Entropy in molecular recognition by proteins

José A. Caro¹,¹, Kyle W. Harpole¹,¹, Vignesh Kasinath¹, Jackwee Lim¹,¹, Jeffrey Granja⁸, Kathleen G. Valentine⁸, Kim A. Sharp⁹, and A. Joshua Wand¹,²

¹Johnson Research Foundation and Department of Biochemistry and Biophysics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104-6059

Edited by G. Marius Clore, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, and approved May 9, 2017 (received for review December 26, 2016)

Molecular recognition by proteins is fundamental to molecular biology. Dissection of the thermodynamic energy terms governing protein–ligand interactions has proven difficult, with determination of entropic contributions being particularly elusive. NMR relaxation measurements have suggested that changes in protein conformational entropy can be quantitatively obtained through a dynamical proxy, but the generality of this relationship has not been shown. Twenty-eight protein–ligand complexes are used to show a quantitative relationship between measures of fast side-chain motion and the underlying conformational entropy. We find that the contribution of conformational entropy can range from favorable to unfavorable, which demonstrates the potential of this thermodynamic variable to modulate protein–ligand interactions. For about one-quarter of these complexes, the absence of conformational entropy would render the resulting affinity biologically meaningless. The dynamical proxy for conformational entropy or “entropy meter” also allows for refinement of the contributions of solvent entropy and the loss in rotational-translational entropy accompanying formation of high-affinity complexes. Furthermore, structure-based application of the approach can also provide insight into long-lived specific water–protein interactions that escape the generic treatments of solvent entropy based simply on changes in accessible surface area. These results provide a comprehensive and unified view of the general role of entropy in high-affinity molecular recognition by proteins.

Significance

Molecular recognition by proteins is a key element of biology. Appreciation of the underlying thermodynamics has been incomplete because of uncertainty in several contributions to the entropy. Here, we demonstrate a way to measure changes in protein conformational entropy using a dynamical proxy provided by NMR relaxation methods. We find that conformational entropy can contribute significantly and variably to the thermodynamics of binding. In addition, we determine the contribution of rotational-translational entropy loss upon forming a high-affinity complex involving a protein. The contribution of solvent entropy is also recalibrated. Thus, a more complete view of entropy in binding has been established and shows that inclusion of conformational entropy is necessary to understanding the origins of high-affinity interactions involving proteins.

Author contributions: A.J.W. designed research; J.A.C., K.W.H., V.K., J.L., K.G.V., K.A.S., and A.J.W. analyzed data; and J.A.C., K.W.H., V.K., K.G.V., and A.J.W. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The NMR chemical shifts and obtained order parameters have been deposited in the BioMagResBank, www.bmrb.wisc.edu (accession nos. 25667, 25670, 25728, 25727, 26983, 26619, and 26620).

¹J.A.C., K.W.H., V.K., and J.L. contributed equally to this work.

²To whom correspondence should be addressed. Email: wand@upenn.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1621154114/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1621154114
PNAS | June 20, 2017 | vol. 114 | no. 25 | 6563-6568
Some time ago, it was realized that fast subnanosecond time scale motion between conformational states might provide access to various thermodynamic features (14), especially conformational entropy (15, 16). Application of this idea has been thwarted by several technical limitations (17, 18), but has nevertheless led to the strong suggestion that dynamical proxies made available by NMR relaxation measurements could provide access to measures of conformational entropy (19). More recently, efforts have been taken to overcome these technical barriers and limitations and to render this approach to protein entropy quantifiable (20, 21). The resulting NMR-based dynamical proxy for conformational entropy or “entropy meter” takes a simple form that requires a few assumptions, particularly about the precise nature of the underlying motion (21):

$$\Delta S_{\text{total}} = s_d \left[ N_{\text{protein}} \Delta (O_{\text{axis}}^2)^{\text{protein}} \right] + \Delta S_{\text{solvent}} + \Delta S_{\text{vdw}} + \Delta S_{\text{other}}.$$  

[2]

