Empirical Entropic Contributions in Computational Docking: Evaluation in APS Reductase Complexes

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Abstract: The results from reiterated docking experiments may be used to evaluate an empirical vibrational entropy of binding in ligand–protein complexes. We have tested several methods for evaluating the vibrational contribution to binding of 22 nucleotide analogues to the enzyme APS reductase. These include two cluster size methods that measure the probability of finding a particular conformation, a method that estimates the extent of the local energetic well by looking at the scatter of conformations within clustered results, and an RMSD-based method that uses the overall scatter and clustering of all conformations. We have also directly characterized the local energy landscape by randomly sampling around docked conformations. The simple cluster size method shows the best performance, improving the identification of correct conformations in multiple docking experiments.

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Introduction

The AutoDock3 and AutoDock4 empirical free energy force fields have been calibrated against a set of several hundred ligand–protein complexes of known structure and binding constants.[1,2] In our experience, this force field has been effective for the prediction of binding constants with tight-binding complexes, but we have noticed two significant problems.

First, we often find an incorrect conformation with slightly more favorable energy than the experimentally observed conformation. However, these incorrect conformations are found with very low frequency when multiple docking experiments are performed: incorrect, low-energy conformations will be found in ~1% of docking experiments, and the correct conformation will be found in 25–100% of the experiments. Thus, in these cases, a simple procedure that chooses the conformation of best energy from a set of multiple docking experiments will yield an incorrect conformation.

Second, the current force field poorly predicts the free energy of binding of weakly interacting molecules. An example from APS reductase (adenosine 5′-phosphosulfate reductase), the subject of this report, highlights the problem. Experimentally, 5′-AMP binds tightly but 3′-AMP, which has a similar number of atoms and functional groups, binds weakly. However, in Auto-Dock both are predicted to bind tightly with similar binding constants. However, by looking at the frequency that a given conformation is found in reiterated docking experiments, a difference may be seen, as shown in Figure 1. When these compounds are docked multiple times, AutoDock finds a consistent conformation for 5′-AMP in many docking experiments, whereas 3′-AMP adopts many different conformations and the low-energy conformations of 3′-AMP are only found in a small fraction of docking experiments.

We have observed this many times in other systems: if a given molecule shows a consistent conformation in many docking simulations, we have far more confidence in the result. Our current hypothesis is that the frequency of finding a given conformation is providing information on the energy landscape of binding, and that a high frequency is a measure of favorable entropy in the binding process. Recent work has shown that the energetic contribution of this vibrational entropy will be high. A recent study by Chang et al.[4] has estimated that the configurational entropy of
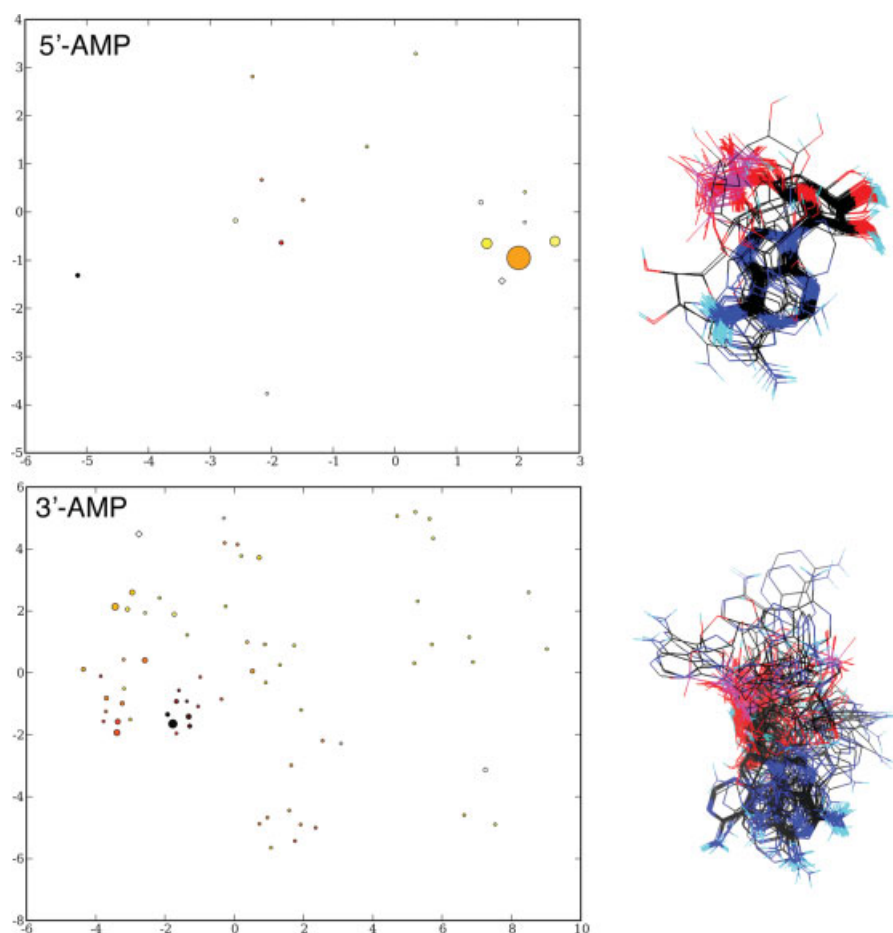

