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Enthalpy−Entropy Compensation upon Molecular Conformational Changes

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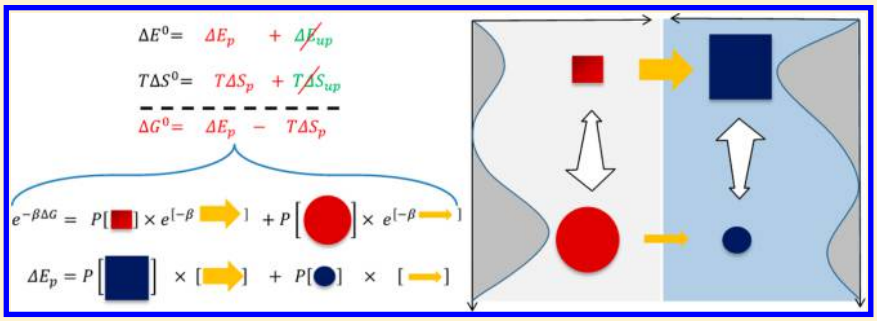

ABSTRACT: The change in free energy is the dominant factor in all chemical processes; it usually encompasses enthalpy−entropy compensation (EEC). Here, we use the free energy perturbation formalism to show that EEC is influenced by the molecular conformational changes (CCs) of the entire system comprising the solute and by the already known solvent reorganization. The internal changes of enthalpy and the entropy due to CCs upon modifying the interactions (perturbation) cancel each other exactly. The CCs influence the dissipation of the modified interactions and their contributions to the free energy. Using molecular simulations, we show that, for solvation of six different HIV-1 protease inhibitors, CCs in the solute cause EEC as large as 10−30 kcal/mol. Moreover, the EEC due to CCs in HIV-1 protease is shown to vary significantly upon modifying its bound ligand. These findings have important implications for understanding of EEC phenomena and for interpretation of thermodynamic measurements.

INTRODUCTION

The change in free energy is a quantity of central importance in biochemistry as well as in other disciplines. It can be used, for example, to estimate ligand affinity and protein stability. The change in the free energy is a result of changes both in enthalpy and in entropy ($\Delta G^0 = \Delta H^0 - T\Delta S^0$). In experimental measurements,[1,2] one consistently observes a cancelation of a substantial portion of the changes in enthalpy and entropy. The extent of enthalpy−entropy compensation was reported to vary widely in magnitude[3] which raises an important question about the mechanisms underlying enthalpy−entropy compensation. Despite the great attention the EEC phenomenon received in the literature, the underlying molecular mechanisms and even the existence of enthalpy−entropy compensation (EEC) are still debated: statistical artifacts pertaining to thermodynamic measurements can lead to correlations between the errors in both enthalpy and entropy and thus produce artificial compensation.[4,5] For example, the large statistical errors in the van't Hoff analysis which was commonly used in earlier times to measure enthalpy and entropy have cast doubts on the existence of EEC.[6] However, the thermodynamic data from Isothermal Titration Calorimetry (ITC) experiments collected recently for more than 400 receptor−ligand complexes provides strong experimental evidence for the existence of EEC.[7] These data exhibit significant correlation between enthalpy and entropy, so that linear fitting of the enthalpy against the entropy gives a slope coinciding with room temperature ($\Delta H = (282.89 \pm 4.71)\Delta S - 8.22 \pm 0.13$ kcal/mol), which was suggested as an indication for EEC[7] as well. However, this observation did not end the continuing discussion in the literature about an experimental proof of EEC. This is due to the point revealed by Krug[8] and more recently by Sharp[4] who suggested that the artificial compensation also leads to a slope equal to the room temperature.[4]

Early experimental observations of EEC suggested that the phenomenon is related to processes in aqueous solution. Important theoretical contributions on the role of water reorganization for the compensation between entropy and enthalpy were made by Ben-Naim[9−11] who studied the changes in the structure of the water caused by a hydrophobic solute. The theoretical framework for the enthalpy−entropy compensation of solvent−solvent interactions along alchemical free-energy calculations was presented by Yu and Karplus who considered the case of solvating a rigid solute molecule.[12] The role of water reorganization in the EEC was also pointed out more recently by Grunwald.[13] The EEC was shown to reflect the flexibility of the surrounding structures in an ideal gas

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systems.[14] Although, the molecular origin of the relation between water reorganization and EEC has been established,[9−12] the specific processes of water reorganization are still heavily debated in the literature.[15] This controversy is partly rooted in the widespread usage of the total entropic changes of the water molecules in the classic iceberg model to explain the hydrophobic interactions.[15]

Recently, the existence of EEC due to water reorganization in the process of ligand−receptor binding has received much interest.[16−19] In a study on the binding of a series of fluorinated benzothiazole sulfonamides to carbonic anhydrase (HCA),[17] ITC measurements and WaterMap[20] calculations suggested that the experimentally observed EEC arises from the reorganization of water molecules in the active site and around the ligand. Extensive EEC was also observed when the desolvation of the pocket of the odorant binding proteins upon binding of a series of aliphatic γ-lactones was studied by X-ray crystallography and NMR.[16] Interestingly, in the latter study, the desolvation of the pocket was reported to have different thermodynamic properties from the desolvation of the ligand.