The first two terms contain the dynamical proxy, where $O_{\text{axis}}$ is a measure of the degree of spatial restriction of the methyl group symmetry axis and ranges between 0, which represents complete isotropic disorder, and 1, which corresponds to no internal motion within the molecular frame (22), and is measured in various ways (17). Numerous models and simulations indicate that changes in entropy will be linearly related to changes in the relevant $O_{\text{axis}}$, varying between 0.1 and 0.9 (14–17, 21, 23). This linearity is the basis for the simple form of the entropy meter (20, 21). Measurement of fast internal side-chain motion in proteins is largely restricted to the methyl group, which, due to its fast rotation, can be used as a relaxation probe in even very large proteins (17). The change in the average methyl group symmetry axis order parameter ($\Delta O_{\text{axis}}$) upon binding a ligand is used as a dynamical proxy, and $s_d$ is the sought-after relationship (conversion) between these measures of fast internal motion and conformational entropy (20, 21). To avoid issues associated with statements about absolute entropy, we restrict this treatment to changes in entropy upon a perturbation (e.g., binding of a ligand). In principle, all internal degrees of freedom of the protein contribute to $\Delta S_{\text{conf}}$, namely, bond lengths, angles, and torsions. In practice, empirical and computational studies show that changes upon binding are largely restricted to the softer, torsional modes (21, 24). Eq. 2 also depends on the total number of torsion angles in the molecule ($N_T$), because the motion of methyl-bearing amino acid side chains reports on side-chain motions across the entire protein molecule. Linear scaling with $N_T$ is expected when weak coupling of side-chain motion with neighbors is present (25). $\Delta S_{\text{solvent}}$ is calculated from the structures of the free and complexed states by scaling changes in apolar and polar accessible surface area with empirically determined solvation entropy coefficients (12).

The conformational entropy meter approach has thus far been applied to only two protein–ligand systems: calcium-activated calmodulin-binding peptides representing the domains of regulated proteins (20) and a series of mutants of the catabolite activator protein binding DNA (26). It is unknown whether this relationship holds generally. We address this issue by examining 28 protein–ligand complexes that span a broad range of binding affinities ($K_d = 10^{-4}$ to $10^{-10}$ M) and ligand types (nucleic acids, enzyme substrates and cofactors, carbohydrates, and polypeptides) (SI Appendix, Table S1).

**Results and Discussion**

We set out to test the hypothesis that the entropy meter is universally applicable (i.e., that the scaling ($s_d$) between changes in fast internal motion and changes in conformational entropy is constant). There are now roughly two dozen published studies of the change in methyl-bearing side-chain dynamics that are sufficiently complete to use to test this idea (SI Appendix, Table S1). Criteria for selection of these complexes included the availability of comprehensive assignments and relaxation data in both free and complexed protein states, as well as a largely dry interface in the complex. We have augmented many of these NMR relaxation studies by measuring the binding thermodynamics using isothermal titration calorimetry (ITC) under corresponding NMR solution conditions (i.e., buffer, pH, temperature). Completely new examples were also examined. The curated dataset is summarized in SI Appendix, Tables S1–S4.

Previously, the empirically determined coefficients relating changes in accessible polar and apolar surface area to the solvation entropies of these two types of surface had to be derived subject to various assumptions about the nature of conformational entropy (12). The unprecedented amount of dynamical data used here now allows us to determine the area coefficients directly. The data on the 28 proteins used here were obtained at somewhat different temperatures (298–308 K). Significant heat capacity changes accompany hydration of apolar and polar groups. Fortunately, the corresponding temperature variation of the desolvation entropy is well described experimentally by the relations $\Delta S_{\text{apolar}} = \Delta C_{\text{polar}} \ln(T/385 K) - \Delta S_{\text{polar}}$ (27) and $\Delta S_{\text{polar}} = \Delta C_{\text{polar}} \ln(T/176 K) - \Delta S_{\text{polar}}$ (28), where $\Delta C_{\text{polar}}$ and $\Delta C_{\text{apolar}}$ are the changes in hydration heat capacities per unit area of apolar ($\Delta S_{\text{apolar}}$) and polar ($\Delta S_{\text{polar}}$) surface, respectively, and 385 K and 176 K are reference temperatures at which the apolar and polar solvation entropies extrapolate to 0 (27, 28). The intercept of Eq. 2 contains the loss in rotational-translational entropy upon formation of the complex. We therefore recast Eq. 2 as:

$$\Delta S_{\text{total}} = s_d \left[ N_{\text{protein}} \Delta (O_{\text{axis}}^2)^{\text{protein}} \right] + \Delta C_{\text{polar}} \ln(T/385 K) \Delta S_{\text{polar}} + \Delta C_{\text{polar}} \ln(T/176 K) \Delta S_{\text{polar}} + [\Delta S_{\text{vdw}} + \Delta S_{\text{other}}].$$  

[3]

where the heat capacity terms are evaluated at the experimental temperature of each complex. When combined with the known changes in apolar and polar accessible surface area, this formulation gives the contributions to solvent entropy. Entropies are given here with respect to a standard state of 1 M.

