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Direct estimation of entropy loss due to reduced translational and rotational motions upon molecular binding

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Direct Estimation of Entropy Loss Due to Reduced Translational and Rotational Motions upon Molecular Binding

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Abstract: *The entropic cost due to the loss of translational and rotational (T–R) degree of freedom upon binding has been well recognized for several decades. Tightly bound ligands have higher entropic costs than loosely bound ligands. Quantifying the ligand's residual T–R motions after binding, however, is not an easy task. We describe an approach that uses a reduced Hessian matrix to estimate the contributions due to translational and rotational degrees of freedom to entropy change upon molecular binding. The calculations use a harmonic model for the bound state but only include the T–R degrees of freedom. This approximation significantly speeds up entropy calculations because only 6×6 matrices need to be treated, which makes it easier to be used in computer-aided drug design for studying many ligands. The methodological connection with other methods is discussed as well. We tested this approximation by applying it to study the binding of ATP, peptide inhibitor (PKI), and several bound water molecules to protein kinase A (PKA). These ligands span a wide range in size. The model gave reasonable estimates of the residual T–R entropy of bound ligands or water molecules. The residual T–R entropy demonstrated a wide range of values, e.g., 4 to 16 cal/K · mol for the bound water molecules of PKA.* © 2005 Wiley Periodicals, Inc. Biopolymers 79: 00–00, 2005

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INTRODUCTION

The association and dissociation of molecules are important and frequent biological events that regulate signal transduction and other biological processes. The binding free energy provides a useful indicator of the extent of an association or dissociation process. Thus, significant efforts have been devoted to the development of methods for calculating free energy changes. The paper by Gilson et al.,[1] for example, gives a thorough review on the theoretical framework for carrying out binding affinity calculations. An important component in calculating an absolute free energy of binding is the estimation of contributions from the loss in translational–rotational (T–R) degrees of freedom (see, for example, Refs. [2–8]). The loss of T–R entropy is due to the restriction of overall translational and rotational motions of the binding molecule in the complex relative to the freely moving state in solution. A rough approximation is to assume a complete loss of the T–R degrees of freedom of the ligand but this approximation does not work too well as recognized in Refs. [2–8]. However, it is difficult to measure the T–R entropy contributions to binding and theoretical estimates vary by more than an order of magnitude.[9]

In principle, one can estimate the entropy of a ligand–receptor complex by including all of the intramolecular degrees of freedom of the complex using models such as normal mode analysis.[5,10,11] Quasiharmonic models have also been introduced to take some anharmonicity into account.[8,12–17] In the quasiharmonic models, one uses coordinate fluctuation covariance matrices obtained from molecular dynamics (MD) simulations to construct effective force constant matrices for harmonic analysis. Several forms of this approach have also been recently discussed.[18] The harmonic and quasiharmonic approaches both require the diagonalization of large Hessian matrices of dimension $3N \times 3N$ where N is the number of atoms in a molecule or molecular complex. The CPU time and memory requirements for such calculations grow rapidly with N. Therefore, it is a common approximation to include only a subset of atoms (e.g., α carbons) to facilitate such calculations. Because the major contributions of the entropy come from the lowest frequency modes, a further approximation that can significantly speed up calculations is to assume both the receptor and the ligand are completely rigid and use their relative motion in the complex to estimate the residual T–R entropy after binding. This is somewhat similar to the separation of T–R motions from the internal motion in Swanson et al.[17] and only requires six degree of freedom to describe. Because these motions are rather restricted in the complex, a normal or quasinormal mode analysis can be carried out, albeit in a reduced six-dimensional space in the relative T–R degrees of freedom. This approximation significantly speeds up calculations because only small 6×6 matrices need to be treated. Therefore, many more entropy calculations can be done quickly, which is useful for computer-aided drug design and for protein–ligand/protein–protein docking. In this article, we derive formulae for calculating entropy changes using this approximation and discuss the methodological connection with several other harmonic and quasiharmonic methods. Finally, we evaluate this approach by applying it to study the binding of ATP, the peptide inhibitor PKI, and several bound water molecules to protein kinase A (PKA).