The EEC has also been acknowledged as a challenging barrier in the lead optimization process.[21] When aiming at improving binding affinity of a drug candidate via introducing chemical modifications that create more interactions with the receptor, increasing the strength of the interactions between the ligand and the receptor often does not lead to a significant improvement in the affinity.[22] It has been hypothesized that EEC encountered in the process of lead optimization may also be caused by the reorganization of water molecules when the strategy of filling the pocket cavity by increasing the size of the ligand is used to improve the affinity.[16,23] A recent study analyzed the differences in binding thermodynamics of two HIV-1 protease inhibitors. Replacing the thioether group in KNI-10033 by a sulfonyl group (KNI-10075) introduced a new hydrogen bond but did not improve the free energy of binding, due to EEC.[21] Although the introduced hydrogen bond resulted in an enthalpic gain of 3.9 kcal/mol, as measured by ITC,[21] the accompanying, entropic changes compensated for this gain leading to an unchanged overall binding affinity. Based on the buried surface area, the authors estimated that only 1.5 kcal/mol of the entropic changes are attributed to the solvation entropy.[21] Another example of EEC in ligand design was presented by DeLorbe[24] who studied peptides binding to the Grb2 SH2 domain. In that study, the structurally modified ligands maintained comparable affinities while their binding enthalpies and entropies changed considerably.[24]

In this paper, we use statistical thermodynamics to revisit the molecular basis of the relation between the enthalpy and the entropy and their compensation. Following the work by Ben-Naim[9−11] and Yu and Karplus[12] on the solvation process of rigid solute molecules, we extended the interpretation of their theoretical findings on the EEC to draw a general concept of EEC that relates the compensation between the enthalpy and the entropy to the conformational changes of the entire system including the solute beside the already known solvent reorganization. To demonstrate the EEC resulting from the conformational changes in a biomolecule, we present results from extensive molecular dynamics simulations that quantify the EEC incurred by the conformational changes in the biomolecules observed in six different HIV-1 protease inhibitors upon solvation and in the wild-type HIV-1 protease upon modifying its bound ligand. Furthermore, the role of conformational changes in free energy changes is illustrated.

THEORETICAL BASIS OF GENERAL EEC

Here we present a brief statistical thermodynamic derivation of such a general EEC framework. We will show that the statistical thermodynamic basis for the enthalpy−entropy compensation due to solvent reorganization that was established by Ben-Naim[9−11] and Yu[12] can be applied straightforwardly to account for the conformational changes in the solute. We consider a system of a total of N molecules, comprising solute and solvent, at a given volume V and temperature T. The total potential energy of the system $U_A(\mathbf{X}^N)$ in state A depends on the conformation $\mathbf{X}^N$ which includes all degrees of freedom of the solvent and the solute molecules. Next, we consider the change in free energy when the system undergoes a reaction which transforms the system to another state B. The reaction is modeled by introducing modifications of interactions within the system and is usually represented by a perturbation potential:

$$U_p(\mathbf{X}^N) = U_B(\mathbf{X}^N) - U_A(\mathbf{X}^N) = U_B(\mathbf{X}^N) - U_{up}(\mathbf{X}^N) \quad (1)$$

Here, we will use the notation $U_{up}(\mathbf{X}^N) = U_A(\mathbf{X}^N)$ for the potential of the initial state A (unperturbed system) to be consistent with the usage of $U_p(\mathbf{X}^N)$. The concept of perturbation is very general and can be used to describe the change in the total potential energy of any reaction including e.g. solute−solvent interaction upon solvation, ligand−protein interaction in the association process, or interactions of the modified atoms with the rest of the system in mutated proteins. The concept of alchemical transformations[25] in free energy calculations is based on the idea of perturbing the potential energy to transform a molecule into another by creation, annihilation, or transformation of the atoms through the modification of their interaction parameters. The change in the free energy $\Delta G^0(A \rightarrow B)$ between states A and B can be calculated by using the free energy perturbation formula which is given by[26]

$$\begin{aligned}\Delta G^0(A \rightarrow B) &= -\beta^{-1} \ln\langle \exp[-\beta U_p(\mathbf{X}^N)]\rangle_A \\ &= \beta^{-1} \ln\langle \exp[\beta U_p(\mathbf{X}^N)]\rangle_B \end{aligned} \quad (2)$$

Here $\beta = 1/kT$; k is the Boltzmann constant. The average $\langle\cdots\rangle_A$ is calculated over the canonical ensemble of state A. Equations for the thermodynamic changes (enthalpy and entropy) can be directly obtained by using the thermodynamic equations.[27] For the enthalpy (internal energy) we have $\Delta H^0 = \Delta E^0 = -T^2\, \partial(\Delta G^0/T)/\partial T$ and for the entropy $T\Delta S^0 = \Delta E^0 - \Delta G^0$:

$$\Delta E^0(A \rightarrow B) = \langle U_B(\mathbf{X}^N)\rangle_B - \langle U_A(\mathbf{X}^N)\rangle_A \quad (3)$$

$$T\Delta S^0(A \rightarrow B) = \langle U_B(\mathbf{X}^N)\rangle_B - \langle U_A(\mathbf{X}^N)\rangle_A - \Delta G^0(A \rightarrow B) \quad (4)$$

Our aim here is to obtain formulas for enthalpy and entropy which can demonstrate EEC and have a molecular interpretation. This can be done by decomposing the formula for the enthalpy into a term resulting from the change of the potential due to the perturbation $\Delta E_p(A \rightarrow B)$ and a term resulting from the potential of the original (unperturbed) system $\Delta E_{up}(A \rightarrow B)$. From eqs 3 and 1 we obtain

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J. Chem. Theory Comput. XXXX, XXX, XXX−XXX

$$\Delta E^0(A \to B) = \Delta E_{up}(A \to B) + \Delta E_p(A \to B)$$
$$\Delta E_{up}(A \to B) = \langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A$$
$$\Delta E_p(A \to B) = \langle U_p(\mathbf{X}^N)\rangle_B \quad (5)$$