Should unaccounted contributions to the binding entropy (i.e., $\Delta S_{\text{other}}$) be insignificant, then $\Delta S_{\text{vdw}}$ will dominate the ordinate and $\Delta S_{\text{vdw}}$ is the sought-after relationship holds generally. We address this issue by examining 28 protein–ligand complexes that span a broad range of binding affinities ($K_d = 10^{-4}$ to $10^{-10}$ M) and ligand types (nucleic acids, enzyme substrates and cofactors, carbohydrates, and polypeptides) (SI Appendix, Table S1).

The conformational entropy meter approach has thus far been applied to only two protein–ligand systems: calcium-activated calmodulin-binding peptides representing the domains of regulated proteins (20) and a series of mutants of the catabolite activator protein binding DNA (26). It is unknown whether this relationship holds generally. We address this issue by examining 28 protein–ligand complexes that span a broad range of binding affinities ($K_d = 10^{-4}$ to $10^{-10}$ M) and ligand types (nucleic acids, enzyme substrates and cofactors, carbohydrates, and polypeptides) (SI Appendix, Table S1).

The fit of Eq. 3 to the dynamical data indicates a strong linear correlation (R = 0.85, $R^2 = 0.72, P < 10^{-3}$) (Fig. 1). To address the stability of the fit, we carried out a jackknife-type analysis, where individual members of the dataset were randomly sampled. A minimum of only five points is required to define $s_d$ with reasonable precision, which smoothly converges as additional points are added (SI Appendix, Fig. S1). The determined values of $s_d$, $\Delta S_{\text{vdw}}$, $\Delta C_{\text{polar}}$, and $\Delta C_{\text{apolar}}$ are listed in Table 1, and further statistics are summarized in SI Appendix, Fig. 2 and Table S5. The parameter of central interest ($s_d$) is well determined and provides a robust and apparently general means to obtain the change in conformational entropy upon protein–ligand association experimentally. It is somewhat smaller than the parameter obtained...
and it is often a large determinant of the thermodynamics of binding (Fig. 2). Indeed, for approximately one-quarter of the complexes used in the calibration of the entropy, the absence of the contribution of conformational entropy to the binding thermodynamics would result in biologically ineffective affinities.

The calibrated entropy meter allows the determination of the contribution of conformational entropy to a binding process using only the dynamical information; that is, for a heterodimer (AB):

\[
\Delta S_{\text{conf}} = s_d \left[ (N_x^A \Delta \langle O_2^A \rangle^A) + (N_x^B \Delta \langle O_2^B \rangle^B) \right];
\]

\[
s_d = -(4.8 \pm 0.5) \times 10^{-3} \text{ kJ mol}^{-1} \text{ K}^{-1}.
\]

Importantly, other entropic contributions to protein–ligand associations are also made accessible by the approach summarized in Fig. 1 and Eq. 3. Eq. 3 allows for other unknown sources of entropy. These sources might include, for example, (de)protonation or (de)solvation of charge associated with binding (29).

Clearly, if \(\Delta S_{\text{other}}\) is both significant and varies between complexes, then the linearity of Eq. 3 will be degraded. The observed linear correlation strongly suggests that such is not the case. Thus, if \(\Delta S_{\text{other}}\) is small relative to \(\Delta S_{\text{conf}}\) and \(\Delta S_{\text{solv}}\), then the ordinate intercept represents the loss in rotational-translational entropy upon formation of high-affinity complexes.

