The Molecular Origin of Enthalpy/Entropy Compensation in Biomolecular Recognition

Jerome M. Fox,1 Mengxia Zhao,2 Michael J. Fink,2 Kyungtae Kang,3 and George M. Whitesides2,4,5

1Department of Chemical and Biological Engineering, University of Colorado, Boulder, Colorado 80309, USA; email: jerome.fox@colorado.edu
2Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, USA; email: mzhao@gmwgroup.harvard.edu, mfink@gmwgroup.harvard.edu, gwhitesides@gmwgroup.harvard.edu
3Department of Applied Chemistry, Kyung Hee University, Yongin, Gyeonggi 17104, Republic of Korea; email: kkang@khu.ac.kr
4Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, Massachusetts 02138, USA
5The Kavli Institute for Bionano Science and Technology, Harvard University, Cambridge, Massachusetts 02138, USA

Keywords
molecular recognition, lead design, protein engineering, isothermal titration calorimetry, water networks, molecular dynamics

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
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 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.

Contents

INTRODUCTION ................................................................. 224
GENERAL SOURCES OF ENTHALPY/ENTROPY COMPENSATION ...... 225
    Experimental Error ..................................................... 225
    Perturbation of a Small Number of Energy Levels ................. 226
MODEL SYSTEMS TO STUDY BIOMOLECULAR RECOGNITION ............. 228
    Biomolecular Recognition ............................................. 228
    Human Carbonic Anhydrase II ......................................... 230
    Human Immunodeficiency Virus 1 Protease .......................... 230
    Other Proteases ........................................................... 230
    Others ................................................................. 230
    Experimental Precautions .............................................. 231
PROTEIN–LIGAND CONTACTS ........................................... 231
    Hydrogen Bonds .......................................................... 231
    Ionic Interactions and Halogen Bonds ............................... 234
WATER ........................................................................ 235
    The Hydrophobic Effect .................................................. 235
    Ionic Interactions and Hydrogen Bonds ............................... 237
    Breaking Enthalpy/Entropy Compensation Caused by Water ......... 239
    Incremental Variations in the Structure of a Ligand ................. 239
    Incremental Variations in the Structure of a Binding Pocket ......... 239
    Asymmetry and Design .................................................. 242
DYNAMICS ................................................................. 242
    The Ligand ................................................................. 242
    The Protein ................................................................. 242
    The Importance of Dynamics ............................................ 243
WHY STUDY ENTHALPY/ENTROPY COMPENSATION? ...................... 244
    Affinity ................................................................. 244
    Activity ................................................................. 244
    Plasticity ................................................................. 244
    Fundamental Biophysics and the Role of Water ....................... 244
CONCLUSION .......................................................... 245
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 com-
monly 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 ($\Delta H^\circ_b$ and $\Delta S^\circ_b$, respectively) are derived from measurements of dissociation
constants ($K_\text{d}$) at different temperatures:

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

The slope and y intercept of linear fits to Equation 1 [i.e., plots of $\ln(K_\text{d})$ versus $1/T$] yield estimates
of $\Delta H^\circ_b$ and $\Delta S^\circ_b$. Such fits assume that values of $\Delta H^\circ_b$ and $\Delta S^\circ_b$ 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 $\Delta H^\circ_b$, which tend to be large relative to the
magnitude of $\Delta G^\circ_b$, give rise to large errors in $\Delta S^\circ_b$ 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 indepen-
dent estimates of $\Delta H^\circ_b$ (from heats of binding) and $\Delta G^\circ_b$ (from nonlinear fits to plots of heat
generated versus the molar ratio of ligand to macromolecule); the difference in these two parame-
ters 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 1a,b* illustrates this argument for a model system with a Gaussian distribution of internal energy levels. *Figure 1a* plots the occupancy probability, \(P(U)\), of energy levels \((U)\) in an unperturbed system (kcal mol\(^{-1}\)) (Equation 3); *Figure 1b* shows changes in mean internal energy \((\Delta U, \text{kcal mol}^{-1})\) and entropy \((T\Delta S, \text{kcal mol}^{-1})\) that result from perturbations

THERMODYNAMIC QUANTITIES IN BIOLOGICAL SYSTEMS

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

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

Enthalpy \((H, \text{kcal mol}^{-1})\), 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:

\[
H = U + pV. \tag{a}
\]

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:

\[
H = \Delta(U + pV) \approx \Delta U. \tag{b}
\]