Figure 1. Clusters analysis of docking for 5′-AMP and 3′-AMP. The graphs on the left use Sammon mapping[3] to preserve the approximate separation in conformational space between clusters. Each circle represents a cluster of conformations within 2 Å RMSD of each other, and the size of the circle is proportional to the number of conformations in the cluster. The expected bound conformation is shown with a diamond. The images on the right show all of the docked conformations. 5′-AMP binds tightly, and many of the docked conformations cluster into one large group at the expected conformation. 3′-AMP, however, binds weakly and shows a wide scattering of small clusters.

binding of amprenavir to HIV-1 protease is 26.4 kcal/mol, of which 1.8 kcal/mol is due to the loss of conformational entropy when the molecule moves from freely flexible in solution to its constrained position in the active site, and the bulk of the penalty is due to loss of vibrational entropy in the restrictive binding site.

Ideally, we would like to quantify the binding energy of the entire range of conformations available to the ligand and protein, and use this explicitly to evaluate the vibrational entropy. However, these types of calculations, such as the Mining Minima calculation employed by Chang et al., are too computationally expensive for typical docking studies. Instead, several laboratories are exploring methods for using information from the docking simulation or from inexpensive approximations of the range of conformations to evaluate this entropic component.

Many of these methods perform multiple docking experiments, cluster the resulting conformations by similarity, and then use a measure of the cluster size to estimate the vibrational

entropy. The assumption is that the docking protocol provides information on the characteristics of the local energy landscape, and that large clusters of conformations are indicative of favorable entropic characteristics of this landscape. In previous experiments using AutoDock, we have used a cluster size measure to discriminate binders from nonbinders in an artificial active site.[5] Bottegoni et al.[6] have explored several clustering methods combined with a number of popular docking methods to identify significantly populated clusters, showing that they tend to be associated with the observed binding mode. Ruvinsky and Kozintsev[7] have used the size of clusters in AutoDock to approximate a vibrational entropy, and, in a later work,[8] to estimate a probability distribution function of conformations from multiple docking experiments. Xiang et al.[9] have used RMSD values between different conformations from a genetic algorithm search to approximate this entropic contribution in protein loop prediction.

In this report, we evaluate these methods for their ability to predict correctly the expected bound conformations of nucleotide analogues in APS reductase (Carroll, K. S. Manuscript in preparation).[10,11] All of these methods seek to characterize the local energy landscape and use this information to estimate an entropic contribution to the binding free energy. The methods perform a sparse sampling of the landscape by reiterated docking or random sampling, making the implicit assumption that the sampled points will represent the features of the entire local landscape. We have found that APS reductase is an excellent test for these methods because experimental binding constants are available for a series of compounds of similar size and chemical composition but with a wide range of binding constants. This provides a more critical test set than the typical databases used in most studies, which typically include a diverse collection of ligand–protein complexes, but all are specific, tight-binding complexes.

