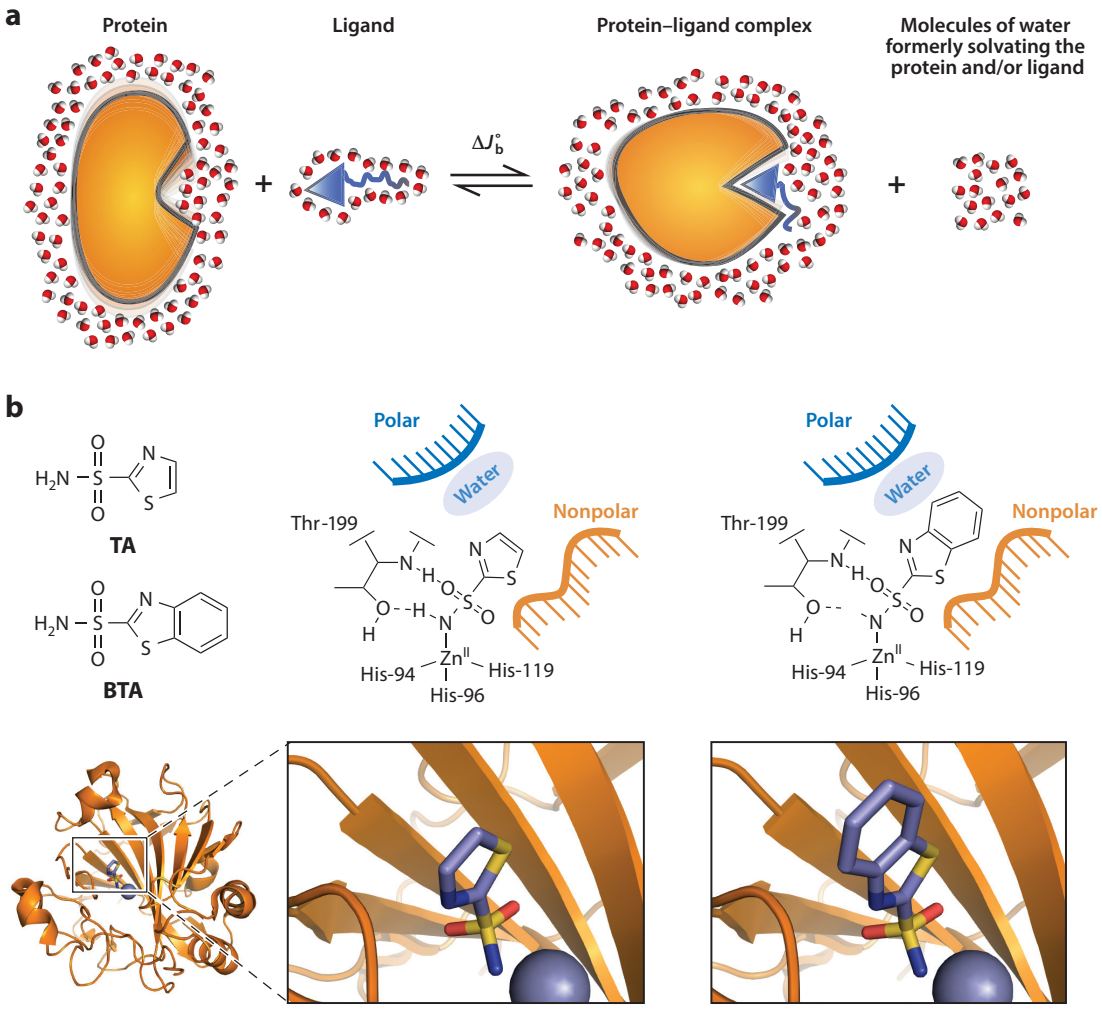


Figure 2

Model systems. (*a*) Biomolecular recognition can be broken down into nine processes: (*i*) the formation of protein–ligand contacts, (*ii*) the rearrangement of water initially solvating the protein, (*iii*) the rearrangement of water initially solvating the ligand, (*iv*) the formation of a hydration structure around the protein–ligand complex, (*v*) changes in the conformation of the protein, (*vi*) changes in the conformation of the ligand, (*vii*) changes in the dynamics of the protein, (*viii*) changes in the dynamics of the ligand, and (*ix*) changes in the organization of—and interactions associated with—buffer ions. This schematic illustrates processes *i–viii*. (*b*) A demonstration of the use of human carbonic anhydrase II to examine the influence of differences in ligand structure—in the absence of differences in protein conformation—on binding. The ligands 1,3-thiazole-2-sulfonamide (TA) and benzo[*d*]thiazole-2-sulfonamide (BTA) differ by a benzene ring but not in binding geometry and, thus, reveal the thermodynamic contribution of the benzene ring to binding. This specific comparison came from our benzo-extension study (see **Figure 5*c,d***). The illustration of bound ligands was adapted with permission from Reference 36. Additional abbreviation: ΔJ°_b, the change in a thermodynamic property J upon binding.

of the protein (i.e., the sampling of multiple protein conformations on multiple timescales), (*h*) changes in the dynamics of the ligand, and (*i*) changes in the organization of—and interactions associated with—buffer ions. Some proteins, as a result of their specific physical attributes (e.g., rigidity), allow a subset of these processes to be neglected and, thus, permit the others to be

studied in detail. Such proteins are, in some respects, exceptional (although, there is no generally agreed upon representative protein), but they are essential tools for exploring the molecular origins of H/S compensation in biomolecular recognition. Here, we review some examples.

Human Carbonic Anhydrase II

Human carbonic anhydrase II (HCAII), a protein that we have used repeatedly, represents a particularly valuable system for detailed biophysical studies (60). It has four principal advantages over (some) other proteins: (*a*) It can be expressed, purified, assayed, and crystallized with ease and, thus, facilitates the collection of large—and statistically significant—sets of biophysical data (32, 33). (*b*) It binds an enormous range of structurally varied sulfonamide ligands with a highly conserved geometry and, thus, permits detailed studies of the thermodynamic influence of differences in ligand structure on binding (**Figure 2*b***) (60). (*c*) It does not undergo significant conformational changes upon binding to structurally varied sulfonamide ligands [i.e., aligned crystal structures with and without sulfonamides bound have root-mean-square deviations of less than 0.3 Å (35, 60)]; it, thus, enables analysis of binding processes in the absence of changes in protein conformation. (*d*) Its binding pocket possesses a Zn^{2+} cofactor, a polar wall [Asn-62, His-64, Asn-67, Gln-92, Glu-206 (29)], and a nonpolar wall [Phe-131, Val-135, Leu-198, Pro-201, Pro-202, Leu-204 (22)] and, thus, permits studies of binding near chemically distinct—and differentially hydrated—surfaces.

Human Immunodeficiency Virus 1 Protease

Human immunodeficiency virus (HIV) 1 protease, a protein of immense pharmaceutical importance [it is the target of 10 drugs approved by the US Food and Drug Administration to treat HIV infection (74)], has a useful combination of attributes: (*a*) It is highly flexible (46, 84); (*b*) can accommodate mutations at multiple sites (4, 34); (*c*) can be expressed, assayed, and crystallized with minimal effort (71); and (*d*) can bind a wide range of readily synthesizable inhibitors (83). These attributes have enabled detailed analyses of the influence of mutation-derived changes in protein structure and dynamics on binding to structurally varied ligands (34, 53, 83, 88).

Other Proteases

Proteases such as thermolysin, thrombin, and trypsin are fairly rigid proteins (relative to HIV-1 protease) that (*a*) possess binding pockets with chemically distinct clefts (13, 16, 56), (*b*) bind a wide range of readily synthesizable peptide mimics (which may differ in cleft-specific substituents) (13, 16, 56), and (*c*) diffract at high resolution (~1.1 Å) (57, 67, 92). These attributes have enabled detailed dissections of structure–affinity relationships (dissections that make use of crystallographically resolvable hydration structures) for congeneric series of ligands (11–13, 16, 28, 49, 56, 109).

Others

Many other proteins—chosen for their tractability (i.e., availability, stability, and crystallizability), their possession of a unique structural characteristic [e.g., the particularly dry binding pocket of mouse major urinary protein (9, 14, 104) or the particularly nonpolar binding pockets of fatty acid binding proteins (40, 43, 76, 103)], and/or their physiological importance—have permitted insightful studies of specific attributes, or specific extremes, of biomolecular recognition.