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

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

Gibbs free energy \((G, \text{kcal mol}^{-1})\) 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:

\[
G = H - TS. \tag{c}
\]
Figure 1
Rationalizations of enthalpy/entropy (H/S) compensation. (a) Occupancy probabilities \( P(U) \) for a system with a Gaussian distribution of energy levels \( (T = 298 \text{ K}; \sigma = 1.5 \text{ kcal mol}^{-1}) \). (b) Correlated changes in internal energy \( \Delta U \) 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: \( D_0 = 5 \text{ kcal mol}^{-1}, r_0 = 2.8 \, \text{Å}, \mu = 18 \, \text{g mol}^{-1}/6.023 \times 10^{23} \text{ molecules mol}^{-1} \). Vibrational entropy \( S_{\text{vib}} \) is calculated from a vibrational partition function as described by Dunitz \( (T = 298 \text{ K}) \) (26); estimates of the entropy of bonding \( 6TS_{\text{vib}} \) assume equal contributions from six vibrational modes (one stretching, two rotational, and three translational modes). Over an intermediate range of dissociation energies \( \text{e.g., } D_0 = -\Delta H^\circ_b = 3.5 - 5.5 \text{ kcal mol}^{-1} \) 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; \( \mu \), reduced mass; \( r_0 \), equilibrium bond length; \( S \), entropy; \( T \), temperature; \( U \), internal energy; \( V \), potential energy.

In Equations 3–5, \( \sigma \) 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 \( \Delta U \) 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.

\[
P(U) = \frac{dU}{\sqrt{2\pi} \sigma^2} e^{\frac{U^2}{2\sigma^2}}
\]

\[\Delta U = (U - U')P(U')dU\]

\[T \Delta S = (U - U' - k_B T)P(U')dU\]

In Equations 3–5, \( \sigma \) 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 \( \Delta U \) 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 ($\Delta H^{\circ}$ becomes more negative) and, thus, “tightens” the bonded system ($-T\Delta S^{\circ}$ becomes more positive). Figure 1c illustrates this argument for a hydrogen bond modeled by a Morse potential (Equations 6–8).

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

6.

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

7.

$$x = \frac{b v}{k_B T}, \quad v = \frac{(f/\mu)^{1/2}}{2\pi}, \quad f = \frac{ml}{(r_0)^2} D_0$$

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$^{-1}$), $v$ is the frequency of stretching (cm$^{-1}$), $b$ is Planck’s constant, $\mu$ 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., $D_0 = -\Delta H^{\circ}_{\text{b}} = 3.5–5.5$ 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 2a): (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
Molecules of water formerly solvating the protein and/or ligand

Protein–ligand complex

Δ$\Delta F_b$

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 benzothiazole-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 5c,d). The illustration of bound ligands was adapted with permission from Reference 36. Additional abbreviation: $\Delta F_b$, the change in a thermodynamic property $F$ upon binding.

of the protein (i.e., the sampling of multiple protein conformations on multiple timescales), (b) changes in the dynamics of the ligand, and (c) 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 par-
ticularly 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
genometry and, thus, permits detailed studies of the thermodynamic influence of differences in
ligand structure on binding (Figure 2b) (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 impor-
tance [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 crystallograph-
cally 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 \( \Delta H^\circ \). 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 3a,b provides examples. Figure 3a 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 3b 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 3a,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 \( \sim 1.5 \) 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 (ΔΔH°b–mut = ΔΔJ°b–mut = ΔΔS°b–mut, 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 Kd 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 4a); a hydrogen bond between this group and a backbone amide lowered ΔH°b by 3.9 kcal mol⁻¹ but raised −TΔS°b 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 4b). [The B-factor is a metric for
protein–ligand complex (note regions of (b) A comparison of B-factors between the two protein–ligand complexes shows that the additional hydrogen bond rigidifies the protease that differ by a sulfonyl group and, thus, in their ability to form a sulfonyl–amide bond with the protein backbone (62).

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 ($\Delta G^\circ$) but large differences in enthalpy and entropy of binding ($\Delta H^\circ$ and $-T\Delta S^\circ$, 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 ($\Delta \Delta H^\circ$ and $-T \Delta \Delta S^\circ$, respectively; each calculated from thermodynamic cycles of cation binding to incrementally varied pairs of residues) suggest strong compensation; free energies of interaction ($\Delta \Delta G^\circ$) 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; $T S$, entropic component of free energy.