THEORY AND METHOD

When two molecules bind, the overall T–R motions of the component molecules become restricted. Here, we focus on deriving equations for estimating the entropy loss resulting from converting two freely translating and rotating molecules into a complex with highly restricted relative motion. We assume that solvation effects can be taken into account by a suitable implicit solvent model so that only the solute degrees of freedom are treated explicitly. To simplify the equations, we first include explicitly only the T–R motion of the ligand. We will consider the contributions from the motion of the receptor later. The motion of the rigid ligand can be described by three translational and three rotational degrees of freedom. We start with the reduced classical molecular partition function for the ligand defined in the six relative T–R degrees of freedom:

$$Z = \frac{1}{h^6} \int dp^6 \, dq^6 \, \exp\left(-\beta H\left(p, q\right)\right) \tag{1}$$

where h is the Planck constant, $\beta = (kT)^{-1}$, k is the Boltzmann constant, T is the absolute temperature, H is the Hamiltonian describing the motion of the ligand, and q and p are the coordinates and their conjugate momenta, respectively. Here, we use three Cartesian coordinates at the center of mass of the ligand to describe its translational motion and three angular coordinates to describe its rotational motion about three axes passing through its center of mass. Also, $H = E_k + U$, where E_k is the kinetic energy and U is the interaction potential of the ligand with its receptor. The interaction potential is zero when the ligand is not bound to the receptor. If one uses the three principal axes of inertia of the ligand as rotation axes, the kinetic energy can be written as

$$E_k = \frac{1}{2m}\left(p_x^2 + p_y^2 + p_z^2\right) + \frac{1}{2I_x} l_x^2 + \frac{1}{2I_{yy}}^2 + \frac{1}{2I_z} l_z^2 \tag{2}$$

where p_x, p_y, p_z and l_x, l_y, l_z are the x, y, z components of the linear and angular momenta respectively, I_x, I_y, and I_z

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are the principal moments of inertia, and m is the mass of the ligand. Now, the integration on the six momenta space contributes a factor $\sqrt{(2\pi)^6\ m^3 I_x I_y I_z/\beta^6}$ to the partition function so that

$$Z = \frac{1}{v}\int dq^6 \exp\left(-\beta U\left(q^6\right)\right) \tag{3}$$

where

$$v = \sqrt{\frac{h^{12}\beta^6}{(2\pi)^6\ m^3\ I_x I_y I_z}} \tag{4}$$

If one takes l_x, l_y, and l_z to be the three generalized momenta, the corresponding generalized coordinates are the three angles φ, θ, and ψ, describing the rotation of the ligand around the three principal axes. In this treatment, the volume element dq^6 in the integration is simply $dxdydzd\varphi d\theta d\psi$.

When the ligand is unbound, U is zero and the configurational integral of Eq. (3) can be evaluated analytically to give

$$Z_{\text{free}} = \frac{1}{v}\left(8\pi^2 V\right) \tag{5}$$

where the subscript *free* is used to denote the unbound state. The integral over the angles gives a contribution of $8\pi^2$ in the full rotational space, and the integral over the translational coordinates gives a factor V that is 1660 Å^3 per molecule in the standard state of 1 M (1 mol/L).

On the other hand, the integral cannot be evaluated analytically in the bound form and needs to be computed numerically. Since the T–R motion of the ligand in the complex is quite restricted, approximations similar to those in a normal mode analysis can be used. One can expand U about a local energy minimum and keep up to second-order terms, noting that the first derivatives of U are zero:

$$U = U_0 + \frac{1}{2}\Delta q^T\ D\Delta q \tag{6}$$

where U_0 is the interaction energy at the local minimum, Δq is the small displacement vector of the six coordinates relative to the receptor, and D is the second derivative matrix with elements $D_{ij} = \partial^2 U/\partial q_i \partial q_j$, which in fact is a reduced Hessian matrix. Because U_0 is a constant, it can be moved out of the integral. The remaining configurational integral is quadratic. The Hessian matrix can be diagonalized by an orthogonal transformation to get the six normal modes. These restricted translational and rotational modes have softer frequencies than the intramolecular modes and therefore account for a large part of the residual entropy. If $kT \gg h\omega$ where ω is one of the six vibrational frequencies, one can treat these modes classically. Since the overall molecular motion is relatively restricted, which implies the exponential factor of the integral decreases rapidly as the coordinates deviate from their equilibrium value, it is a good approximation to set the limits of integration from negative to positive infinity. The integral can then be computed analytically so that Eq. (3) becomes

$$Z = \frac{\exp(-\beta U_0)}{v}\left(\sqrt{2\pi}\right)^6 (kT)^3\ \det(D)^{\frac{1}{2}} \tag{7}$$

where $\det(D)$ is the determinant of the Hessian matrix D. The free energy of the complex can then be written as

$$F = -kT\ \ln\ Z = U_0 + \frac{kT}{2}\ln\frac{\det(D)(h\beta)^{12}}{m^3\ I_x I_y I_z} \tag{8}$$

where $h = h/2\pi$. Because $F = E - TS$, where E is the energy and S is the entropy, comparing Eq. (8) with the corresponding expression for classical harmonic oscillators, $E = U_0 + 6kT$, yields

$$S = -\frac{k}{2}\ \ln\frac{\det(D)(h\beta)^{12}}{m^3\ I_x I_y I_z} + 6k \tag{9}$$