Experimental Precautions

Model proteins are not immune to common sources of error in thermodynamic measurements, but, by virtue of their experimental tractability (and the existence of comparable data from multiple independent laboratories), they often allow error to be minimized. In our studies of HCAII, we reduced experimental error with three important precautions: (*a*) We used a single stock solution of ligand for each study (and for each ligand); (*b*) we carried out many repeated measurements (usually, $n \geq 7$ for each combination of ligand and protein); and (*c*) we used a physical-organic approach to experimental design (113). The first two precautions are motivated by a common problem: With ITC, errors in the concentration of ligand (titrant), which is assumed to be exact in most procedures for fitting thermograms, lead to proportional errors in estimates of K_a and ΔH_b° (21, 82). Accordingly, within a given study, the use of one stock solution of ligand for all experiments (which might differ from one another in temperature, buffer conditions, or protein) and the collection of many repeated measurements (which supply reliable averages and p values with which to compare them) allowed us to reduce sources of error that can cause physically uninteresting manifestations of H/S compensation.

Our third precaution represents an experimental approach. We focused our studies on incremental variations in the structures of ligands and/or proteins; such variations allowed us to observe trends in thermodynamic binding parameters that were inconsistent with—and, thus, insensitive to—experimental error. **Figure 3*a*,*b*** provides examples. **Figure 3*a*** plots measurements of free energy, enthalpy, and entropy of binding for bovine carbonic anhydrase (BCAII; the bovine analog of HCAII) and benzenesulfonamides substituted with chains of oligoglycine, oligosarcosine, and oligoethylene glycol. In this study, we observed that increased chain length correlated with less favorable enthalpies of binding and more favorable entropies of binding for each series of ligands. As experimental error—whether random or systematic—is unlikely to correlate with a structural variable (chain length), it is an improbable source of the observed trends. **Figure 3*b*** plots the influence of mutations in the binding pocket of HCAII on the enthalpy and entropy of binding for HCAII and benzo[*d*]thiazole-2-sulfonamide (BTA). When mutations were combined, changes in enthalpy and entropy (which nearly compensated) were either preserved or enhanced; error should not obey such conservation/additivity. The data in **Figure 3*a*,*b***, thus, illustrate how HCAII can be used to collect data uncontaminated by the types of errors that commonly compromise estimates of enthalpy and entropy. With carefully designed experiments, HCAII and other important model systems have yielded numerous examples of H/S compensation (81, 85, 90).

PROTEIN–LIGAND CONTACTS

To begin our discussion of the molecular origins of H/S compensation, we use a simplified, water-free description of binding: A protein and ligand, initially separated in a vacuum, bind one another. This description, while clearly overly simplistic, focuses attention on interactions between binding partners; we return to it shortly.

When two molecules form a complex, enthalpically favorable contacts between them can reduce their conformational, rotational, and/or translational freedom. This trade-off between enthalpy and entropy, when averaged over entire molecules, is clearly incomplete, or binding would not occur. Over specific regions of noncovalent association, however, it can bring about nearly perfect compensation.

Hydrogen Bonds

Hydrogen bond donors or acceptors offer a potential means of increasing the affinity of ligands for proteins. A favorable hydrogen bond worth ~1.5 kcal mol^{-1} in free energy, for example, should

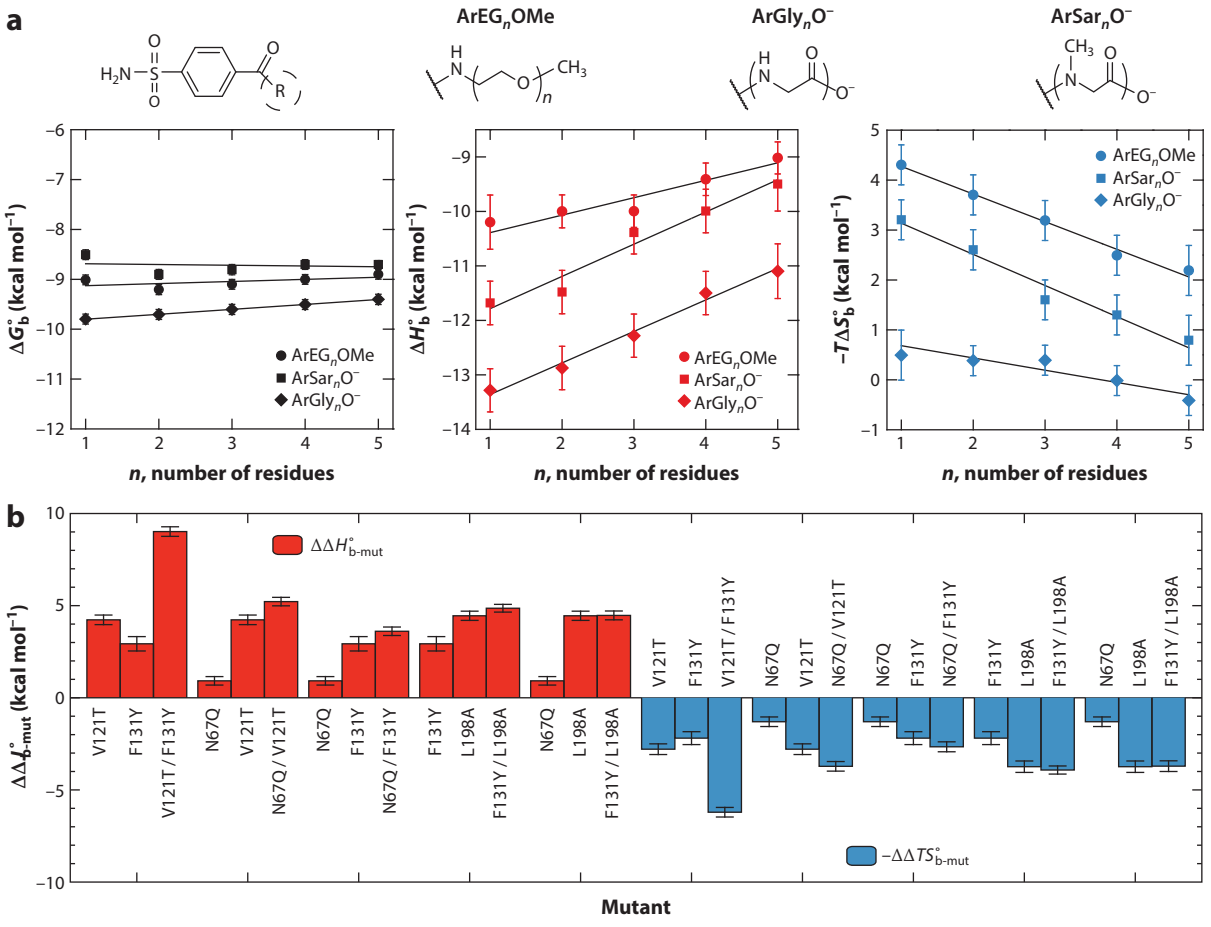
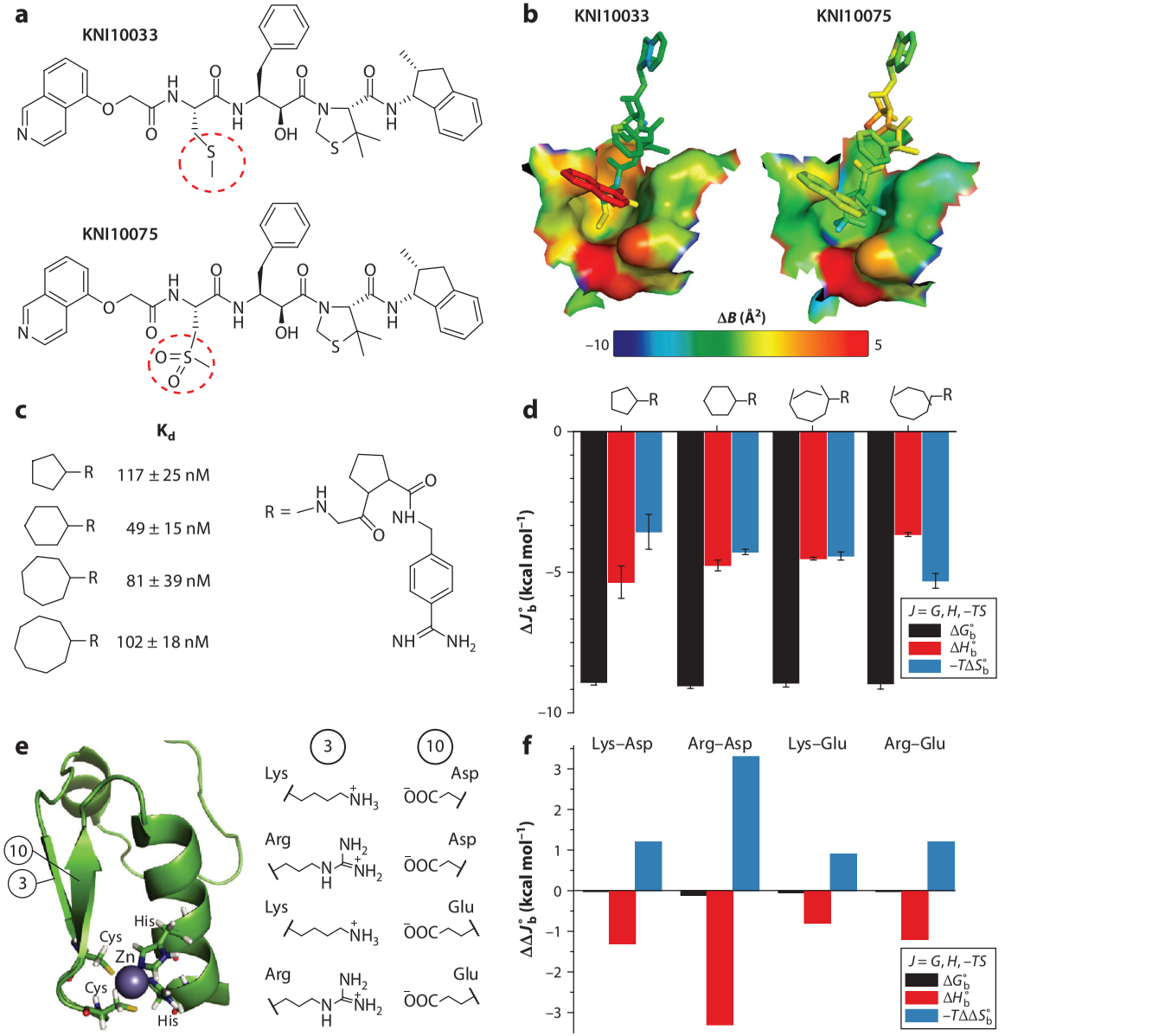