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 ($\Delta G^\circ$) but large differences in enthalpy and entropy of binding ($\Delta H^\circ$ and $-T\Delta S^\circ$, 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 ($\Delta \Delta H^\circ$ and $-T \Delta \Delta S^\circ$, respectively; each calculated from thermodynamic cycles of cation binding to incrementally varied pairs of residues) suggest strong compensation; free energies of interaction ($\Delta \Delta G^\circ$) 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; $T S$, entropic component of 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$_3$Ser$_{10}$, Ser$_3$Asp$_{10}$, Lys$_3$Ser$_{10}$, and Lys$_3$Asp$_{10}$, 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 4e,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 5a) (78). X-ray crystal structures, which showed the same binding geometry for nine out of ten ligands (Figure 5b), 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 arylo sulfonamide ligands (see Figure 2b 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 5c) (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)
In our study of the binding of anions to the Zn\(^{2+}\) cofactor of HCAII, we found that this effect 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 (\(\Delta G_{\text{hydration}}^*\), the free energy change associated with the transfer of one mole of ion from the gas phase to a solvent of water). 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 (\(\Delta G_{\text{hydration}}^*\), the free energy change associated with the transfer of one mole of ion from the gas phase to a solvent of water).
Figure 6
The binding of fluorinated ligands to HCAII. (a) Structures of partially fluorinated benzo[thiazole-2-sulfonamide ligands (17).
(b) Differences in the thermodynamic binding parameters of fluorinated ligands and BTA (e.g., $\Delta H_b = \Delta H_{b,BTA} - \Delta H_{b,FBA}$, 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 $\Delta G_b$ 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[thiazole-2-sulfonamide; $\Delta G_b$, 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 S2' pocket (one of three distinct clefs 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 P2' substituents (Figure 7a), the authors linked differences in the organization of water near the S2' 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 P2' 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 7b). 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 8a). 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 (Δ$G_b$, 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: Δ$G_b$, the change in a thermodynamic property $J$ upon binding; $G$, Gibbs free energy; $H$, enthalpy; $TS$, entropic component of free energy.
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: \( \Delta \Delta J^\circ_b \) = \( \Delta J^\circ_b^{\text{mut}} - \Delta J^\circ_b^{\text{WT}} \). Most mutations cause \( \Delta H^\circ_b \) 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). \( H^\circ_{\text{water}}^\text{molec} \) and \( TS^\circ_{\text{water}} \) 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, benzothiazole-2-sulfonamide; TA, 1,3-thiazole-2-sulfonamide; \( \Delta J^\circ_b \), 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 3a). 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., 1H-15N or 1H-13C) 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 9a). 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 (ΔΔJb, where J = G, H, or TS) associated with transitions between conformational clusters: C1 to C0, and C1 to C2. Panel b adapted with permission from Reference 31. Abbreviations: BPTI, bovine pancreatic trypsin inhibitor; CAP, catabolite activator protein; ΔΔJb, 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 9b). For example, two conformational clusters, denoted C2 and C1, were equally stable within 0.5 kcal mol⁻¹ yet possessed large differences in configurational entropy (−19 kcal mol⁻¹, C2 relative to C1) 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 monoxygenase, 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.

ACKNOWLEDGMENTS

The authors acknowledge support from the following sources: the University of Colorado, Boulder (J.M.F., startup), the John Templeton Foundation (M.Z., award no. 48423), the Erwin Schrödinger fellowship from the Austrian Science Fund (M.J.F., award no. J3771-N28), the National Research Foundation of Korea (K.K., award no. 2016R1C1B2011414), and the National Science Foundation (G.M.W., award no. CHE-1152196).

LITERATURE CITED

105. Tanford C. 1979. Interfacial free energy and the hydrophobic effect. PNAS 76(9):4175–76
Contents

Structural Basis for G Protein–Coupled Receptor Signaling
Sarah C. Erlandson, Conor McMahon, and Andrew C. Kruse ............................................. 1

Collapse Transitions of Proteins and the Interplay Among Backbone, Sidechain, and Solvent Interactions
Alex S. Holehouse and Robit V. Pappu ................................................................. 19

Measuring Entropy in Molecular Recognition by Proteins
A. Joshua Wand and Kim A. Sharp ........................................................................... 41

Assembly of COPI and COPII Vesicular Coat Proteins on Membranes
Julien Béthune and Felix T. Wieland ......................................................................... 63

Imaging mRNA In Vivo, from Birth to Death
Evelina Tutucci, Nathan M. Livingston, Robert H. Singer, and Bin Wu .................... 85