This decomposition is useful for quantifying the effect of newly introduced interactions (via perturbation) and for understanding their impact on the compensation. We apply an analogous decomposition to the entropy:

$$\begin{aligned} T\Delta S^0(A \to B) &= \Delta E^0(A \to B) - \Delta G^0(A \to B) \\ &= \langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A + \langle U_p(\mathbf{X}^N)\rangle_B \\ &- \beta^{-1}\ln\langle\exp[\beta U_p(\mathbf{X}^N)]\rangle_B = [\langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A] \\ &- \beta^{-1}\ln\langle\exp[\beta\{U_p(\mathbf{X}^N) - \langle U_p(\mathbf{X}^N)\rangle_B\}]\rangle_B \\ &= T\Delta S_{up}(A \to B) + T\Delta S_p(A \to B) \end{aligned}$$
$$T\Delta S_p(A \to B) = -\beta^{-1}\ln\langle\exp[\beta\{U_p(\mathbf{X}^N) - \langle U_p(\mathbf{X}^N)\rangle_B\}]\rangle_B$$
$$T\Delta S_{up}(A \to B) = \langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A \quad (6)$$

A comparison between eqs 5 and 6 shows that the energetic changes which are related to the unperturbed potential (the original system) $\langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A$ are the same in enthalpy $\Delta E_{up}(A \to B)$ and the entropy $T\Delta S_{up}(A \to B)$:

$$\begin{aligned} \Delta G_{comp}(A \to B) &= \Delta E_{up}(A \to B) = T\Delta S_{up}(A \to B) \\ &= \langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A \end{aligned} \quad (7)$$

Equation 7 reveals the exact compensation of the energy term ΔG_{comp} occurring both as a contribution of the unperturbed potential $U_{up}(\mathbf{X}^N)$ (initial system) to the enthalpy $\Delta E_{up}(A \to B)$ and to the entropy $\Delta S_{up}(A \to B)$ when summed in the free energy ($\Delta G^0 = \Delta H^0 - T\Delta S^0$). This compensation can be interpreted highly insightfully. The contribution $\langle U_{up}(X^N)\rangle_B - \langle U_{up}(X^N)\rangle_A$ accounts for changes in the internal energy of the original system and is related to the conformational changes in the original system. The relation between the compensated energy ΔG_{comp} and the conformational changes can be better explained by obtaining a new equation for the compensated energy ΔG_{comp} starting from the relation between the probability of a given conformation $\boldsymbol{X}^N$ in state A and the partition function $Q(A)$:[27]

$$P_A(\mathbf{X}^N) = \frac{\exp[-\beta U_{up}(\mathbf{X}^N)]}{Q(A)}$$
$$U_{up}(\mathbf{X}^N) = -\beta^{-1}\ln[Q(A)] - \beta^{-1}\ln[P_A(\mathbf{X}^N)] \quad (8)$$

Using eqs 7 and 8, we get:

$$\begin{aligned} \Delta G_{comp}(A \to B) &= \langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A \\ &= \int d\mathbf{X}^N[P_B(\mathbf{X}^N) - P_A(\mathbf{X}^N)]U_{up}(\mathbf{X}^N) \\ &= \int d\mathbf{X}^N[P_B(\mathbf{X}^N) - P_A(\mathbf{X}^N)]\{-\beta^{-1}\ln[Q(A)] \\ &- \beta^{-1}\ln[P_A(\mathbf{X}^N)]\} \end{aligned}$$
$$\Delta G_{comp}(A \to B) = -\beta^{-1}\int d\mathbf{X}^N[P_B(\mathbf{X}^N) - P_A(\mathbf{X}^N)]\ln[P_A(\mathbf{X}^N)] \quad (9)$$

The last equation shows that the sign and the magnitude of the compensated energy are controlled by the conformational changes which are defined by the changes in the probabilities of the conformations $[P_B(\mathbf{X}^N) - P_A(\mathbf{X}^N)]$. The compensation can be interpreted as a conformational relaxation without a direct contribution to the free energy change. The change of the energy related to the conformational changes is termed relaxation of the system: the system relaxes to adapt to the new changes similarly to how the water reorganization is interpreted in hydrophobic interactions.[9] The molecular basis of the enthalpy−entropy compensation associated with the water reorganization upon hydrophobic interaction was previously discussed[11,12] for the solvation of a rigid solute. The above derivation is performed for the general case of free energy changes, and the compensated terms in this general case include the contribution due to the internal energy of the solute in addition to the interaction energy between the water molecules. Therefore, the concept of EEC due to water reorganization during the solvation process of a rigid solute[11,12] can be extended to all types of interactions: the conformational changes in the solute contribute to EEC as does the water reorganization. Equation 7 can be generalized to systems with an arbitrary number of components and still remains correct if we use the internal energy of any component of the unperturbed system instead of the total unperturbed potential. In other words, the contributions to the enthalpy and to the entropy of the unperturbed potential of every component of the system exactly cancel each other. All types of unperturbed interactions can be regarded as conformational changes taking place in different components of the system. Examples include the reorganization of the water molecules in the case of solvating a rigid solute in a solvent,[10−12] conformational changes in any molecule upon solvation, conformational changes in a ligand and a receptor upon binding of the ligand, and the conformational changes in a protein upon mutations.

Another method for computing the compensated energy can be found in analogy to the equation used by Yu and Karplus[12] based on the thermodynamic integration formalism. The thermodynamic integration[28] approach uses a switching variable λ to switch from the canonical ensemble of state A to that of state B by gradually changing $U_p(\mathbf{X}^N; \lambda) = \lambda U_p(\mathbf{X}^N)$ from $(\lambda = 0)$ for state A to $(\lambda = 1)$ in state B via a series of intermediate steps from $\lambda = 0$ to $\lambda = 1$:

$$U_\lambda(\mathbf{X}^N) = U_A(\mathbf{X}^N) + \lambda U_p(\mathbf{X}^N) = U_{up}(\mathbf{X}^N) + U_p(\mathbf{X}^N; \lambda) \quad (10)$$

The total change in the unperturbed energy can be computed via the numerical integration of its derivative:[12]