Figure 3

Experimental evidence of enthalpy/entropy (H/S) compensation. Experimental replicates—when carried out with carefully prepared stock solutions of ligands—reveal examples of H/S compensation that are inconsistent with experimental error. (*a*) Benzenesulfonamides substituted with chains of oligoglycine, oligosarcosine, and oligoethylene glycol exhibit affinities for BCAII that are insensitive to chain length, but they show compensating differences in enthalpies and entropies of binding that increase and decrease, respectively, with chain length (59). (*b*) Mutations in the binding pocket of HCAII influence the enthalpy and entropy of binding of BTA ($\Delta\Delta J^\circ_{b-mut} = \Delta J^\circ_{b-mut} - \Delta J^\circ_{b-WT}$, where $J = G$, H, or TS) in a compensating manner that is either preserved or enhanced when mutations are combined (35). Abbreviations: BCAII, bovine carbonic anhydrase II; HCAII, human carbonic anhydrase II; BTA, benzo[*d*]thiazole-2-sulfonamide; *G*, Gibbs free energy; *H*, enthalpy; *TS*, entropic component of free energy.

lower K_d by a factor of ten (at 298 K). In practice, however, additional hydrogen bonds between ligands and proteins often yield enthalpic and entropic contributions to binding that nearly cancel, leaving affinity unaltered. Freire and colleagues (62) observed such an effect when they attempted to increase the affinity of an inhibitor of HIV-1 protease by incorporating a sulfonyl group (**Figure 4*a***); a hydrogen bond between this group and a backbone amide lowered ΔH_b° by 3.9 kcal mol^{-1} but raised $-T\Delta S_b^\circ$ by an equal and opposite amount. Subsequent analysis of the B-factors of protein–inhibitor complexes indicated that both the protein and inhibitor became more rigid near the sulfonyl–amide bond (**Figure 4*b***). [The B-factor is a metric for



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Enthalpy/entropy compensation resulting from the entropic cost of enthalpically favorable contacts. (*a*) Two inhibitors of HIV-1 protease that differ by a sulfonyl group and, thus, in their ability to form a sulfonyl–amide bond with the protein backbone (62). (*b*) A comparison of B-factors between the two protein–ligand complexes shows that the additional hydrogen bond rigidifies the protein–ligand complex (note regions of *yellow* and *orange* on the left that become *blue* and *green* on the right). (*c*) Peptide mimics with different cycloalkyl moieties (16). (*d*) The ligands bind trypsin with similar free energies (ΔG°_b) but large differences in enthalpy and entropy of binding (ΔH°_b and $-T\Delta S^\circ_b$, respectively). Molecular dynamics simulations suggest that favorable entropies correspond to ligands that are more "mobile" in the binding pocket. (*e*) A zinc finger peptide on which pairs of charged residues at positions 3 and 10 (i.e., sites numbered in accordance with their position in the peptide sequence) were varied (15). (*f*) Enthalpies and entropies of interaction between charged residues ($\Delta\Delta H^\circ_b$ and $-T\Delta\Delta S^\circ_b$, respectively; each calculated from thermodynamic cycles of cation binding to incrementally varied pairs of residues) suggest strong compensation; free energies of interaction ($\Delta\Delta G^\circ_b$) vary little between pairs. Computational analysis of the most enthalpically stable pair indicates that it is also the most rigid. Abbreviations: *B*, B-factor; *G*, Gibbs free energy; *H*, enthalpy; *TS*, entropic component of free energy.

temperature-dependent atomic vibrations in X-ray crystal structures (91)]. The authors, thus, attributed most of the entropic penalty to conformational restrictions imposed by the hydrogen bond on the protein–ligand complex.

Klebe and colleagues (16, 54) observed similar thermodynamic trade-offs in their analysis of hydrogen bonds between peptide mimics and trypsin. Ligands with different cycloalkyl moieties proximal to a hydrogen bond donor—an amino group—exhibited indistinguishable binding affinities, but large differences in ΔH°_b and $-T\Delta S^\circ_b$ (**Figure 4*c*,*d***). Crystal structures of protein–ligand complexes revealed two poses: one in which a hydrogen bond between the amino group of the ligand and a backbone amide on the protein forced the cycloalkyl moiety outside of the hydrophobic S_3 pocket, and one in which the cycloalkyl moiety bound to the S_3 pocket, preventing a hydrogen bond from forming. Molecular dynamics (MD) simulations suggested that the first pose, which was more enthalpically favorable, reduced the mobility of the bound ligand, while the latter, which was more entropically favorable, gave the ligand greater conformational flexibility. The two poses (the former adopted by small ligands, and the latter by large ligands), thus, enabled different enthalpies and entropies of binding without changing free energy.

Ionic Interactions and Halogen Bonds

Ionic interactions and halogen bonds offer the same enthalpic promise of hydrogen bonds but often suffer from similar entropic penalties. Berg and colleagues studied the interaction of pairs of oppositely charged residues on zinc finger peptides by measuring the binding of cations to those pairs (for example, analysis of the binding of Cu^{2+} to Ser_3Ser_{10}, Ser_3Asp_{10}, Lys_3Ser_{10}, and Lys_3Asp_{10}, which constitute a thermodynamic cycle, can be used to study the binding of Lys_3 and Asp_{10} to one another; see Reference 15 for a detailed description of this approach). Their results suggested that the enthalpies and entropies of residue–residue association varied strongly between pairs of residues, while the free energies varied only slightly (**Figure 4*e*,*f***). MD simulations suggested, not surprisingly, that the most enthalpically favorable pair (Asp–Arg) suffered the largest loss in conformational entropy when it formed.

Ho and colleagues (18) observed a similar result in their study of Holliday junctions that contained halogen bonds between halogenated uracil bases and nonhalogenated adenine bases. [Holliday junctions are cross-shaped structures formed by four double-stranded segments of DNA in which each DNA molecule participates in two segments (112)]. An analysis of B-factors from X-ray crystal structures suggested that the most enthalpically favorable interactions (i.e., interactions involving highly polarizable anions) incurred strong entropic penalties that resulted from reduced intermolecular mobility.