$6kT$ results from the average harmonic potential and kinetic energy in the six degrees of freedom. For comparison, the absolute T–R entropy of the ligand in the free state S_{free} obtained from Eq. (5) is

$$S_{\text{free}} = -\frac{k}{2}\ln\frac{h^{12}(2\pi\beta)^6}{m^3\ I_x I_y I_z (8\pi^2 V)^2} + 3k \tag{10}$$

The factor $3kT$ results from the kinetic energy terms of the free ligand. Using Eqs. (5) and (8), the free energy change of the binding process can be written as

$$\Delta F = -kT\ \ln\frac{Z}{Z_{\text{free}}} = U_0\ - kT\ \ln\frac{\det(D)^{-\frac{1}{2}}(2\pi)^3}{8\pi^2 V\beta^3} \tag{11}$$

From Eqs. (9) and (10), the entropy change upon binding is then

$$\Delta S = k\ \ln\frac{\det(D)^{-\frac{1}{2}}(2\pi)^3}{8\pi^2 V\beta^3} + 3k \tag{12}$$

And the entropy difference between two docked conformations, 1 and 2, can be obtained with the following expression:

$$\Delta\Delta S = \Delta S_2\ - \Delta S_1 = S_2\ - S_1 = -\frac{k}{2}\ln\frac{\det(D_2)}{\det(D_1)} \tag{13}$$

So far, we have not written out the receptor coordinates explicitly. However, including these coordinates does not affect the formula for calculating entropy changes, although it affects the expression for calculating the absolute entropies of the unbound species and the complex, as we now show.

When the receptor coordinates are also considered explicitly, we have 12 instead of 6 degrees of freedom. Using similar arguments as before, the integral over all the momenta now gives a factor of $v' = \sqrt{\frac{h^{24}\ \beta^{12}}{(2\pi)^{12}(m_A m_B)^3\ I_{Ax} I_{Ay} I_{Az} I_{Bx} I_{By} I_{Bz}}}$, where A and B denote molecule A and molecule B, respectively. In the remaining configurational integral, we have three more Cartesian coordinates to describe the transla-

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tional motion and three more rotational angles to describe the rotational motion of the receptor in addition to the six degrees of freedom that we used earlier to describe the T–R motion of the ligand. Since the interaction potential is only dependent upon the relative position between the two molecules and this interaction has already been taken into account in the above treatment, the contributions from the receptor can simply be obtained by integrating over its six degrees of freedom in the configurational integral in a field-free environment. For the complex, the integration yields a factor of $8\pi^2 V_{AB}$ where V_{AB} is the volume of a solution of the complex AB in the standard state. Combining this with the previous treatment of the ligand gives the following expression for the free energy of the complex:

$$F = -kT\ \ln\ Z = U_0 + \frac{kT}{2}\ln\frac{\det(D)h^{24}\ \beta^{18}(2\pi)^{12}}{(m_A m_B)^3 I_{Ax}I_{Ay}I_{Az}I_{Bx}I_{By}I_{Bz}(8\pi^2 V_{AB})^2} \tag{14}$$

When the free energies of the free molecules A and B are subtracted to obtain a free energy change, the following formula results:

$$\Delta F = U_0\ - kT\ \ln\frac{\det(D)^{-\frac{1}{2}}(2\pi)^3 V_{AB}}{8\pi^2 V_A V_B \beta^3} \tag{15}$$

where V_A and V_B are the volume of the solutions containing species A and B, respectively. V_A, V_B, and V_{AB} can be also expressed as the inverse of the concentration of each species if, as we have assumed, the size of the molecules can be negligible compared to the volume of the solution in the concerned state. Therefore, for the case that A and B have the same concentrations, V_A, V_B, and V_{AB} are the same, and then Eq. (15) becomes Eq. (11). If the volumes of the three solutions are different, especially for different concentrations of A, B, and AB, one can take them into account by using Eq. (15) directly instead of Eq. (11). The formulae derived above show that the main part that needs to be computed numerically is the determinant of the Hessian matrix, which describes the curvature of the potential well at a local minimum and should be positive in general. This determines within the harmonic approximation the residual entropy after binding.