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J. Chem. Theory Comput. XXXX, XXX, XXX−XXX

$$\frac{\partial}{\partial\lambda}\langle U_{up}(\mathbf{X}^N)\rangle_\lambda = \beta\left\{\langle U_{up}(\mathbf{X}^N)\rangle_\lambda\left\langle\frac{\partial U_\lambda(\mathbf{X}^N)}{\partial\lambda}\right\rangle_\lambda - \left\langle U_{up}(\mathbf{X}^N)\left(\frac{\partial U_\lambda(\mathbf{X}^N)}{\partial\lambda}\right)\right\rangle_\lambda\right\}$$

$$\frac{\partial}{\partial\lambda}\langle U_{up}(\mathbf{X}^N)\rangle_\lambda = \beta\int d\mathbf{X}^N P(X^N;\lambda)U_{up}(\mathbf{X}^N) \times [\langle U_p(\mathbf{X}^N)\rangle_\lambda - \langle U_p(\mathbf{X}^N)\rangle] \quad (11)$$

$$\Delta G_{comp}(A\rightarrow B) = \langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A = \int_0^1\left[\frac{\partial}{\partial\lambda}\langle U_{up}(\mathbf{X}^N)\rangle_\lambda\right]d\lambda = \beta\int_0^1 d\lambda\left\{\langle U_{up}(\mathbf{X}^N)\rangle_\lambda\left\langle\frac{\partial U_\lambda(\mathbf{X}^N)}{\partial\lambda}\right\rangle_\lambda - \left\langle U_{up}(\mathbf{X}^N)\left(\frac{\partial U_\lambda(\mathbf{X}^N)}{\partial\lambda}\right)\right\rangle_\lambda\right\} \quad (12)$$

where the average $\langle\cdots\rangle_\lambda$ is taken over the canonical ensemble sampled at a fixed λ value using the partially coupled potential $U_\lambda(\mathbf{X}^N)$. A computation using this equation is expected to be less accurate than the computation of the direct difference $\langle U_{up}(\mathbf{X}^N)\rangle_B - \langle U_{up}(\mathbf{X}^N)\rangle_A$ in eq 7 due to the propagation of the errors from the intermediated states and the additional errors in the computation of $\partial U_\lambda(\mathbf{X}^N)/\partial\lambda$, in contrast to the error in the computation of $\langle U_{up}(\mathbf{X}^N)\rangle$ being the only source of the error in the direct difference.

It was also shown that the entropic changes ΔS_p originating from the perturbed potential can be computed using thermodynamic integration analogously to eq 12:[12]

$$\Delta S_p(A\rightarrow B) = \frac{1}{kT^2}\int_0^1 d\lambda\left\{\langle U_p(\mathbf{X}^N;\lambda)\rangle_\lambda\left\langle\frac{\partial U_\lambda(\mathbf{X}^N)}{\partial\lambda}\right\rangle_\lambda - \left\langle U_p(\mathbf{X}^N;\lambda)\frac{\partial U_\lambda(\mathbf{X}^N)}{\partial\lambda}\right\rangle_\lambda\right\} \quad (13)$$

The term on the right side of eq 13 can be shown to be related to the fluctuation of U_p by using eq 10:

$$\Delta S_p(A\rightarrow B) = \frac{1}{kT^2}\int_0^1\lambda\, d\lambda\{[\langle U_p(\mathbf{X}^N)\rangle_\lambda]^2 - \langle[U_p(\mathbf{X}^N)]^2\rangle_\lambda\} = -\frac{1}{kT^2}\int_0^1\lambda\, d\lambda\{\langle[U_p(\mathbf{X}^N)]^2\rangle_\lambda - [\langle U_p(\mathbf{X}^N)\rangle_\lambda]^2\} \le 0 \quad (14)$$

This is the same equation as presented by Frenkel and Smit (p 171),[29] where ΔS_p is actually the second derivative of ΔG^0.

It is worth mentioning that the sign of ΔS_p is defined to be nonpositive. The sign of the entropic change from the unperturbed potential ΔS_{up}, on the other hand, is difficult to predict (see eqs 9, 11, and 12). ΔS_{up} can either increase or decrease as we will show below in the examples from simulations.

COMPUTATION OF EEC RELATED TO CONFORMATIONAL CHANGES

Now, we consider examples of the EEC due to conformational changes of the solute upon its solvation. We performed computational studies of solvating several HIV-1 protease inhibitors to show the magnitude of EEC due to conformational changes in these biomolecules. We consider the solvation process of a biomolecule in N water molecules. The total potential energy of this system can be written explicitly:

$$U(\mathbf{X}^N) = U_w(\mathbf{X}^N) + U_s(\mathbf{P}_s) + B_{sw}(\mathbf{X}^N,\mathbf{P}_s) \quad (15)$$

$U_w(\mathbf{X}^N)$ is the interaction energy between the water molecules. $U_s(\mathbf{P}_s)$ is the internal potential energy of the solute which is dependent on its conformation $\mathbf{P}_s$. Here

$$U_{up}(\mathbf{X}^N,\mathbf{P}_s) = U_w(\mathbf{X}^N) + U_s(\mathbf{P}_s) \quad (16)$$

$B_{sw}(\mathbf{X}^N,\mathbf{P}_s) = U_p(\mathbf{X}^N,\mathbf{P}_s)$ is the change of the potential due to the perturbation and, in this case, represents the interactions between the water molecules and the solute. The solvation process is defined as the transfer of a molecule from a fixed position in an ideal gas phase (state A ; $B_{sw} = U_p = 0$) into a fixed position in the liquid phase (state B) where the interactions between the solute and the solvent (perturbation) $B_{sw}(\mathbf{X}^N,\mathbf{P}_s) = U_p(\mathbf{X}^N,\mathbf{P}_s)$ are turned on.[30] The compensated energy includes both the contributions from the reorganization of the solvent molecules ΔG_{complw} and contributions due to the conformational changes inside the solute ΔG_{compls}:

$$\Delta G_{complw} = \langle U_w(\mathbf{X}^N)\rangle_{sol} - \langle U_w(\mathbf{X}^N)\rangle_{gas} \quad (17)$$

$$\Delta G_{compls} = \langle U_s(\mathbf{P}_s)\rangle_{sol} - \langle U_s(\mathbf{P}_s)\rangle_{gas} \quad (18)$$