The observations of Freire, Klebe, Berg, and Ho [and many others (58, 66, 94)] are qualitatively consistent with the simplified description of H/S compensation initially proposed by Williams and Dunitz: When molecules bind tightly (favorable enthalpy), they incur conformational constraints (unfavorable entropy). This succinct description clearly helps rationalize some binding phenomena. By ignoring the influence of water, however, it is incomplete and generally insufficient for explaining H/S compensation in aqueous systems.

WATER

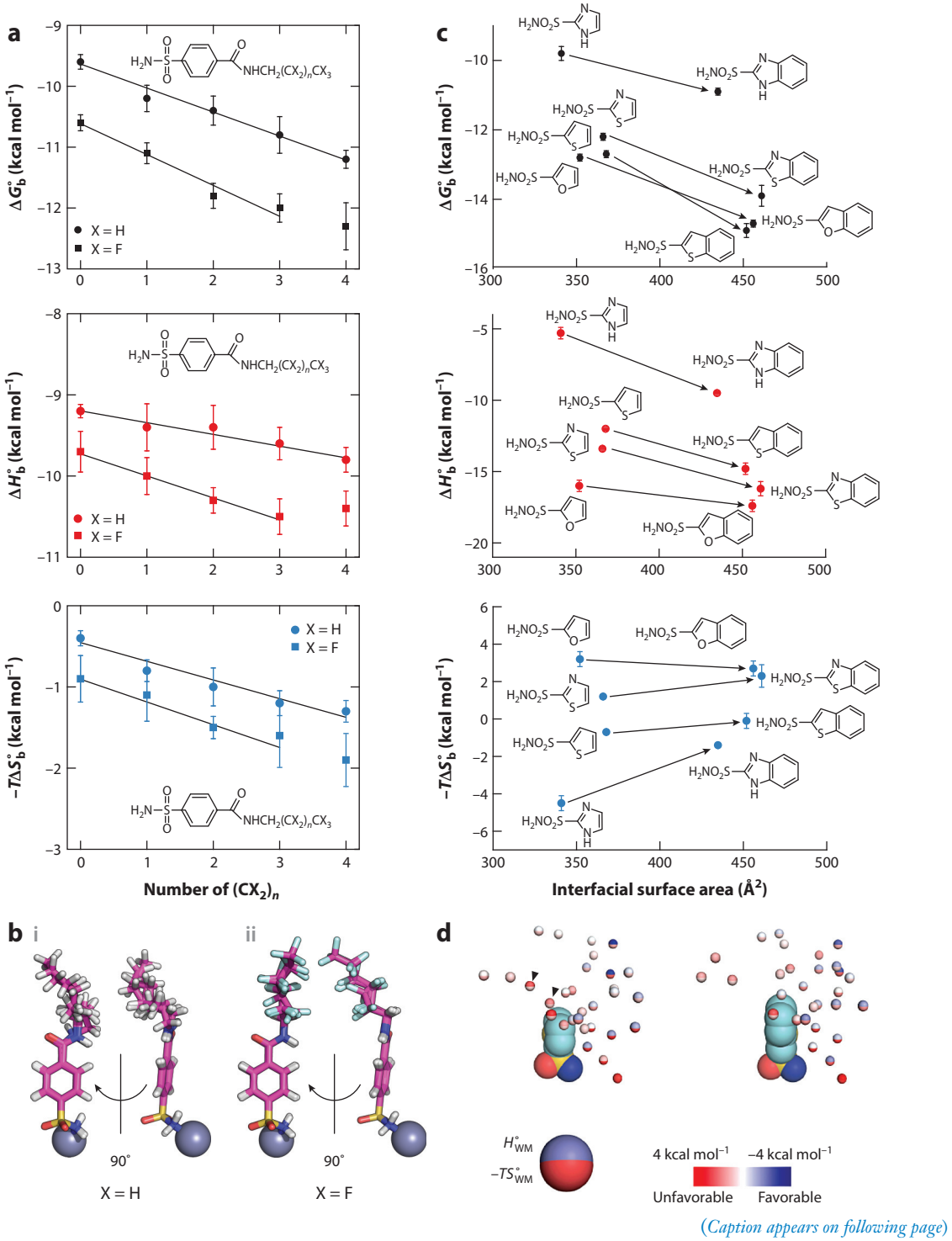
When proteins and ligands associate, water initially solvating each entity rearranges, yielding enthalpic and entropic contributions to binding that are still—despite advances in techniques for simulation—difficult to predict (65). In the hydrophobic effect, these contributions combine to yield a net favorable change in free energy. The way in which they combine, however, and their response (often compensating) to structural perturbations of ligand, protein, and other interacting entities—nonpolar or otherwise—is controversial (7).

The Hydrophobic Effect

The classical description of the hydrophobic effect—the explanation developed by Frank, Kauzmann, Tanford, and others (37, 52, 105)—suggests that it should be accompanied by H/S compensation: When two nonpolar surfaces associate with each other, ordered molecules of water solvating each surface are released to the bulk (i.e., the region of water where molecules do not "feel" the presence of solutes); binding, thus, yields a favorable change in entropy (ordered water becomes less ordered) and an unfavorable change in enthalpy (molecules of water formerly engaged in strong hydrogen bonds at nonpolar surfaces engage in weaker hydrogen bonds in the bulk). This description, while commonly invoked, is inconsistent with many—if not most—hydrophobic interactions in biological systems, where nonpolar entities are topologically complex and chemically varied. H/S compensation, when it occurs, does so through more than one mechanism.

Let us first point out that hydrophobic interactions between biomolecules need not bring about H/S compensation of any kind. In our analysis of the binding of HCAII to para-substituted benzenesulfonamides with alkyl and fluoroalkyl tails (para-substituents sometimes referred to as greasy tails), both sets of tails contributed favorably to the enthalpy and entropy of binding (**Figure 5*a***) (78). X-ray crystal structures, which showed the same binding geometry for nine out of ten ligands (**Figure 5*b***), suggested that these tails bound to the nonpolar wall of HCAII through (*a*) the enthalpically favorable release of nonoptimally bonded molecules of water that hydrate the unliganded binding pocket and (*b*) the entropically favorable release of tightly bound (by comparison with the bulk) molecules of water that hydrate the free ligand. For both sets of tails, surface area (not polarizability) determined the magnitude of the hydrophobic effect. This study motivated two questions. Does an increase in the nonpolar surface area of a ligand always increase its affinity for the binding pocket of HCAII? If so, does the mechanism of enhancement resemble that observed with greasy tails?

Our analysis of what we call benzo-extended ligands allowed us to begin answering these questions. The addition of a benzene ring to arylsulfonamide ligands (see **Figure 2*b*** for a depiction of the strategy) enhanced their affinity for the nonpolar wall of HCAII through an enthalpically favorable and (slightly) entropically unfavorable hydrophobic effect (**Figure 5*c***) (101). The addition of cyclohexyl rings yielded the same enthalpy-derived enhancement, which indicated that favorable van der Waals contacts were not the cause [a finding that contrasted with previous studies of enthalpy-driven hydrophobic effects (14, 104)]. Explicit-water calculations suggested an



(*Caption appears on following page*)

Figure 5 (*Figure appears on preceding page*)

Human carbonic anhydrase II (HCAII) and the hydrophobic effect. (*a*) Thermodynamic parameters (i.e., ΔJ°_b, where $J = H$ or TS) describe the binding of HCAII to para-substituted benzenesulfonamides with alkyl and flouroalkyl tails; both sets of tails contribute favorably to the enthalpy and the entropy of binding (78). (*b*) Aligned X-ray crystal structures of protein–ligand complexes. Results of this study suggest that ligands bind to the nonpolar wall of HCAII through (*i*) the enthalpically favorable release of nonoptimally bonded molecules of water that hydrate the unliganded binding pocket and (*ii*) the entropically favorable release of tightly bound molecules of water that hydrate the free ligand. (*c*) Thermodynamic binding parameters describe the binding of arylsulfonamides and their benzo-extended analogs to HCAII; see **Figure 2*b*** for an example of a representative protein–ligand complex from this study (101). (*d*) Results of WaterMap calculations for HCAII complexed with (*left*) thiophene-2-sulfonamide and (*right*) 1-benzothiophene-2-sulfonamide. Spheres represent molecules of water colored according to their enthalpies (*top*) and entropies (*bottom*) of hydration, relative to bulk water. The benzene ring expels two enthalpically favorable molecules of water (see *arrows*), giving rise to an enthalpy-driven hydrophobic effect. Panel *d* adapted with permission from Reference 101. Abbreviations: ΔJ°_b, the change in a thermodynamic property upon binding; *G*, Gibbs free energy; *H*, enthalpy; *TS*, entropic component of free energy.

alternative origin: an enthalpically favorable rearrangement of molecules of water that hydrate the nonpolar wall (**Figure 5*d***). This study, thus, supported the general contention that the hydrophobic effect results from differences in the thermodynamic characteristics of water in the bulk and water close to surfaces but suggested that those characteristics (which differed, slightly, between benzo-extensions and greasy tails) are highly dependent on the structures of interacting entities and, thus, difficult to generalize.