The contribution of solvent entropy to processes involving proteins is generally derived from changes in solvent accessible surface area. Before this work, such approaches have required strong assumptions regarding conformational entropy (12), which are not required here because all four parameters of the entropy terms of Eq. 3 can be directly determined. We find that burial of both apolar and polar surfaces upon binding produces a positive (favorable) change in entropy. This positive change occurs because hydration of polar groups, like hydration of apolar groups, has negative entropy of hydration, in agreement with a wide range of thermodynamic data on solute, ion, and protein hydration (28, 31, 32). We determined the surface area coefficients for apolar (\(a_{\text{apolar}}\)) and polar (\(a_{\text{polar}}\)) desolvation entropy at 298 K to be \(-0.096 \pm 0.029\text{ J mol}^{-1} \text{ K}^{-1}\text{ Å}^{-2}\) and \(-0.027 \pm 0.005\text{ J K}^{-1} \text{ Å}^{-2}\), respectively (Table 1). The corresponding hydration heat capacity coefficients are also listed in Table 1. Burial of the hydrophobic area stabilizes the complex through the

Table 1. Calibration of the entropy meter

| Parameter | Value |
|-----------|-------|
| Intercept \(\Delta S_{\text{apolar}} + \Delta S_{\text{other}}\) kJ mol\(^{-1}\) K\(^{-1}\) | \(-0.10 \pm 0.01\) |
| Slope \(s_d\) kJ mol\(^{-1}\) K\(^{-1}\) | \(-4.8 \pm 0.5\) |
| \(\Delta C_{\text{polar}}/10^{-4}\) kJ mol\(^{-1}\) K\(^{-1}\) Å\(^{-2}\) | \(+3.7 \pm 1.1\) |
| \(\Delta C_{\text{polar}}/10^{-5}\) kJ mol\(^{-1}\) K\(^{-1}\) Å\(^{-2}\) | \(-5.2 \pm 1.0\) |
| \(a_{\text{apolar}}\) (298 K) kJ mol\(^{-1}\) K\(^{-1}\) Å\(^{-2}\) | \(-9.6 \pm 2.9\) |
| \(a_{\text{polar}}\) (298 K) kJ mol\(^{-1}\) K\(^{-1}\) Å\(^{-2}\) | \(-2.7 \pm 0.5\) |

Calibration of the entropy meter is derived from a global fit for the parameters \(\Delta C_{\text{apolar}}, \Delta C_{\text{polar}}, \Delta S_{\text{apolar}},\) and \(s_d\) of Eq. 3 using the experimental binding entropy changes, order parameter changes, and known experimental temperatures for the 28 complexes summarized in Fig. 1 \((R = -0.85, R^2 = 0.72, P < 10^{-13})\). Uncertainties were determined by Monte Carlo sampling. SDs are shown. The average covariance matrix is provided in SI Appendix, Table 56. Values for the hydration entropy coefficients per unit area are also tabulated at the standard temperature of 298 K. Calibration is performed with reference to a standard state concentration of 1 M.