The average $\langle\cdots\rangle_{gas}$ is calculated over the canonical ensemble of the solute in the gas phase and the pure water, and $\langle\cdots\rangle_{sol}$ is calculated over the canonical distribution of the solute in water. The corresponding thermodynamic integration equation for computing the compensated energy inside the solute is

$$\Delta G^{TI}_{compls} = \beta\int_0^1\left\{\langle U_s(\mathbf{P}_s)\rangle_\lambda\left\langle\frac{\partial U_\lambda(\mathbf{X}^N,\mathbf{P}_s)}{\partial\lambda}\right\rangle_\lambda - \left\langle U_s(\mathbf{P}_s)\frac{\partial U_\lambda(\mathbf{X}^N,\mathbf{P}_s)}{\partial\lambda}\right\rangle_\lambda\right\}d\lambda \quad (19)$$

The changes in the compensated energy in the solute are computed using the direct difference method (eq 18) and the thermodynamic integration method (eq 19) separately. We chose to study the solvation of six HIV-1 protease inhibitors: Tipranavir, Darunavir, KNI-10075, KNI-10033, Amprenavir, and Saquinavir (see Figure 1 and the details of the simulations). These molecules are relatively small, so that their internal energies U_s fluctuate moderately (3−6 kcal/mol) in comparison to the fluctuation in U_w of about 60 kcal/mol. Therefore, we can expect to achieve reasonable accuracy for the relaxation of the internal energy and entropy of the solute according to eq 18. The accuracy in the calculation of both terms in eq 18 is dependent on U_s and its fluctuations, which can incur substantial error when both of them are large. The error in

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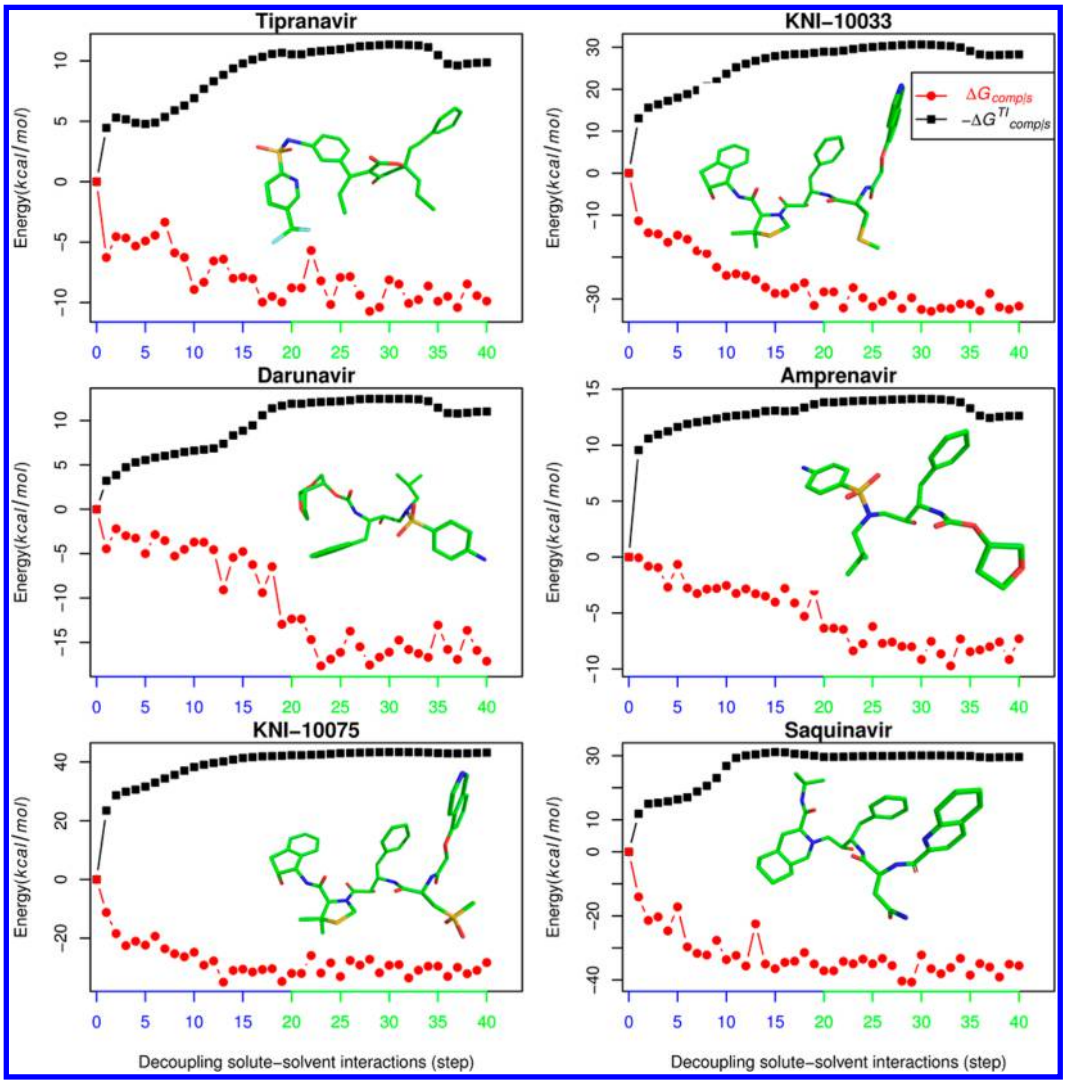