Our analysis of the binding of fluorinated benzothiazole sulfonamide ligands to HCAII suggested that modifications of the structures of nonpolar ligands can yield yet another outcome: complete compensation (17, 72). We observed that ligands with different fluorination patterns had different enthalpies and entropies of binding but indistinguishable free energies (with the exception of one ligand, where an enhanced affinity resulted from a more favorable free energy of desolvation) (**Figure 6*a*,*b***). As with greasy tails, ligands bound with a well-conserved—although, in two cases, a flipped—geometry. Computational estimates of changes in the thermodynamic properties of water during binding yielded trends similar to those exhibited by binding parameters (**Figure 6*c***); the similarity of these trends suggested that fluorination patterns brought about H/S compensation by reorganizing—and, thus, changing the enthalpic and entropic properties of—networks of water in the protein–ligand complex.

Our analyses of a hydrophobic association with the nonpolar wall of HCAII has, thus, illustrated a variety of thermodynamic mechanisms—some marked by H/S compensation and others devoid of it. Analyses of hydrophobic effects in other proteins have revealed similarly varied origins (2, 9, 14, 45). Such studies highlight the importance of understanding the context dependence of the hydrophobic effect—the way in which specific nonpolar regions of the ligand, protein, and protein–ligand complex affect the thermodynamic properties of proximal water networks—in efforts to exploit this effect in the design of high-affinity ligands.

Ionic Interactions and Hydrogen Bonds

The competing enthalpic and entropic contributions of aqueous reorganization to binding are not limited to hydrophobic interactions; they are, in fact, particularly pronounced in the formation of ion pairs. In our study of the binding of anions to the Zn^{2+} cofactor of HCAII (a binding process that is predominantly ionic, despite the ability of two of the eight anions studied to form hydrogen bonds with amino acids that neighbor Zn^{2+}), we observed that enthalpies and entropies of binding decreased and increased, respectively, with the chaotropicity of anions (36). Free energies of binding, by contrast, differed little between them and varied inversely with their affinity for water ($\Delta G^\circ_{hydration}$, the free energy change associated with the transfer of one mole of ion from the gas

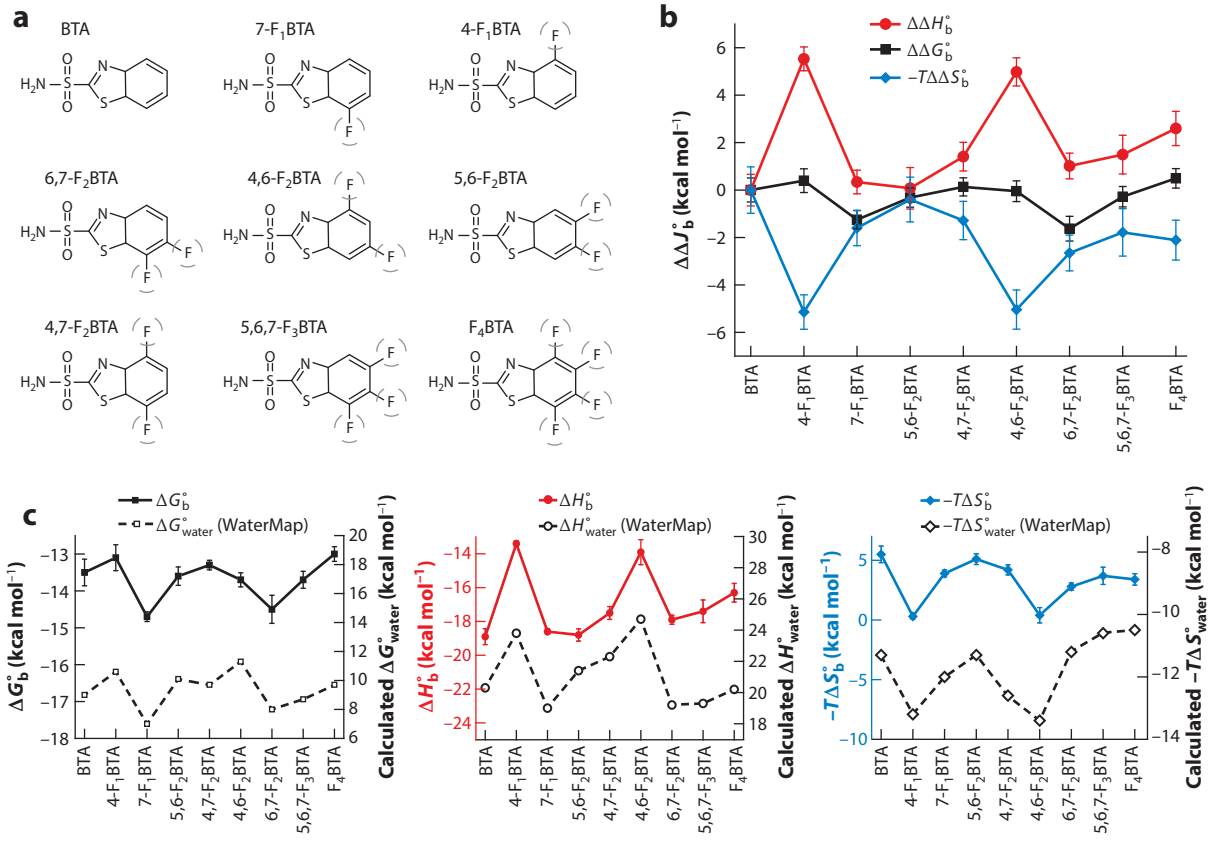


Figure 6

The binding of fluorinated ligands to HCAII. (*a*) Structures of partially fluorinated benzothiazole sulfonamide ligands (17). (*b*) Differences in the thermodynamic binding parameters of fluorinated ligands and BTA (e.g., $\Delta\Delta J^\circ_b = \Delta J^\circ_{b-BTA} - \Delta J^\circ_{b-FBTA}$, where $J = G$, H, or TS); changes in enthalpy are compensated by equal and opposite changes in entropy. (*c*) Estimates of binding parameters based on WaterMap-predicted hydration sites (i.e., binding parameters that account only for rearrangements in molecules of water) follow the same trends as binding parameters based on isothermal titration calorimetry and, thus, suggest that differences in $\Delta\Delta J^\circ$ between ligands result from differences in the organization—and thermodynamic properties—of water solvating the protein–ligand complexes. Figure adapted with permission from Reference 17. Abbreviations: BTA, benzo[*d*]thiazole-2-sulfonamide; ΔJ°_b, the change in a thermodynamic property J upon binding; G, Gibbs free energy; H, enthalpy; HCAII, human carbonic anhydrase II; TS, entropic component of free energy.

phase to water at standard state). This trend, in light of explicit-water calculations showing that water in various protein–anion complexes had similar thermodynamic properties, suggested that differences in binding resulted primarily from differences in the enthalpic and entropic costs of partially desolvating the anion.

Thermodynamic trade-offs between solute–solute and solute–water association may also play a role in the H/S compensation commonly observed in hydrogen bonds (H-bonds). In a recent study, the Savidge group (20) evaluated the H-bonding capability of individual atoms in protein–ligand complexes by pairing a theoretical model for the formation of hydrogen bonds with estimates of H-bonding capability based on water/hexadecane partition coefficients (differences in partition coefficients of functionalized hydrocarbons and saturated hydrocarbons of the same molecular surface area indicate the H-bonding capability of the functional group by which they differ).

Their analysis suggested that hydrogen bonds enhance protein–ligand affinity when both the donor and acceptor have significantly weaker or significantly stronger H-bonding capabilities than the hydrogen and oxygen atoms of water; a mismatch yields compensation. This observation could help explain why H-bonding functionalities often fail to improve the affinity of ligands and proteins in aqueous environments.