Comparisons with Other Methods

Our approach can be extended to approximately take anharmonic effects into account by using similar arguments as in quasiharmonic calculations except that we only focus on the lowest-frequency restricted T–R modes and assume them to be uncoupled to the intramolecular modes. Thus, our formulae can still be compared to those obtained for quasiharmonic analysis. The quasiharmonic method assumes that the fluctuation of the atomic coordinates Δx around their equilibrium positions can be described by multivariable Gaussian distributions:[12]

$$P(\Delta x) \propto\ \exp\left(-\frac{1}{2}\Delta x^T\left(\sigma_{ij}^{-1}\right)\Delta x\right) \tag{16}$$

where σ_{ij} is the positional fluctuation covariance matrix. Comparing this with Eq. (6), one can find the relation

$$\sigma_{ij}^{-1} = \beta(D_{ij}) \tag{17}$$

This relation has also been derived based on linear response theory.[19,20] One can obtain similar formulae in various quasiharmonic models by substituting Eq. (17) into Eq. (9).[14,15,18] For example, Schlitter's formula[14] can be written as

$$S = \frac{k}{2}\ln\ \det\left(\frac{kTe^2}{h^2}M\sigma + 1\right) \tag{18}$$

where $e = \exp(1)$ is the Euler number, and $\mathbf{M}$ and $\mathbf{1}$ are the mass and unit matrices, respectively. This is similar to our Eq. (9) except that the mass matrix is replaced by a six by six matrix containing masses and moment of inertia, and there is an extra unit matrix in the Schlitter formula. The unit matrix in Eq. (18) results from a heuristic treatment of quantum oscillators. This suggests that we can include quantum effects by adding a similar unit matrix to our formulae. This unit matrix also does not show up in Andricioaei and Karplus' classical expression for the entropy.[18] The constant Euler number e in Schlitter's formula is also present in our formula; it comes from the kinetic energy term [see Eqs. (9) and (12)]. However, this term cancels out in calculating entropy changes $\Delta\Delta S$ (see Eq. 12).

One may add intramolecular contributions within the approximation of negligible couplings between restricted T–R degrees of freedom and intramolecular motion. Here, the Hessian matrix can be divided into parts corresponding to different types of motions in the complex and the free molecules. Thus, the free energy can be estimated from

$$\Delta F = U_0 - kT \ln\frac{\left[\det(D)\det\left(D_A^{ibnd}\right)\det\left(D_B^{ibnd}\right)\right]^{-\frac{1}{2}}(2\pi)^3 V_{AB}}{\left[\det\left(D_A^{ifree}\right)\det\left(D_B^{ifree}\right)\right]^{-\frac{1}{2}}8\pi^2 V_A V_B \beta^3} \tag{19}$$

If one substitutes each Hessian matrix by $kT\sigma^{-1}$, where σ is the corresponding covariance matrix, Eq. (19) becomes similar to Eqs. (5) and (12) in the work of Luo and Sharp[15] except for a few differences. In Luo and Sharp's work, orientational motion is assumed to be isotropic about the axes φ and ψ but quasiharmonic over small magnitudes of χ. Finkelstein and Janin's treatment of T–R entropy loss upon binding[4] was even simpler. They also assumed that the probability distribution of the T–R motion was uniform within their allowed range; this is equivalent to setting the exponential term to unity in the integrals of Eq. (3). On the other hand, our treatment uses a more realistic Boltzmann distribution.

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System Preparation

Calculating the entropy of binding from Eqs. (8)–(12) requires computation of the Hessian matrix. We first carried out energy minimization to locate the local energy minimum of a complex structure. The calculation of the second derivative matrix with respect to the three translational degrees of freedom is trivial. The formulae for calculating the elements of the second derivative matrix with respect to the three rotational degrees of freedom are summarized in the Appendix. The interaction potential between two molecules in a complex included electrostatic and van der Waals contributions obtained by using the AMBER force field.[21] A distance-dependent dielectric function ($\varepsilon = r_{ij}$) was used in the electrostatic calculations to approximate solvent screening effects.