Figure 1. EEC due to the structural changes in the solute upon desolvation. The changes in the internal energy and entropy of the six HIV-1 protease inhibitors are monitored while decoupling the interactions between the solute and the solvent. The decoupling of the Coulomb interactions was performed in the first 20 windows followed by another 20 windows for the decoupling of the van-der-Waals interactions. The total changes in the compensated energy computed by the thermodynamic integration ($-\Delta G^{TI}_{compls}$) or by the direct differences of the solute internal energy to the initial state $\Delta G_{compls} = \langle U_s(\mathbf{P}_s)\rangle_\lambda - \langle U_s(\mathbf{P}_s)\rangle_{\lambda=0}$ are colored black and red, respectively. The final results obtained from the 40 steps are reported in Table 1. The two ways of computing the compensated energy are plotted in opposite directions to ease the comparison of the results. A stick representation of each molecule is included in the corresponding diagram.

the computed values via the thermodynamic integration in eq 19 depends on both $U_\lambda(\mathbf{X}^N, \mathbf{P}_s)$ and $U_s(\mathbf{P}_s)$.

The computation of the compensated energies was performed using simulations of the intermediate states along the pathway of turning off (decoupling) the interactions between the solute and the solvent (desolvation). The results (Table 1 and Figure 1) illustrate the magnitude of the compensation between the enthalpy and the entropy due to the conformational changes in the biomolecules. In 5 out of 6 studied systems, we observed quantitative agreement between the values from the direct difference method and those of the thermodynamic integration method. Only one system (KNI-10075) does not show quantitative agreement within the simulated time interval. This may have resulted from poor convergence of the computation based on the thermodynamic integration eq 19 which is known to converge about 2 orders of magnitude more slowly than free energies when it is used to compute the total entropy.[31,32] The errors in the computations are computed by taking the standard deviation of five calculations after breaking the total simulation time into five equal-size windows. The error in the direct computation (eq 18) of the relaxation energy is mostly less than 5 kcal/mol and is related to the errors in the computation of the internal energies of the solute at the initial and the final steps (see Table 1). The errors in the computation of the relaxation energy via the thermodynamic integration (eq 19) vary between 3−30 kcal/mol throughout the decoupling pathway. These large numbers can be explained by the propagation of the errors of the two highly fluctuating quantities—the internal energy and the derivative of the potential energy. Therefore, the calculation based on the thermodynamic integration (eq 19) is not expected to converge quickly in comparison with the direct computation method (eq 18). A similar convergence problem was reported by Smith and van Gunsteren when they computed the total entropy using an equation similar to eq 19.[31] The most important result from studying these systems is the fact

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that even a small molecule can produce a large contribution to EEC (10−30 kcal/mol) in a simple process like solvation (see Table 1).

Two additional examples of EEC due to the conformational changes in biomolecules are presented in Figure 2. These examples exemplify energy compensation due to the conformational changes in a protein when its bound ligand is modified. The compensated energy due to the conformation changes in the wild-type HIV-1 protease was computed along 20 steps of modifying the electrostatic interactions of two ligands (Tipranavir and Darunavir). The modifications of the ligands are performed via the gradual decoupling of the electrostatic interactions of the ligand with the rest of the system while keeping the van-der-Waals interactions unchanged. The changes in the compensated energy and the corresponding changes in the free energy are presented for every step of the transformation. Although the changes in the ligand at each step are considered relatively simple, the changes of the compensated energies are shown to be highly variable in magnitude and direction. The corresponding changes in the free energy are considerably smaller and do not exhibit noticeable fluctuations. The changes of the sign and the magnitude of the compensated energy as presented in Figure 2 represent the difficulties in predicting the compensated energy as was pointed out above (see eqs 9, 11, and 12). It is important to notice that while ΔS_p is always nonpositive (see eq 14), ΔS_{up} can increase or decrease which in turn masks the contribution of ΔS_p to the free energy. Therefore, the large differences in the compensated energies upon perturbing the same system highlight the difficulties in the interpretation of the thermodynamic changes upon ligand modification where the noisy changes in the compensated energy may hide the changes which are related to the free energy and the affinity.

DISCUSSION

In this work, we have shown how the interpretation of previous theoretical findings related to the EEC due to the reorganization of water molecules can be extended to draw a general molecular basis for the relation between changes in the enthalpy and entropy and to establish the molecular basis of the well-known phenomenon of enthalpy−entropy compensation (EEC). We showed that EEC results from exactly canceling energetic changes due to the conformational changes in the solute, on the one hand, and reorganization of the solvent, on the other hand. Examples were presented that quantify the contributions of the conformational changes to EEC upon solvation of six different HIV-1 protease inhibitors, using extensive molecular dynamics simulations. The computation showed that the conformational changes of these relatively small molecules lead to significant entropic and enthalpic changes in the solute of 10−30 kcal·mol^{-1} in magnitude. Two additional examples were presented in which the EEC due to the conformational changes in wild-type HIV-1 protease can be large and variable in direction and magnitude upon only slight modification in the ligand.

Table 1. Magnitude of the EEC between Internal Enthalpic and Entropic Changes in the Solute Due to the Conformational Changes upon Decoupling of the Solvent−Solute Interactions

molecule	ΔG^{TI}_{compls} (kcal·mol^{-1})	ΔG_{compls} (kcal·mol^{-1})[a]
Tipranavir	−10.07	−9.45 ± 4.35
Darunavir	−10.82	−14.64 ± 4.34
KNI-10033	−28.43	−30.24 ± 3.16
KNI-10075	−42.24	−32.43 ± 7.88
Amprenavir	−12.62	−9.06 ± 3.15
Saquinavir	−29.65	−33.51 ± 2.67

[a]The values represent the average ± error.