Breaking Enthalpy/Entropy Compensation Caused by Water

Our discussion of water motivates an important question: How do we "break" H/S compensation associated with rearrangements in molecules of water? Several studies have addressed this question with comprehensive thermodynamic and structural analyses of incrementally varied model systems. The observations, while not yet generalizable, are informative.

Incremental Variations in the Structure of a Ligand

In an important series of papers, Klebe and colleagues (11, 12, 56) used thermolysin to determine how water near the surface of a protein–ligand complex contributes to the thermodynamics of binding. Thermolysin has an S_2' pocket (one of three distinct clefts in its active site) that is both solvent exposed and capable of accommodating different nonpolar functional groups. By using phosphonamidate-type ligands with incrementally varied nonpolar P_2' substituents (**Figure 7*a***), the authors linked differences in the organization of water near the S_2' pocket of the protein–ligand complex (determined from high-resolution X-ray crystal structures) to differences in the enthalpy and entropy of protein–ligand association (determined via ITC). (The authors focused their analysis on the final state of binding—the protein–ligand complex—for two reasons: (*a*) The binding affinities of different ligands did not correlate with the buried nonpolar surface area of their P_2' substituents, an observation that suggested that differences in free energies of ligand desolvation—differences in the initial states of the hydrated ligands—were not the source of differences in affinity; and (*b*) the initial state of the hydrated protein was the same for each ligand.) In short, ligands that stabilized networks of water on the surface of the protein–ligand complex had more favorable enthalpies of binding, and less favorable entropies of binding, than ligands that destabilized those networks (**Figure 7*b***). Stabilization was apparent in an increase in the number of crystallographically observed fixed waters, an increase in the number of water–water hydrogen bonds (i.e., the number of pairs of crystallographically observed fixed waters separated by a distance of 3.5 Å or less), a decrease in the length of water–water hydrogen bonds, and/or a decrease in the B-factors of fixed waters; destabilization correlated with the opposite effects. Interestingly, the ligand with the greatest binding affinity was not the one that buried the largest amount of nonpolar surface area—a result that might be predicted by a classical description of the hydrophobic effect—but, rather, the one that yielded a particularly stable (and enthalpically favorable) hydration pattern around the protein–ligand complex.

Incremental Variations in the Structure of a Binding Pocket

Taking an approach complementary to that of Klebe, we used mutants of HCAII to determine how changes in the organization of water within a binding pocket influence the thermodynamics of protein–ligand association (35). Our study made use of the polar and nonpolar walls that line the binding pocket of HCAII; we used mutations to modify the size and/or polarity of these walls and, thus, the organization of water hydrating them (with no detectible changes in protein conformation) (**Figure 8*a***). ITC allowed us to analyze the influence of mutations on the thermodynamics

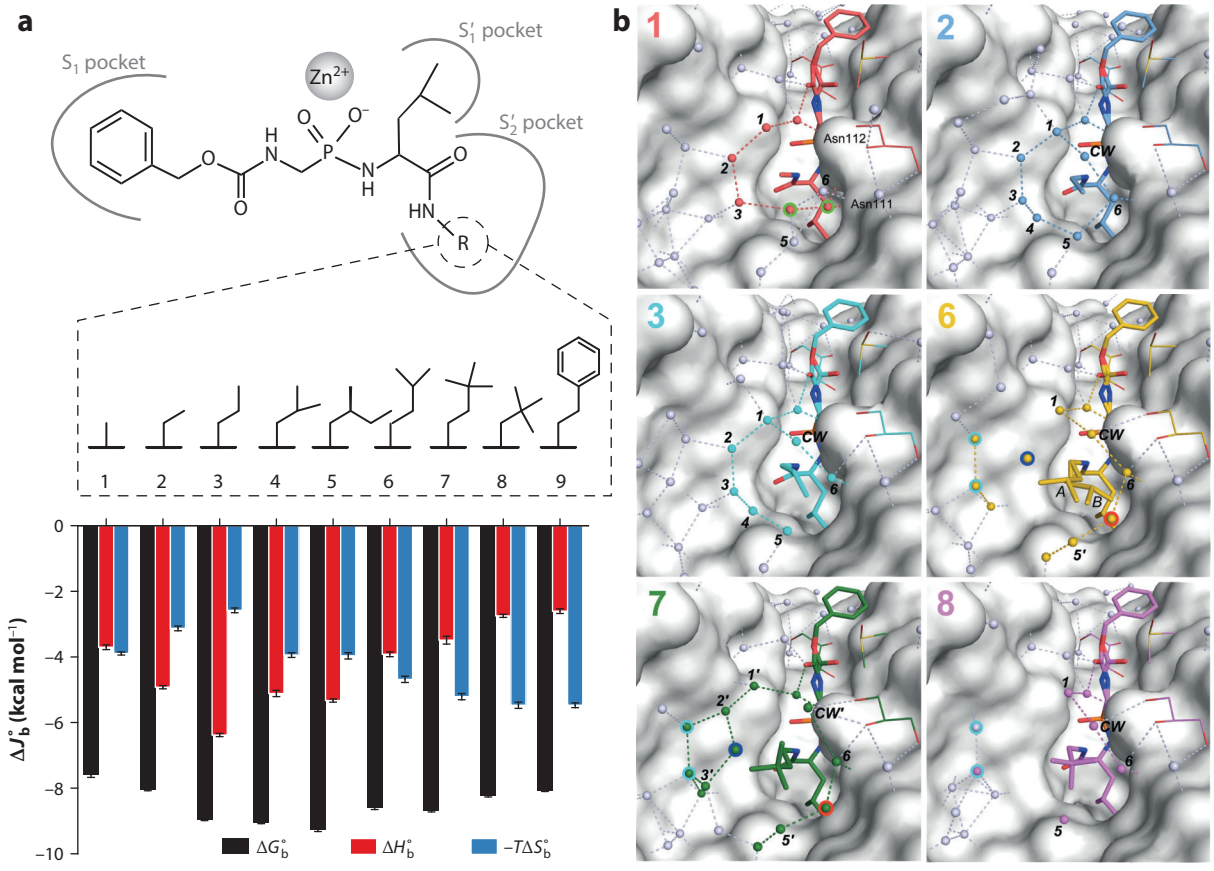


Figure 7

Water networks on the surface of a protein. (*a*) Schematic of phosphonamidate-type inhibitor in the substrate-binding cleft of thermolysin. Bottom: thermodynamic binding parameters (ΔJ°_b, where $J = H$ or TS) for different inhibitors. (*b*) High-resolution crystal structures show ligands with varied P_2' substituents bound to the S_2' pocket. Networks of water near ligands with particularly favorable enthalpies—and free energies—of binding (e.g., ligand 3) feature more molecules of water, more water–water hydrogen bonds, and/or shorter hydrogen bond lengths than networks of water near ligands with particularly favorable entropies of binding (e.g., ligands 7 and 8). Similar molecules of water near different P_2' substituents are labeled with numbers; molecules of water that exhibit a slight shift in their position are labeled with an apostrophe. A capping water (CW) appears over the P_2' substituent. Adapted with permission from Reference 56. Abbreviations: ΔJ°_b, the change in a thermodynamic property J upon binding; G, Gibbs free energy; H, enthalpy; TS, entropic component of free energy.

of protein–ligand association; X-ray crystallography and explicit-water simulations permitted an assessment of their impact on the structures of protein–ligand complexes (small) and on patterns of hydration (large). Our results suggested that most mutations strengthened local water networks and reduced binding affinity by increasing the enthalpic cost and, to a lesser extent, the entropic benefit of rearranging those networks during binding (**Figure 8*b*,*c***). Interestingly, a comparison of the thermodynamic profiles of two arylsulfonamide ligands that differed by a benzene ring (1,3-thiazole-2-sulfonamide, or TA, and BTA) indicated that the organization of water filling the binding pocket could determine whether the hydrophobic interactions in which it engaged were enthalpy driven or entropy driven.