*Entropic contribution of the backbone is not directly included (as discussed in the main text).
hydrophobic effect. Concomitantly, burial of the polar area also stabilizes the complex via release of its hydrating water into the bulky, less ordered state (31). The coefficients are smaller than obtained previously (12, 33) and, in fact, reflect the impact of inadequately assessing the contribution of residual side-chain entropy in the folded state of proteins in prior work. It is also interesting to note that the smaller magnitude likely arises from a difference in the nature of solvation. Prior solvation entropies have been estimated from group transfers between water and organic solvents or global unfolding of globular proteins (e.g., refs. 12, 27, 36), which involves complete solvation of side chains. In contrast, the studies here focus on the (de)solvation of more extended protein surfaces and may reflect differences in length scale, contrast, the studies here focus on the (de)solvation of more extended protein surfaces and may reflect differences in length scale. In addition to exploring the role of conformational entropy in protein function, the entropy meter can be used to illuminate other important manifestations of entropy. For example, the complexes used for calibration of the entropy meter largely have dry interfaces. The presence of retained “structural” water at the buried surface comprising the interface would affect the solvent entropy but would be masked by the standard method to calculate it based on accessible surface area. Following calibration (Fig. 1), the entropy associated with the organization of water within a protein complex can be explored. To illustrate this view, we examined the complex between the extracellular ribonuclease barnase of Bacillus amyloliquefaciens and an oligonucleotide dCGAC model substrate. Barnase is a 110-residue ribonuclease and is inhibited by the 89-residue barstar to suppress its potentially lethal activity inside the cell. The barnase/dCGAC complex has nine fully buried and crystalllographically well-defined structural water molecules at the interface (47) (Fig. 3). Determination of the binding thermodynamics by direct titration monitored by NMR spectroscopy and ITC indicates that the free energy of binding of dCGAC to barnase is essentially provided by a gain in entropy (SI Appendix, Figs. S3 and Table S2). Dynamic analysis indicates a corresponding increase in side-chain motion upon binding of the oligonucleotide that corresponds to a favorable contribution to the binding entropy (SI Appendix, Figs. S3 and Table S4). An inventory of the entropy contributions as outlined by Eq. 3 results in good agreement, which is in apparent contradiction to the implications of the structure of the complex noted above. This contradiction arises because rigidification of nine water molecules is not accommodated by the solvation term of Eq. 3. Using the entropy of fusion of water (−22 J·mol⁻¹·K⁻¹) (48), the rigidification of these waters in the complex is be predicted to result in a distinct deviation from the calibration line (Fig. 4, gray and blue diamonds). Thus, either the structural waters retain considerable S tethered to the protein. Thus, the rigidly held water by free barnase appears to nullify the entropic cost of using water as a structural element in the complex. A second example of the further insights that can be garnered from the entropy meter centers on the idea that the loss of rotation-translational entropy will diminish for weaker interactions (i.e., K₈ > 100 μM) due to significant residual motion in the complex. To explore this idea, we examined the interaction of (43, 44), and a calmodulin–peptide complex (45). Early NMR studies suggested that the backbone contributed less to the heat capacity of folded proteins than side chains (41). Using the entropy meter, we find that the amino acid side chains contribute only a small fraction (~5–6%) to the total heat capacity measured by differential scanning calorimetry of the latter two proteins (SI Appendix, Table S6). This finding reinforces the proposal that most of the heat capacity comes from solvent–protein interactions (10, 40, 46).
histamine and serotonin with the histamine-binding protein (HBP) from the *Rhipicephalus appendiculatus* tick. HBP is a member of the lipocalin family of proteins, which have been shown to bind to histamine and serotonin (52). These heterocyclic molecules serve as primary mediators of the inflammatory response upon tissue damage (53). HBP is a 171-residue, β-barrel protein that, unlike most other lipocalin family proteins, has evolved to possess two histamine-binding sites, with one having high affinity (H-site) and one having low affinity (L-site) (52). The tick secretes HBP to interfere with the defensive inflammatory response of the host. For our studies, we have used the D24R mutation located in the L-site that largely abolishes the binding of the second histamine molecule while retaining the primary high-affinity histamine binding (H-site) (54). ITC established that histamine binds with very high affinity to HBP(D24R) [Kd = 3.2 ± 0.7 nM, consistent with earlier measurements using trace radiolabeling (52)] and is accompanied by a large favorable change in enthalpy and an unfavorable total change in entropy (SI Appendix, Table S2). NMR relaxation analysis indicates a heterogeneous response of the protein to the binding of histamine (SI Appendix, Fig. S4). These results give a datum very close to the consensus fitted line of the entropy meter. Titration of the HBP(D24R)-histamine complex with serotonin reveals a weak binding site largely centered on residues Y30, V49, V51, A53, F67, E82, which are near the L-site (SI Appendix, Fig. S5). This finding could be indicative of residual binding activity at the L-site. ITC reveals no detectable heat, indicating that the modest binding free energy (−13.7 kJ/mol) is driven entirely by entropy. NMR relaxation analysis indicates an increase in side-chain dynamics that corresponds to a very modest ∆Sconf of 3 ± 1 J mol⁻¹ K⁻¹. Interestingly, when completing the inventory of entropy, one finds only a small deviation from the entropy predicted (Fig. 1). This deviation is likely traced to an uncertainty in the solution parameters for this complex and perhaps to a small residual Sres of the serotonin ligand in the complex. Thus, ∆Srot estimated in Fig. 1 is likely valid down to relatively low affinities corresponding to dissociation constants in the millimolar range.

In summary, the coefficients relating changes in fast protein side-chain motion to changes in conformational entropy, changes in accessible surface area to changes in solvent entropy, and the loss of rotational-translational entropy in high-affinity complexes have been determined. The range of ligand types used here demonstrates that the relationship between fast internal side-chain motion and the underlying conformational entropy is universal and represents a fundamental property of soluble proteins. It is demonstrated that conformational entropy has a highly variable role in the formation of complexes involving proteins: It can favor, disfavor, or have no impact on the free energy of binding.