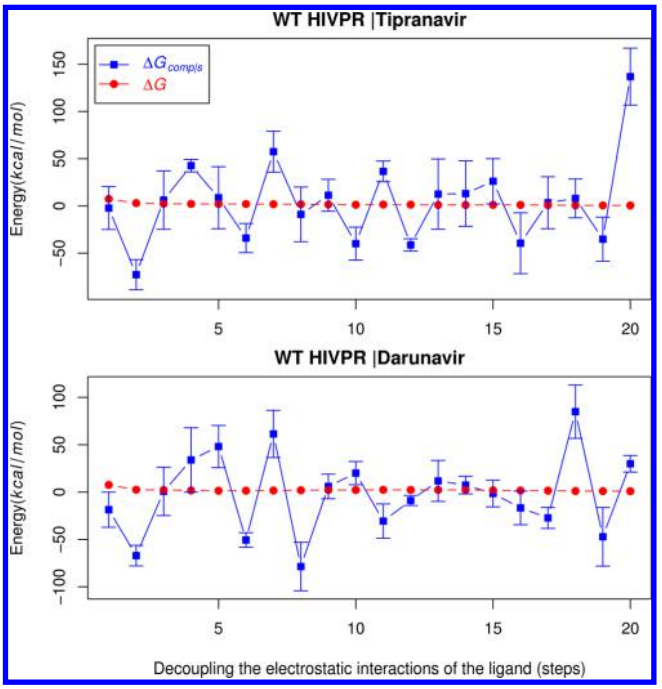

Figure 2. EEC due to conformational changes in wild-type HIV-1 protease upon changing the bound ligand. The changes in compensated energy and the corresponding changes in free energy are displayed for every step in the process of turning off the electrostatic interaction of the ligands.

Our observations are important in context of the common usage and interpretation of thermodynamic data (entropy and enthalpy) in thermodynamically guided lead optimization[19,33−35] and in protein stability analysis.[36] The large entropic changes observed upon the bimolecular binding processes are usually interpreted as the fingerprints of hydrophobic interactions.[19,33−35] However, the contributions of both ΔS_{up} and ΔS_p to the total entropy (eq 6) make it difficult to interpret the total entropic changes because one does not know whether they are related to EEC (ΔS_{up}) or to the change of free energy (ΔS_p). We emphasize that the reorganization of the water network around the ligand and in the binding pocket should be interpreted carefully to be able to distinguish EEC from the actual energetic contribution to the free energy. Also, we argue that in the literature the traditional concept of hydrophobic effect is often confused with EEC, so that the reorganization of the "ordered" water molecules around a hydrophobic solute is incorrectly assumed to be the driving force for hydrophobic interactions. Some typical misconceptions of this field are discussed in ref 15. The difficulties in the interpretation of the thermodynamic changes were highlighted in the recent review of EEC by Chodera and Mobley.[5] The interplay between enthalpy and entropy in different conformations of the protein bovine pancreatic trypsin inhibitor was also observed by Fenley and Gilson,[37] which led them to conclude that the measured enthalpy and entropy in protein folding are unreliable indicators of the actual driving

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J. Chem. Theory Comput. XXXX, XXX, XXX−XXX

forces.[37] The concept of EEC obtained from the present work shows that the EEC always takes place in all types of biochemical changes.

The analysis and discussion above manifest exact compensation between the enthalpy and the entropy due to the conformational changes in the original system (unperturbed part). Thus, the conformational changes do not directly contribute to the free energy and the energetic changes in the original system (unperturbed) are exactly compensated. The compensation between the total changes in the enthalpy and the entropy are known to vary widely.[3] In his analysis of the thermodynamic data of ligands binding to 32 diverse proteins, Olsson observed that EEC is significant and diverse in magnitude, where substantial compensation was observed in 22% of the cases.[3] These variations in the observed compensation can be explained by the additional partial compensation between the contributions from the perturbed part of the potential ΔE_p and $T\Delta S_p$, which define the change in the free energy. The free energy changes are related to the contribution from the enthalpy and the entropy due to the perturbation as can be shown using 5, 6, and 7:

$$\Delta G^0(A \rightarrow B) = \Delta E_p(A \rightarrow B) - T\Delta S_p(A \rightarrow B) \quad (20)$$

The compensation between these two contributions can be shown to be due to the conformational changes if we notice that the free energy change as given by the perturbation formula 2 $\Delta G^0(A \rightarrow B) = -kT \ln\langle \exp[-\beta U_p(\mathbf{X}^N)]\rangle_A$ is related to the probability of each conformation in the initial state A. The change of enthalpy due to the perturbation, on the other hand, is related to the probability of the conformations in the final state B: $\Delta E_p(A \rightarrow B) = \langle U_p(\mathbf{X}^N)\rangle_B$. The conformational changes that are defined by the changes in the probabilities of the conformations take place when an unfavorable conformation in the initial state is more favorable for the interaction in the final state. Therefore, the conformational changes contribute to the change of enthalpy due to the perturbation (ΔE_p) by improving the probability of the favorably interacting conformations. Increasing the probability of a certain conformation due to the favorable interactions from the perturbation improves its contribution to $\Delta E_p = \langle U_p\rangle_B$ (see eq 5), but this does not necessarily improve its contribution to the free energy changes $\Delta G^0 = -kT \ln\langle \exp[-\beta U_p]\rangle_A$ (see eq 2) which is dependent on the probability in the initial state. Rather, the changes in the enthalpy are compensated for in the free energy changes. The compensation between the changes in enthalpy and entropy due to the perturbation is not total, as in the case of the unperturbed potential, and is related to the difference in the probabilities of the conformations between the initial and the final state (see Figure 3).