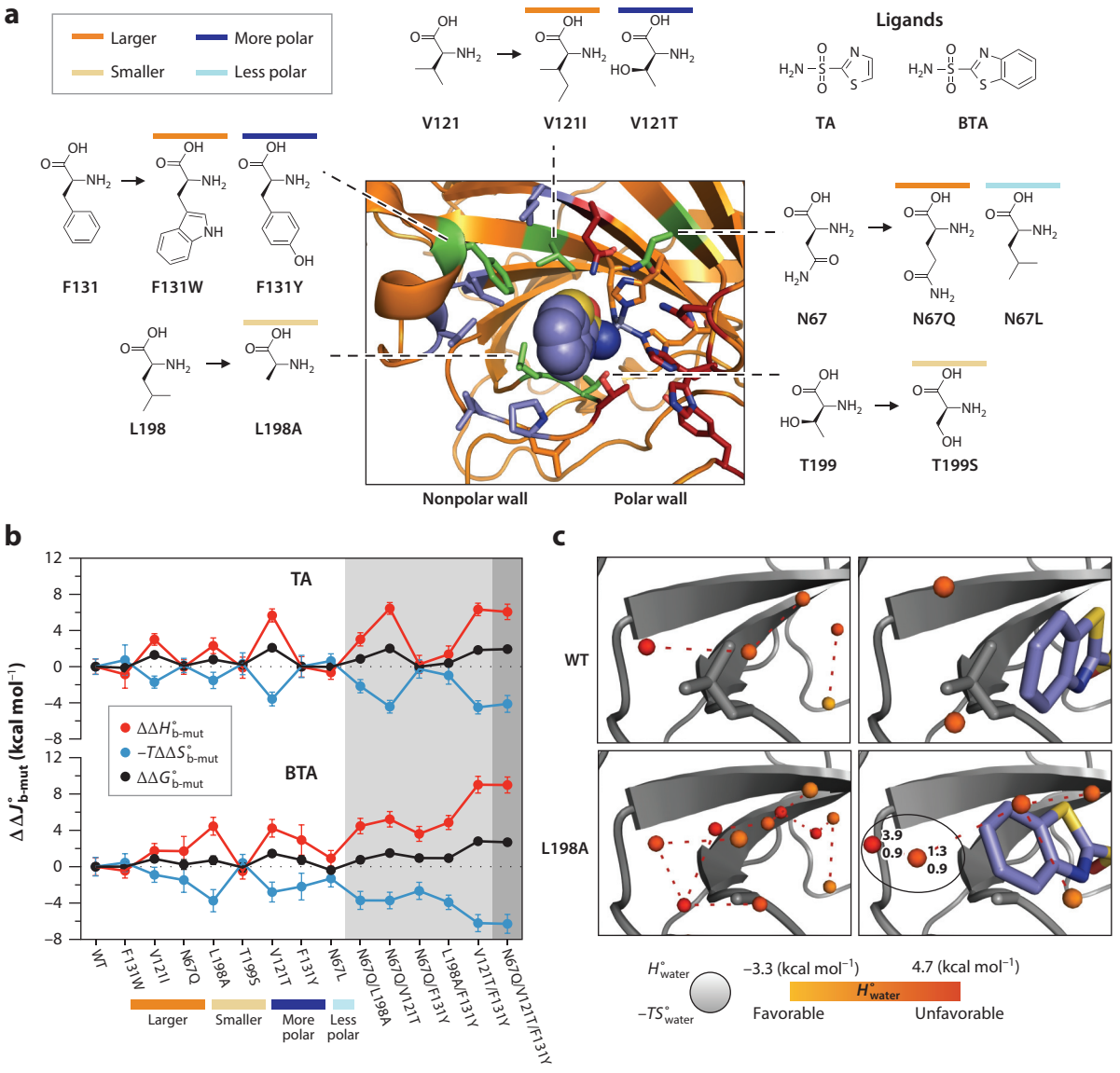


Figure 8

Water-restructuring mutations in a binding pocket. (*a*) The center image depicts the structure of the active site of HCAII complexed with BTA (PDB ID: 3S73): nonpolar wall (*purple*), polar wall (*red*), and mutation sites (*green*). BTA and TA appear on the upper right. (*b*) Differences in the thermodynamic binding parameters of mutants and wild-type HCAII: $\Delta\Delta J^\circ_{b-mut} = \Delta J^\circ_{b-mut} - \Delta J^\circ_{b-WT}$. Most mutations cause ΔH°_b to become more positive and $-T\Delta S^\circ_b$ to become more negative in a nearly compensating fashion. (*c*) WaterMap-predicted hydration sites show the influence of L198A on the thermodynamic properties of water. The leucine-to-alanine mutation strengthens a network of water near the nonpolar wall. During HCAII–BTA association, this network undergoes an enthalpically unfavorable rearrangement (*circle*). H°_{water} and TS°_{water} represent the WaterMap-based estimates of the enthalpy and entropic component of free energy, respectively, of a water molecule, relative to bulk water. Adapted with permission from Reference 35. Additional abbreviations: HCAII, human carbonic anhydrase II; BTA, benzo[*d*]thiazole-2-sulfonamide; TA, 1,3-thiazole-2-sulfonamide; ΔJ°_b, the change in a thermodynamic property upon binding; *G*, Gibbs free energy; *H*, enthalpy; *TS*, entropic component of free energy; WT, wild-type.

Asymmetry and Design

Our analysis of HCAII, and Klebe's analysis of thermolysin, suggested a similar thermodynamic asymmetry: Binding events associated with enthalpically favorable rearrangements of water—binding events that displaced so-called unstable networks in the unliganded pocket or that stabilized networks in the liganded pocket—were stronger than those associated with entropically favorable rearrangements of water. The results of these studies emphasize a seemingly obvious, yet inconsistently appreciated, conclusion: Biomolecular design strategies that accurately account for the thermodynamic repercussions of rearranging molecules of water over different regions of a binding pocket (e.g., polar or nonpolar, flat or concave) are likely to be more capable of improving the affinity of interacting biomolecules than are design strategies that treat water as a uniform medium (and the hydrophobic effect as one effect).

DYNAMICS

Molecules in solution wiggle, rotate, and translate on multiple timescales; changes in their dynamics during binding represent another possible source of H/S compensation. The entropic cost of forming a dynamically constrained protein–ligand complex may offset gains in enthalpically favorable interactions between binding partners (and water), while the entropic benefit of relieving a strained—or so-called caged state—can compensate a loss of enthalpic stability (34, 110). Such trade-offs can result from local or global structural perturbations.

The Ligand

Our analysis of the binding of BCAII to benzenesulfonamides substituted with chains of oligoglycine, oligosarcosine, and oligoethylene glycol demonstrates the potential influence of ligand dynamics (**Figure 3*a***). This study had three unexpected results: (*a*) The binding affinity was constant over all chain lengths examined for each series of ligands; (*b*) longer chain lengths yielded less favorable enthalpies of binding and more favorable entropies of binding than shorter chain lengths; and (*c*) changes in heat capacity, a metric for the molecular surface area buried during binding, were independent of chain length. These results suggested that H/S compensation resulted from differences in the so-called tightness of protein–ligand complexes: Longer chains, when bound, engaged in fewer van der Waals contacts with the protein (less favorable enthalpy) but possessed greater residual mobility (more favorable entropy) than shorter chains.

The Protein

The contribution of protein dynamics—and associated entropic adjustments—to binding is most easily examined with nuclear magnetic resonance (NMR) spectroscopy. For example, nuclear spin relaxation (e.g., ^{1}H-^{15}N or ^{1}H-^{13}C) experiments carried out under two different field strengths permit analysis of the dynamics of individual residues on subnanosecond timescales and, thus, enable estimates of the configurational entropy of a protein (39, 80, 108, 111). A recent study by Tzeng & Kalodimos (110) used NMR spectroscopy to assess the role of conformational entropy in determining the affinity of catabolite activator protein (CAP) for DNA. They analyzed 11 mutants of CAP, which populated active/inactive conformations to varying degrees (2% to 100%). Upon binding DNA, the mutants exhibited large changes in conformational entropy (both positive and negative, spanning a range of 40 kcal mol^{-1}) but nearly imperceptible differences in free energy (**Figure 9*a***). Changes in enthalpy were, thus, compensatory (although the authors did not investigate the origin of those changes).