The calculations were applied to study PKA, which is one of the most studied protein kinases. The crystal structure (pdb code: 1ATP) of the C subunit of PKA was selected. This structure contains ATP, a peptide inhibitor (PKI), two Mn ions (changed to Mg ions in the calculation to mimic the reactant state before phosphoryl transfer), and bound crystal water molecules. The charges of the phosphorylated residues were obtained by using Gaussian (6-31 + G* basis set) together with the RESP method implemented in AMBER. The polyphosphate parameters for ATP were from the work of Meagher et al.[22] developed for the AMBER force field. We took ATP, PKI, and 11 bound crystal water molecules as the ligand in the T–R entropy calculations and the rest of the system as the receptor. Seven of the selected 11 water molecules were conserved water molecules found in all the crystal structures (with or without substrates or their analogs) described in Shaltiel et al.[23] The other four nonconserved water molecules were selected for comparison; they were more loosely bound. The standard state of ATP and PKI was taken to be 1 *M* and that for water was 55.6 *M*. The calculation was based on structures obtained by carrying out 5000 steps of conjugate–gradient energy minimization. For comparison, we also carried out quasiharmonic-like analysis by using results from a MD simulation of the complex. In setting up the MD simulation, the system was first relaxed by energy minimization, followed by heating from 0 to 300 K in 20 ps. The system was then equilibrated for 100 ps at 300 K followed by 500 ps of production run to generate a trajectory (snapshots saved every 1 ps) for structural fluctuation analysis, which was used in the quasiharmonic calculations. During the simulation, the nonbonded cut-off distance was set to 9.0 Å, and SHAKE[24] was used to constrain bonds involving hydrogen atoms.

RESULTS AND DISCUSSION

T–R Entropy, Frequencies, and Fluctuations

The entropy and other property calculation results are listed in Table I. T1

Equations (9) and (12) were used to calculate the residual T–R entropy, S, and entropy change upon binding, ΔS. To estimate the rigidity of binding, the formula $\frac{1}{4}\lambda_i A_i^2 = \frac{1}{2}kT$, where λ as the eigenvalue of the Hessian matrix, is used to get the vibrational

Table I Residual Translational–Entropy and Properties of Ligands[a]

Ligand	Mass (amu)	S	ΔS(cal/k · mol)	ΔS_{couple}	$\overline{A}_{\text{t}}$	$\overline{A}_{\text{r}}$	Frequency range (cm^{-1})
ATP	503	16.1	−57.4	1.4	0.035	0.011	123–259
PKI	2194	27.6	−60.0	1.3	0.043	0.049	61–138
Bound water							
a	18	8.1	−22.2	0.5	0.18	0.22	153–816
b[b]	18	7.2	−23.4	0.3	0.15	0.21	128–655
c	18	10.2	−20.1	0.3	0.25	0.24	63–636
d	18	4.0	−26.3	0.1	0.11	0.19	163–827
e1	18	7.4	−22.9	0.2	0.14	0.28	175–845
e2	18	4.5	−25.8	0.1	0.11	0.22	195–824
f	18	6.8	−23.5	0.1	0.15	0.21	170–752
471	18	15.6	−14.7	0.6	0.36	0.55	113–600
459	18	16.5	−13.8	0.2	0.34	0.57	51–375
523	18	11.6	−18.7	0.1	0.26	0.26	56–587
412	18	12.2	−18.1	0.1	0.24	1.99	51–375

[a] S, residual translational–rotational entropy of ligand; ΔS, entropy change upon binding at 300 K; ΔS_{couple}, the Contribution to entropy from the translation–rotation cross-term (see text); $\overline{A}_t$ and $\overline{A}_r$, the mean vibrational amplitudes (see text) of T–R Motion; and the frequency range of the T–R modes shown by the lowest and highest wave number.

[b] Reference data obtained with a constant dielectric to avoid the negative eigenvalue in the calculation using distance-dependent dielectric.

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(fluctuating) amplitude (A_i for the ith normal mode). It is worth noting that it has been recognized that the amplitude calculated from normal mode cannot be compared directly to that obtained from MD simulations;[8] it only has relative meaning when comparing different modes of motion. Table I gives the mean translational amplitude, denoted as A_t and the mean rotational amplitude, denoted as A_r over all corresponding normal modes determined by the reduced Hessian matrix for each bound molecule.

It is found that ATP gives a smaller S_{free} (73.5 cal/K · mol) and residual entropy (16.1 cal/K · mol) relative to the PKI. One reason is that ATP has less mass (503 amu) and size than PKI (2194 amu); the other reason is the tighter binding of ATP relative to PKI. The amplitude in the translational (0.035 Å) and rotational (0.011 radian) motion of ATP is smaller than that of PKI (0.043 Å and 0.049 radian). This is reasonable because ATP is deeply buried in the binding pocket and surrounded by the protein, whereas PKI is more solvent exposed.