There are no obvious structural correlates apparent for this behavior. The connection between structure, the enthalpy that it represents, conformational entropy, and internal motion presents an immediate challenge to our current understanding of protein thermodynamics and function. In this vein, the view emerging from crystallographic analysis of minor conformers in proteins (55–57), combined with the dynamical proxy validated here, provides a means to quantify the role of conformational entropy in protein structure and function.

**Methods**

Various proteins and their complexes (47, 52, 58–61) were prepared as described in detail in SI Appendix. Some published NMR relaxation and thermodynamic studies were used without further analysis (SI Appendix, Table S1). Macromolecular rotational correlation times and backbone O−H relaxation were determined (62) from 15N relaxation obtained at two magnetic fields. Model-free parameters (22) were determined using a grid search approach (63) using a version of Richn2A (64) implemented in the C++/AMPP language. The 13N-relaxation analysis used an effective N-H bond length of 1.04 Å (65) and a general 15N tensor breadth of 170 ppm (66). Tumbling models were identified through standard statistical analysis (67). Methyl group O−H parameters were determined from measured (68) deuterium T1, T2, and Tr relaxation. A quadrupolar coupling constant of 167 kHz was used (69). Analysis of NMR relaxation in these systems is summarized in SI Appendix, Tables S3, S4, and S7. Changes in polar and apolar accessible surface area were calculated using AREAIMOL (70) as described previously (20) (SI Appendix, Table S4). BioMagResBank accession numbers are summarized in SI Appendix, Table S8. Studies of protein hydration dynamics used 3D 15N-resolved 1H–15N NOE spectroscopy and ROE spectroscopy (7.2-KHz spin lock field) experiments (51, 71). Perdeuterated barnase prepared in 25 mM imidazole (pH 6.2) and 10 mM KCl was encapsulated by defined volume injection (72) into 75 mM deuterated hexadecyltrimethylammonium bromide (CTAB) and 190 mM deuterated hexanol in deuterated pentane to a molar ratio of water to CTAB (W0 or water loading) of 20. Structural fidelity of encapsulated barnase was confirmed by comparison of 15N-HSQC spectra. These experiments were performed at 25 °C and 500 MHz (1H).

**ACKNOWLEDGMENTS.** We thank Professor Mark Greene for generous access to the isothermal titration calorimeter. We thank Veronica Moorman and Kendra Frederick for preliminary calorimetry experiments and initial cura-
tion of published studies. This work was supported by the Mathers Foundation (A.J.W.), NIH Grants GM102447 and GM100910 (to A.J.W.), and National Science Foundation Grant MCB-1158038 (to A.J.W.). K.A.S. acknowledges support from the Johnson Research Foundation. J.L. gratefully acknowledges a Biomedical Research Council (A*STAR) Singapore postdoctoral fellowship. J.A.C. gratefully acknowledges an NIH postdoctoral fellowship (GM117878).