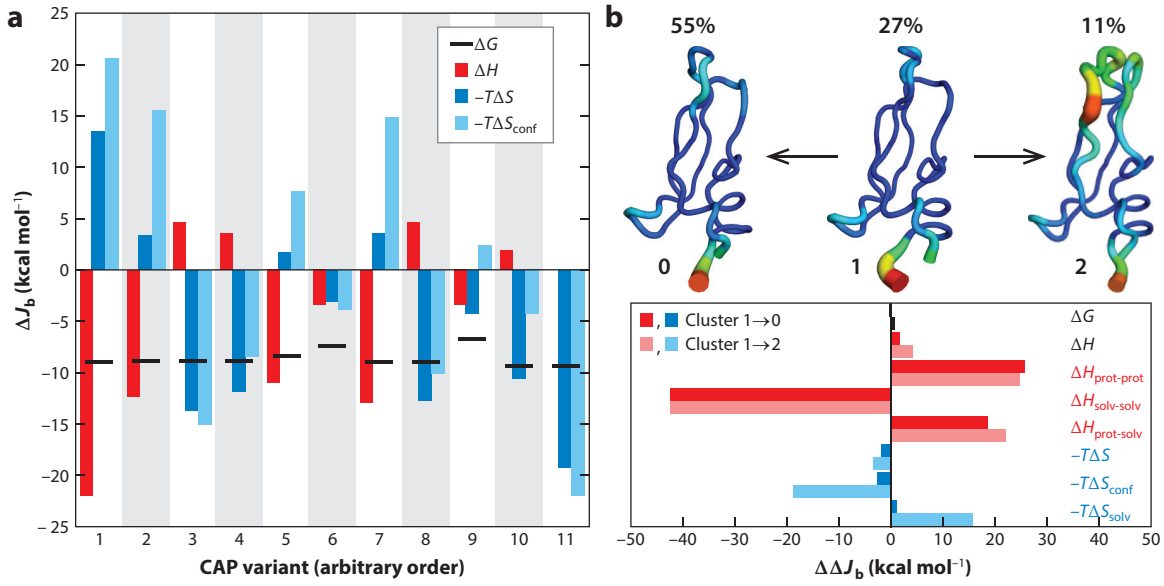


Figure 9

Enthalpy/entropy compensation resulting from changes in protein dynamics. (*a*) Thermodynamic parameters describe the binding of different mutants of CAP to DNA. Changes in conformational entropy upon binding differ significantly between mutants; affinities, however, remain similar, and, thus, suggest compensation (110). (*b*, *top*) Backbone traces of the three main conformational clusters of BPTI, labeled with their relative occupancies; the root-mean-square fluctuations of the backbone are indicated by the thickness and color of the traces (*narrow blue*, 0.4 Å; *thick red*, 3.7 Å). Clusters are equally stable, despite large differences in the enthalpic and entropic properties. (*b*, *bottom*) Changes in thermodynamic parameters ($\Delta\Delta J^\circ_b$, where $J = G$, H, or TS) associated with transitions between conformational clusters: C_1 to C_0, and C_1 to C_2. Panel *b* adapted with permission from Reference 31. Abbreviations: BPTI, bovine pancreatic trypsin inhibitor; CAP, catabolite activator protein; ΔJ°_b, the change in a thermodynamic property J upon binding; G, Gibbs free energy; H, enthalpy; TS, entropic component of free energy.

Gilson and coworkers (31) carried out a particularly detailed analysis of the contribution of protein dynamics to H/S compensation by using long-time (1-ms) MD simulations of bovine pancreatic trypsin inhibitor (BPTI) alone in solution. Their analysis of BPTI revealed equally stable clusters of conformations that differed dramatically in thermodynamic character (**Figure 9*b***). For example, two conformational clusters, denoted C_2 and C_1, were equally stable within 0.5 kcal mol^{-1} yet possessed large differences in configurational entropy (−19 kcal mol^{-1}, C_2 relative to C_1) and enthalpies for intraprotein (25 kcal mol^{-1}), intrasolvent (22 kcal mol^{-1}), and protein–solvent (−42 kcal mol^{-1}) interactions. Surprisingly, a single control variable (e.g., an interresidue distance or a torsional angle) could select for one conformation over another. Their results, thus, suggested that small structural perturbations to BPTI, by changing the enthalpic and entropic profile of its initial state, could yield large and compensating differences in enthalpy and entropy of binding to trypsin. They termed their principal observation (i.e., compensating changes in enthalpy and entropy between different protein conformations) entropy–enthalpy transduction.

The Importance of Dynamics

A number of detailed biophysical studies (both experimental and theoretical) suggest that binding-induced changes in the structure or dynamics of proteins and ligands—changes that are often

difficult, if not impossible, to decouple from interactions among protein, ligand, and solvent—contribute to H/S compensation. The nature of this contribution—and approaches for controlling it—represents a major challenge of efforts to engineer inhibitors of flexible proteins [e.g., HIV-1 protease (19, 34), BRAF kinase (5), and others (25)].

WHY STUDY ENTHALPY/ENTROPY COMPENSATION?

If different combinations of enthalpy and entropy can yield the same free energy, what is the use in understanding where they come from? We believe that a detailed understanding of the molecular origin of H/S compensation could provide new opportunities in biomolecular design. Here, we highlight a few examples.

Affinity

The modification of bimolecular affinity requires changes in molecular structures that "break" compensation. Such changes often follow trends (e.g., the affinity of alkyl-substituted benzenesulfonamides for HCAII increases with chain length, and the affinity of HCAII for benzo-extended arylsulfonamides decreases with the stability of water networks hydrating its binding pocket) and, thus, provide a structural scale for fine-tuning affinity. Strategies for identifying compensation-breaking modifications—developed from an understanding of the molecular basis of such breaks—might, thus, facilitate the design of tight or weak-binding molecules for applications in pharmaceutical science, diagnostics, and synthetic biology.

Activity

Biocatalysis requires enzymes and substrates to associate with the correct orientation. When a substrate binds to the active site of cytochrome P450 monooxygenase, for example, different orientations can yield different products (115). Studies of H/S compensation suggest that different poses of bound ligand often have different enthalpic and entropic signatures but similar free energies of binding (16, 17, 35, 56). An understanding of the link between the orientation of a bound substrate and its thermodynamic profile could, thus, enable better methods to change—or, at least, detect—that orientation in efforts to design biocatalysts.

Plasticity

By enabling many routes to the same change in free energy, H/S compensation can give proteins broad binding specificities (i.e., the ability to bind many different types of ligands). An analysis of odorant binding protein OBP by Portman and colleagues (87) provides evidence of this function: They observed that aliphatic γ-lactones of different sizes associated with different nonpolar patches on OBP, triggering desolvation processes with distinct but compensatory enthalpic and entropic signatures. An understanding of molecular features that cause H/S compensation could, thus, enable the exploitation of those features in the design of receptors or ligands that bind a broad range of targets.

Fundamental Biophysics and the Role of Water

Early examinations of biomolecular recognition focused on intermolecular contacts, relegating the role of water to the periphery [a status consistent with the common pictorial depiction of

biomolecules as colored structures placed on white, water-free backgrounds (41)]; the large contribution of water to enthalpies and entropies of binding between biomolecules, however, highlights its active role in controlling the strength of biomolecular association. A detailed understanding of H/S compensation in aqueous environments—that is, an understanding of the mechanisms by which rearrangements of water that occur during binding can cause H/S compensation for some interactions and break it for others—is, thus, essential to understanding how water enables living systems to function.

CONCLUSION

Enthalpy/entropy compensation is an undeniably important phenomenon for which quantitative rationalizations are essential for predicting how—and how strongly—biomolecules interact. Statistical mechanical analyses have suggested plausible physical origins for it, and carefully chosen model systems have helped illuminate its molecular basis, but manifestations of H/S compensation, for the most part, remain surprising—a result indicative of the general inadequacy of the current state of knowledge of biomolecular recognition. Nonetheless, similar observations in different biophysical studies suggest an important conclusion: Water and molecular motions—more so than intermolecular contacts—represent common sources of enormous (and potentially compensating) differences in enthalpy and entropy between similar binding processes. Such differences are incompatible with the classic lock-and-key model of biomolecular recognition [where two rigid complements assemble in a vacuum (48)] or with common approaches to molecular docking [which rely on strict conformational constraints and implicit water (30, 42)]. Future efforts to improve predictive capabilities in biomolecular recognition must, thus, focus on the systematic dissection, system-to-system comparison, and eventual parameterization of contributions of water and molecular dynamics to the enthalpy and entropy of binding; importantly, as the studies detailed in this review demonstrate, binding affinity is an information-poor—and, thus, insufficient—experimental observable for studying recognition processes. We cannot, after all, claim to truly understand interactions between biomolecules—or hope to engineer those interactions—until their full thermodynamic characteristics (their enthalpies, entropies, and free energies of binding) are no longer surprising.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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