To compare with experimental results, we also calculated the entropy loss of the ATP congener inhibitor balanol upon binding with PKA (complex PDB code: 1bx6). The ΔS is about -51.6 cal/K · mol. If one uses the binding energy obtained from a more sophisticated Poisson model, -26 kcal/mol, which includes nonpolar and electrostatic contributions except for the loss of T–R entropy,[25,26] and adds to it the entropy change obtained here, the resulting binding free energy of -10.5 kcal/mol is very close to the experimental result of about -10 kcal/mol.[27]

We also selected 11 water molecules in the crystal structure to study their entropy of binding to PKA. The first 7 water are conserved water molecules at the active site in the ternary crystal structure 1ATP. The active site consists of an extended network of interactions that weave together the kinase core. The neighbors of each water molecule and their properties are presented in Table II of Shaltiel et al.[23] The other 4 nonconserved water molecules (numbered according to the PDB file) are selected for comparison. These 4 water molecules are more loosely bound. Our calculation shows that the 7 conserved water molecules (a–f) contribute entropy ranging from 4.0 to 10.2 cal/K · mol while the other 4 loosely bound water molecules show an entropy range between 11.6 and 16.5 cal/K · mol. Such T–R entropy values and ranges are large enough to affect binding processes and/or to modulate the function and dynamics of the system. Dunitz[28] estimated that each firmly bound water molecule in solid hydrates contributes around or less than 10 cal/K · mol to the entropy while a weakly bound water molecule in a protein contributes more, about 14 to 15 cal/K · mol but hardly greater than 17 cal/K · mol. Fischer et al.[29] estimated by normal mode analysis that the entropy contributed by the librational modes corresponding to the translational and rotational motions of a water molecule was 9.4 cal/K · mol in bovine pancreatic trypsin inhibitor (BPTI). The standard entropy of ice at its freezing point is 9.9 cal/K · mol.[28] The conserved water molecules in PKA strongly interact with their surrounding residues and/or cations (magnesium) and hence adopt well-defined equilibrium positions and orientations. The three internal vibrational modes have frequencies so high that they have little contribution to entropy at 300 K.[28] Fischer's normal mode calculations (also using distance-dependent dielectric) also support this statement (see Table II in Fischer et al.[29]). The frequencies of the T–R motion obtained with the present model for the 7 conserved water molecules are in the range $60 \sim 850$ cm^{-1} (see Table I), very close to the range ($100 \sim 630$ cm^{-1}) of the six lowest frequencies of a water molecule buried in BPTI obtained by Fischer et al.[29] using a more expensive model. Fischer et al.'s work also showed that the three highest frequencies corresponding to the internal motion were much larger (1776, 3357, and 3434 cm^{-1}, respectively) and similar to those of an isolated water molecule (1737, 3323, and 3370 cm^{-1} for TIP3P model of water). Thus, the entropy content of the internal high frequency modes is nearly negligible (less than 0.1 of the total entropy of a water molecule). The entropy calculated by the present reduced Hessian matrix method adequately accounts for the entropy of a water molecule. Table I gives a range in free energy contribution from the T–R entropy of bound water molecules of about 3.1 kcal/mol. According to the residual entropy, we can sort the 7 conserved water molecules by their tightness of binding as: d, e2, f, b, e1, a, c. From the structure,[23] it is found that water molecule d (denoted as W-d and similar for the others) interacts with the inhibitory metal ion Mg_2 (Mn in the original crystal structure), ATP(O_2G), and ATP(ribose 3′OH). Both W-e2 and W-e1 interact with Mg1, ATP, and Asp-184. With the strong interaction with metal ions, the motion of these water molecules is very restricted and similar to the case of solid hydrates. W-f is found at a hydrogen bond–forming distance from the hydroxyl group of Tyr-330, the side chain of Glu-127, and the 2′OH of the ribose ring of ATP. W-a, b, c interact directly with one of the conserved residues Lys-72, Glu-91, Asp-184 at the active site cleft, respectively, in which W-b is the most buried one. The other four nonconserved water molecules W-471, 459, 523, and 412 show larger entropy, consistent with their loose bind-

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ing. These four water molecules reside further away from the active site cleft and contact at most one PKA residue.