Finally, the presented new insight that the general EEC affords interpretation of the relation between the enthalpy and entropy on the molecular scale is of crucial importance in many fields. The observation of the very large ECC is tightly connected to understanding the resistance of HIV-1 protease inhibitors[38] and the amino-acid conformational preferences in the unfolded peptides.[39] It is also interesting to notice that the free energy perturbation formula avoids the computation of relaxation terms and affords direct computation of the free energy. This is beneficial for the practical aspects of computation, because including the relaxation terms as it is usually performed in the end-points free energy calculation methods[40] introduces a large error due to the largely fluctuating

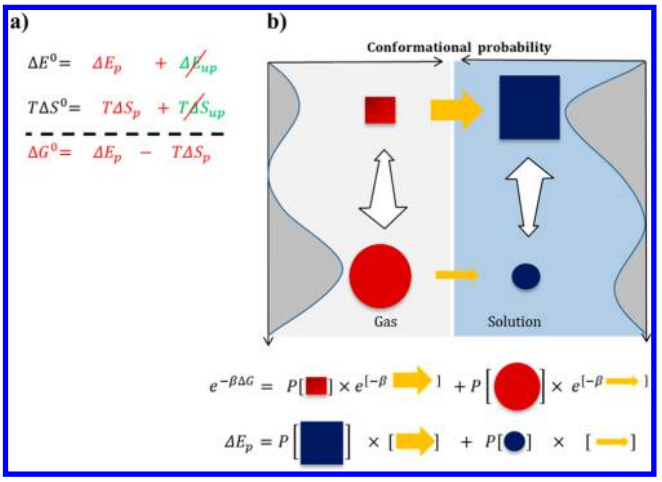

Figure 3. Schematic representation of the origin of enthalpy−entropy compensation due to the conformational changes upon the solvation (perturbation) of a flexible molecule. (a) The complete compensation (relaxation) between the enthalpic changes due to the change in the internal energy of the molecule (unperturbed) ΔE_{up} and the corresponding entropic changes $T\Delta S_{up}$ eq 7. (b) The compensation of the enthalpic changes due to perturbation (solvation): The conformation ● is the dominating conformation in the gas phase. The conformational changes induced by the interactions (arrow) upon the solvation result in the conformation ■ becoming the dominating conformation in solution. The change of the probability of the conformation ■ implies that this conformation has a favorable interaction (enthalpy) in the final state (solution), and at the same time, a lower probability in the initial state (gas) which in turn reduces the contribution of this conformation to the free energy changes.

total potential. Since the compensated energetic terms cancel in the free energy of the system, including them in the free energy calculation using the end-point methods is a possible source of errors.

■ SIMULATION DETAILS

The compensating energy terms were calculated from simulations along the pathway of decoupling the interactions between the solute and solvent using the Gromacs 4.6.5 simulation package.[41] The starting structures of Tipranavir, Darunavir, KNI-10075, KNI-10033, Amprenavir, and Saquinavir were extracted from the crystal structures of their complexes with HIV-1 protease (RCSB Protein Data Bank[42] codes: 1D4Y, 1T3R, 2PK5, 2PK6, 3EKP, and 3EKQ, respectively; see Figure 1). The general AMBER force field[43] was used for the solutes. The force field parameters and AM1-BCC partial charges were obtained by using Antechamber.[44] The TIP3P water model[45] was used for the solvent. Periodic boundary conditions were used and the solutes were solvated in a rectangular box such that water extended at least 1.4 nm beyond the solute surface. 500 steps of steepest-descent energy minimization followed by 1000 steps of conjugate gradient optimization were used to energetically minimize each system. The systems were equilibrated using a 100 ps molecular dynamics simulation with harmonic position restraints using a force constant of 1000 $kJ{\cdot}mol^{-1}{\cdot}nm^{-2}$ for the heavy atoms in the solutes for each system. The production simulations were performed using a leapfrog stochastic dynamics integrator.[46] The isothermal−isobaric ensemble (1 atm and 300 K) was used by maintaining the pressure through a Berendsen pressure bath.[47] Long-range electrostatic interactions were computed by the particle-mesh

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J. Chem. Theory Comput. XXXX, XXX, XXX−XXX

Ewald method.[48] van-der-Waals interactions and short-range electrostatic interactions were computed within a 1.2 nm cutoff. A time step of 2.0 fs was used. The decoupling was performed in 20 steps for the Coulomb interactions followed by 20 steps for the decoupling of the Lennard-Jones interactions. Each simulation was performed for 25 ns in the simulations of solvating the small molecules. The derivative of the Hamiltonian and the energies were collected every 25 steps. The first 250 ps were not used for the analysis. The compensated energy due to the conformational changes in the solute were computed using both of the direct difference and the thermodynamic integration methods separately as given in eqs 18 and 19. The average values of the internal energy of the biomolecules $\langle U_s(\mathbf{P}_s)\rangle_\lambda$ were computed over the trajectories and the differences to the initial state $\langle U_s(\mathbf{P}_s)\rangle_\lambda - \langle U_s(\mathbf{P}_s)\rangle_{\lambda=0}$ were computed. The computation of the compensated energy using the thermodynamic integration method was performed by numerical integration using the trapezoidal rule.[49] The errors in the direct difference method were computed by breaking the total simulation time into five windows and taking the standard deviation of the resulting values. The computations of the EEC due to the conformational changes in the wild-type HIV-1 protease were performed using simulations of 10 ns for each of the 20 steps of decoupling the electrostatic interactions of the ligands with the rest of the system. The compensated energies are computed using the direct difference method (eq 18). The free energy changes are computed using the Bennett Acceptance Ratio method using the Gromacs g_bar tool.[41]

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Author Contributions

M.A. performed the experiments and analyzed the results. M.A., T.L., and O.V.K. designed the experiments and analyzed the results. M.A. and V.H. formulated the theoretical derivations. All authors contributed to writing the manuscript and approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

EEC = enthalpy−entropy compensation
MD = molecular dynamics
HIV = human immunodeficiency virus
CCs = conformational changes

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