Caro et al. PNAS | June 20, 2017 | vol. 114 | no. 25 | 6567
1. Motlagh HH, Wrbal JO, Li J, Hilsel VJ (2014) The ensemble nature of allosterily. Nature 408:311-315.
2. Clackson T, Wells JA (1995) A hot spot of binding energy in a hormone-receptor interface. Science 267:383-386.
3. Wodak SJ, Janin J (2002) Structural basis of macromolecular recognition. Adv Protein Chem 61:19-73.
4. Zhou HX, Gilson MK (2000) Theory of free energy and entropy in noncovalent binding. Chem Rev 109:4092-4107.
5. Welch GR, Somogyi B, Damjanovich S (1982) The role of protein fluctuations in enzymatic catalysis: a review. Prog Biophys Mol Biol 38:109-146.
6. Cooper A, Dryden DTF (1984) Allostery without conformational change. A plausible model. Eur Biophys J 11:103-109.
7. Gilson MK, Given JA, Bush BL, McCammon JA (1997) The statistical-thermodynamic basis for computation of binding affinities: A critical review. Biophys J 72:1047-1069.
8. Luo H, Sharp K (2002) On the calculation of absolute macromolecular binding free energies. Proc Natl Acad Sci USA 99:10399-10404.
9. Singh N, Warshel A (2010) A comprehensive examination of the contributions to the binding energy of protein-ligand complexes. Proteins 78:1724-1735.
10. Sturmevant JM (1972) Heat capacity and entropy changes in processes involving proteins. Proc Natl Acad Sci USA 74:2236-2240.
11. Dill KA (1990) Dominant forces in protein folding. Biochemistry 29:7133-7155.
12. Privalov PL, Makhatadze GI (1993) Contribution of hydration to protein folding. Nature 400:26-33.
13. Steinberg IZ, Scheraga HA (1963) Entropy changes accompanying association reactions of proteins. J Biol Chem 238:172-181.
14. Akke M, Brocchiellier R, Palmer AG (1993) NMR order parameters and free-energy of an artificial protein and its application to cooperative Ca2+ binding by calbindin D9K. J Mol Biol 195:8932-9333.
15. Li Z, Raychaudhuri S, Wand AJ (1996) Insights into the local residual entropy of proteins from protein liquid. Protein Sci 5:2667-2705.
16. Yang D, Kay LE (1996) Contributions to conformational entropy arising from bond vector fluctuations measured from NMR-derived order parameters: Application to protein folding. J Mol Biol 263:369-382.
17. Igumenova TI, Frederic KK, Wand AJ (2000) Characterization of the fast dynamics of protein amino acid side chains using NMR relaxation in solution. Chem Rev 106:1672-1699.
18. Wand AJ (2013) The dark energy of proteins comes to light: conformational energy and its role in protein function revealed by NMR.Curr Opin Struct Biol 23:75-81.
19. Frederik KK, Marlow MS, Valentine KG, Wand AJ (2007) Conformational entropy in molecular recognition by proteins. Nature 448:325-329.
20. Marlow MS, Dogan J, Frederik KK, Valentine KG, Wand AJ (2010) The role of conformational entropy in molecular recognition by calmodulin. Nat Biol 6:352-358.
21. Kasinath V, Sharp KA, Wand AJ (2013) Microscopic insights into the NMR relaxation-based protein conformational entropy meter. J Am Chem Soc 135:15092-15100.
22. Lipari G, Szabo A (1982) Model-free approach to the interpretation of nuclear magnetic-resonance relaxation in macromolecules.1. Theory and range of validity. J Am Chem Soc 104:4546-4559.
23. Lee AL, Sharp KA, Kranz JK, Song XJ, Wand AJ (2002) Temperature dependence of the internal dynamics of a calmodulin-peptide complex. Biochemistry 41:13814-13825.
24. Karplus M, Ichiye T, Pettitt BM (1987) Configurational entropy of native proteins: An application to the interpretation of nuclear magnetic resonance relaxation in macromolecules.2. Theory and range of validity. J Am Chem Soc 104:4546-4559.
25. Karplus M, Ichiye T, Pettitt BM (1987) Configurational entropy of native proteins. Biochemistry 26:2246-2259.
26. Karplus M, Ichiye T, Pettitt BM (1987) Configurational entropy of native proteins: 1. Theory and range of validity. J Am Chem Soc 109:2164-2175.
27. Lipari G, Szabo A (1982) Model-free approach to the interpretation of nuclear magnetic-resonance relaxation in macromolecules.1. Theory and range of validity. J Am Chem Soc 104:4546-4559.
28. Lee AL, Sharp KA, Kranz JK, Song XJ, Wand AJ (2002) Temperature dependence of the internal dynamics of a calmodulin-peptide complex. Biochemistry 41:13814-13825.
29. Karplus M, Ichiye T, Pettitt BM (1987) Configurational entropy of native proteins. Biochemistry 26:2246-2259.
30. Karplus M, Ichiye T, Pettitt BM (1987) Configurational entropy of native proteins: 1. Theory and range of validity. J Am Chem Soc 109:2164-2175.
31. Lipari G, Szabo A (1982) Model-free approach to the interpretation of nuclear magnetic-resonance relaxation in macromolecules.1. Theory and range of validity. J Am Chem Soc 104:4546-4559.
32. Lee AL, Sharp KA, Kranz JK, Song XJ, Wand AJ (2002) Temperature dependence of the internal dynamics of a calmodulin-peptide complex. Biochemistry 41:13814-13825.
33. Lipari G, Szabo A (1982) Model-free approach to the interpretation of nuclear magnetic-resonance relaxation in macromolecules.1. Theory and range of validity. J Am Chem Soc 104:4546-4559.