The positional and orientational fluctuation analysis from the MD simulation trajectory also shows that the conserved water molecules have small fluctuations from their average positions and orientations relative to the four weakly bound waters. For example, the average fluctuation in the three translational directions ($\bar{\Delta}x$) of W-a is 3.72 Å, and its average fluctuation in three rotational directions ($\bar{\Delta}\theta$) is 0.36 radian. For W-e1, $\bar{\Delta}x$ is 2.66 Å, $\bar{\Delta}\theta$ 0.41 radian. For W-523, $\bar{\Delta}x$ is 1.89 Å, $\bar{\Delta}\theta$ 1.19 radian. The most flexible water molecule, W-459, has $\bar{\Delta}x = 30.04$ Å, $\bar{\Delta}\theta =$ 1.27 radian. In fact, this water molecule moves around freely during the simultion and the calculated entropy of 16.5 cal/K · mol approaches the standard entropy (16.7 cal/K · mol at 298 K) of liquid water.[28] The ligand ATP or PKI has 10-fold smaller positional and orientational fluctuations than the water molecules. These large ligand molecules are more difficult to move or rotate inside the protein. However, it is found that the fluctuation obtained with MD simulation is generally larger than that calculated from the Hessian matrix both for the water molecules and the ligands. Therefore, the entropy loss calculated with the quasiharmonic approximation [Eq. (18)] is generally larger than that calculated by using the harmonic potential approximation. This was also shown in the analysis of MD results by Lazaridis et al.[8] This is due to the more extensive conformational sampling by their MD simulation and also comes from the anharmonicity of the potential surface of intermolecular interaction. The fluctuations of some water molecules obtained by the Hessian matrix method did not match those from MD simulation too well. For example, W-f showed small residual T–R entropy in our model but large fluctuation in the MD simulation. This is because the residual T–R entropy calculations did not include the contributions from the protein. For example, the neighboring residue Tyr-330 of W-f is located near the C terminal ''tail'', Glu-127 at the linker region, and Leu-49 at the Gly-rich-loop and these three regions are all very flexible in PKA.

A natural extension of our model is to use an effective Hessian matrix obtained from a MD simulation as in quasiharmonic analysis but these calculations are significantly more expensive to do due to the extra costs in running MD simulations.

In the frequency analysis, we also found that the frequencies of the T–R modes for the ligands ATP and PKI distributed in a narrow range, indicating that the entropy contributions from translation were not far from rotation. On the other hand, for the small water molecules, the frequency ranges were much wider and generally had higher frequencies compared to the larger ligands.

Coupling Between Translation and Rotation

We also calculated the effects due to the coupling between the translational and rotational motion by including the cross-terms between the rotational and translational motion in the Hessian matrix. If the coupling effects are negligible, the cross-terms between the translational and rotational motion can be ignored. Accordingly, the 6 × 6 Hessian matrix can be divided into two 3 × 3 matrices, one corresponding to the translational motion and the other to the rotational one. Denoting the entropy obtained by the latter method as S_{decouple}, the entropy difference resulting from the coupling effects can be calculated as $\Delta S_{\text{couple}} = S - \Delta S_{\text{decouple}}$. These contributions are listed in Table I. One can see that the coupling effects are small, of the order of 0–1.5 cal/K · mol and less than 10% of the residual T–R entropy. Therefore, the coupling between the translational and rotational motions is normally weak, especially for the small bound water molecules. This supports treatments, such as those in Swanson et al.'s[17] work, that separate these two types of motions. In addition, the coupling between the translational and rotational motion in our calculations all gave positive contributions to the entropy of the bound form for all the cases studied here. As a result, the coupling effects decrease the entropy change of binding somewhat.

Dielectric Effect

The constant dielectric model was also tested in our calculations. We found that, for ATP and PKI, the calculated entropies differ by less than 1 cal/K · mol from those obtained by using distance-dependent dielectric. For water molecules, the differences are also generally less than 2 cal/K · mol.