Nanodiscs: A Controlled Bilayer Surface for the Study of Membrane Proteins
Mark A. McLean, Michael C. Gregory, and Stephen G. Sligar ................................. 107

The Jigsaw Puzzle of mRNA Translation Initiation in Eukaryotes:
A Decade of Structures Unraveling the Mechanics of the Process
Yaser Hashem and Joachim Frank ........................................................................... 125

Hemagglutinin-Mediated Membrane Fusion: A Biophysical Perspective
Sander Boonstra, Jelle S. Blijleven, Wouter H. Roos, Patrick R. Onck,
Erik van der Giessen, and Antoine M. van Oijen ................................................. 153

Cryo-EM Studies of Pre-mRNA Splicing: From Sample Preparation to Model Visualization
Max E. Wilkinson, Pei-Chun Lin, Clemens Plaschka, and Kiyoshi Nagai .................. 175

Structure and Dynamics of Membrane Proteins from Solid-State NMR
Venkata S. Mandala, Jonathan K. Williams, and Mei Hong ................................... 201

The Molecular Origin of Enthalpy/Entropy Compensation in Biomolecular Recognition
Jerome M. Fox, Mengxia Zhao, Michael J. Fink, Kyungtae Kang,
and George M. Whitesides .................................................................................. 223
Po-Yi Ho, Jie Lin, and Ariel Amir ................................................................. 251

Macroscopic Theory for Evolving Biological Systems Akin to Thermodynamics
Kunihiko Kaneko and Chikara Furusawa .................................................. 273

Photoreceptors Take Charge: Emerging Principles for Light Sensing
Tilman Kottke, Aihua Xie, Delmar S. Larsen, and Wouter D. Hoff ............. 291

High-Resolution Hydroxyl Radical Protein Footprinting: Biophysics Tool for Drug Discovery
Janna Kiselar and Mark R. Chance ................................................................. 315

Dynamic Neutron Scattering by Biological Systems
Jeremy C. Smith, Pan Tan, Loukas Petridis, and Liang Hong ....................... 335

Hydrogel-Tissue Chemistry: Principles and Applications
Viciana Gradinaru, Jennifer Treweek, Kristin Overton, and Karl Deisseroth .... 355

Serial Femtosecond Crystallography of G Protein–Coupled Receptors
Benjamin Stauch and Vadim Cherezov .......................................................... 377

Understanding Biological Regulation Through Synthetic Biology
Caleb J. Bashor and James J. Collins ............................................................... 399

Distinct Mechanisms of Transcription Initiation by RNA Polymerases I and II
Christoph Engel, Simon Neyer, and Patrick Cramer .................................... 425

Dynamics of Bacterial Gene Regulatory Networks
David L. Shis, Matthew R. Bennett, and Oleg A. Igoshin ............................... 447

Molecular Mechanisms of Fast Neurotransmitter Release
Axel T. Brunger, Ucheor B. Choi, Ying Lai, Jeremy Leitz, and Qiangjun Zhou .... 469

Structure and Immune Recognition of the HIV Glycan Shield
Max Crispin, Andrew B. Ward, and Ian A. Wilson ........................................ 499

Substrate-Induced Formation of Ribosomal Decoding Center for Accurate and Rapid Genetic Code Translation
Michael Y. Pavlov and Måns Ebrenberg ....................................................... 525

The Biophysics of 3D Cell Migration
Pei-Hsun Wu, Daniele M. Gilkes, and Denis Wirtz ........................................ 549

Single-Molecule View of Small RNA–Guided Target Search and Recognition
Viktorija Glibyte, Sung Hyun Kim, and Chirlmin Joo ..................................... 569
Behavioral Variability and Phenotypic Diversity in Bacterial Chemotaxis  
Adam James Waite, Nicholas W. Frankel, and Thierry Emonet ....................... 595

Mechanotransduction by the Actin Cytoskeleton: Converting Mechanical Stimuli into Biochemical Signals  
Andrew R. Harris, Pamela Freij, and Daniel A. Fletcher ................................. 617

The Physical Properties of Ceramides in Membranes  
Alicia Alonso and Félix M. Goñi ........................................................................ 633

The Physics of the Metaphase Spindle  
David Oriola, Daniel J. Needleman, and Jan Brugués ................................. 655

Indexes  
Cumulative Index of Contributing Authors, Volumes 43–47 ......................... 675

Errata  
An online log of corrections to Annual Review of Biophysics articles may be found at http://www.annualreviews.org/errata/biophys