CONCLUSIONS

An approximate approach for estimating translational and rotational entropy loss upon protein–ligand and protein–protein complexation is proposed and tested for the binding of ATP, peptide inhibitor, and bound water molecules to protein kinase A. The calculations gave reasonable estimates for the entropy change with significantly less computational costs because only 6 × 6 matrices (or 3 × 3 matrices when coupling between translational and rotational degrees of freedom

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is ignored) need to be diagonalized. This work shows that the T–R entropy loss upon binding can vary greatly, by tens of cal/K · mol, for different ligands or different binding sites. For lighter small ligands, such as water molecules, the T–R entropy was more dependent on intermolecular interactions. This result contrasts that of Finkelstein and Janin[4] in which the T–R entropy loss upon binding is always roughly half of the total T–R entropy. Generally, for rigid tight protein–ligand binding, our reduced Hessian approach appears to be a good approximation. For flexible protein–ligand complexation this method may underestimate the residual T–R entropy of the ligand, because anharmonic effects may become significant. Here, we used a relatively simple distance-dependent dielectric model for electrostatic calculations but more sophisticated implicit-solvent models such as the Generalized Born and Poisson–Boltzmann models can also be used. The analyses on bound waters also suggest a potential application of this approach to protein–protein/ligand docking or to drug design. A tightly bound water is likely to be less mobile and thus presents a small residual T–R entropy. This was the case when we compared our results between the conserved water molecules and the loosely bound water molecules, and a similar question was addressed in Yu et al.[30] If tightly bound water molecules indeed have small residue T–R entropy, our reduced Hessian matrix approach could provide a rapid means of identifying these water molecules from a crystal structure. These sites are useful to consider in designing inhibitors to target a protein. The approach presented here can also be applied to study molecules with multiple domains, in which one can obtain the residual T–R entropy of each domain upon complex formation. It can also be extended to construct a higher dimensional, i.e., larger than 6×6, Hessian matrix to study the residual entropy in multiple domain structures.

APPENDIX

The following symbols were used: A_1, A_2, A_3

x	Cartesian coordinates of an atom of the ligand
x^c	Cartesian coordinates of an atom relative to the center of mass of the ligand
A_1, A_2, A_3	three rotational axes, parallel to the x (x_1), y (x_2), z (x_3) axes, through the center of mass of the ligand
V	interaction potential between the receptor and the ligand
V'_i	the partial derivative $\partial v/\partial x$
V''_{ij}	the second derivative $\partial^2 v/\partial x_i \partial x_j$
θ_i	a rotational angle about one of the A_i axes, $i = 1, 2, 3$

The calculation of the first and second derivatives of V, V'_i, and V''_{ij} with respect to the translational degrees of freedom are trivial. The first derivatives with respect to the rotation angles are calculated as

$$\frac{\partial V}{\partial \theta_i} = \sum\left(\sum_{j=1,2,3} \frac{\partial x_j}{\partial \theta_i}\frac{\partial V}{\partial x_j}\right) = \sum\left(\sum_{j=1,2,3} \frac{\partial x_j^c}{\partial \theta_i} V'_j\right) = \sum\left(\sum_{j,k=1,2,3} \varepsilon_{ijk} x_j^c V'_k\right) \quad (20)$$

where the first sum is taken over all the atoms in the ligand. ε_{ijk} is the Levi–Cevita symbol, which equals 1 when $\{ijk\}$ is an even permutation of $\{123\}$, -1 when $\{ijk\}$ is an odd permutation of $\{123\}$, and 0 otherwise. The second derivatives of V are calculated as

$$\frac{\partial^2 V}{\partial \theta_i \partial \theta_j} = \sum\left(x_i^C V'_j - x_k^C x_k^C V''_{ij} + x_i^C x_k^C V''_{jk} + x_j^C x_k^C V''_{ik} + x_i^C x_j^C V''_{kk}\right) \quad (21)$$

$$\frac{\partial^2 V}{\partial \theta_i \partial \theta_i} = \sum\left(-x_j^C V'_j - x_k^C V'_k + x_j^C x_j^C V''_{jj} + x_k^C x_k^C V''_{kk} - 2x_j^C x_k^C V''_{jk}\right) \quad (22)$$

where the sum is taken over all the atoms in the ligand, and i, j, and k are any ordered set of 1, 2, and 3 but different from each other. The expression $\frac{\partial^2 V}{\partial \theta_i \partial \theta_j}$ indicates that the Hessian matrix is not symmetric in general because rotation operations are not commutative. However, it is the case at a local minimum when the torque is zero. We therefore only calculated $\frac{\partial^2 V}{\partial \theta_i \partial \theta_j}$ and made $\frac{\partial^2 V}{\partial \theta_j \partial \theta_i} = \frac{\partial^2 V}{\partial \theta_i \partial \theta_j}$.

The cross term is

$$\frac{\partial^2 V}{\partial \theta_i \partial x_j} = \sum\left(\sum_{k,l=1,2,3} \varepsilon_{ikl} X_k^c V''_{ij}\right) \quad (23)$$

and can be shown to be symmetric at a local minimum.

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