Methods

Motivation

In the most general case, we seek to evaluate the vibrational contribution to the free energy of binding through use of a configurational integral[7]:

$$\Delta G = T \ln\left(\frac{\sigma_l \sigma_p}{\sigma_{pl}} \frac{c_0 N_a}{8\pi^2 (2\pi)^{ntor}} V_B\right) \quad (1)$$

where the configurational integral V_B is

$$V_B = \int_\Gamma \exp\left[-(U_{pl}(\mathbf{r}, \Omega) - E_{pl})/RT\right] d\mathbf{r}\, d\Omega \quad (2)$$

In these equations, the σ terms account for any symmetry in the molecules, with values of 1 for asymmetric molecules, $c_0 = 1$ mol/L, N_a is Avogadro's constant, ntor is the number of torsional degrees of freedom in the ligand, $U_{pl}(\mathbf{r}, \Omega)$ is the energy of each complex conformation, Γ is the region of integration (typically a small space that includes conformations with similar binding modes), and E_{pl} is the minimum energy of the complexes within the region Γ. The vectors $\mathbf{r}$ and Ω define the three translational and the $3 + $ ntor rotational motions of each complex. As noted in the Ruvinsky presentation, these calculations are appropriate for relative protein–ligand motions.

In this manuscript, we test several simple approximations to this integral, based on conformations obtained in reiterated AutoDock docking experiments and by directly sampling the local energy landscape. Our goal is to provide an efficient empirical method for estimating this entropic contribution. We seek to improve the estimation of binding constants by rescoring trial docked conformations, combining this estimated vibrational entropy, which is derived from reiterated docking experiments, with predicted enthalpic and desolvation contributions used during the docking simulation of each conformation.

In all of these methods, we begin with a set of conformations obtained from docking simulation or from random sampling, and

we assume that these sparse samples may be used to characterize the entire local energy landscape. It is important to keep in mind that the evolutionary search method used in AutoDock, which combines a genetic algorithm with a local search,[1] is not designed to be a uniform (Monte Carlo) sampling process, but instead to be successful at finding extreme (minimum) values of the energy function. Thus, it is not directly giving the information needed to estimate the configurational integral, but may be used to infer properties of the energy landscape and vibrational entropies. Note also that: (1) the method is heuristic and stochastic, and thus does not guarantee convergence, and so the search must be repeated multiple, statistically independent times; and (2) it generates a history of the search process as a byproduct. Both of these properties provide opportunities and limitations for use in estimation of entropic contributions, and help to motivate our random sampling experiments, described later.

Method of Ruvinsky and Kozintsev

Ruvinsky and Kozintsev have reported a method for using the results from multiple docking experiments to evaluate a vibrational entropy.[7] We will refer to this as the "RK" method. They begin by clustering the docked conformations, and then they evaluate the entropy based on the conformational space spanned within the cluster. They choose the lowest energy conformation in each cluster as the representative position, and assume that the members of the cluster provide a representative snapshot of the motions available to representative position. For each cluster i, they estimate this configurational integral $V_B(r_i, \Omega_i)$ by evaluating the maximum and minimum values in each of the dimensions of r and Ω, and then calculating the product of these maximum-to-minimum intervals:

$$V_B(r_i, \Omega_i) \cong V_{RK}(r_i, \Omega_i) = \prod_{j=1}^{3} \left(\max(r_i^j) - \min(r_i^j)\right)$$
$$\times \prod_{k=1}^{3+Ntor} \left(\max(\Omega_i^k) - \min(\Omega_i^k)\right) \quad (3)$$

Rather than explicitly incorporating a dependence on inter-sample distances, as in the Colony method or the weighted RMSD method described later, the RK method is evaluated within 1 or 2 Å clusters.

This method has one potential conceptual limitation: in cases where the number of conformations is small, the value of V_B may be more an indication of the sample size than the extent of the favorable conformational space. We performed a random value test (see Fig. 2) to determine the possible influence of the small number of conformations in a typical cluster on the value of this volume. It was performed by picking random values for the max and min values of each conformational variable within a constant volume of conformational space. For the experiment, random values were chosen within uniform bounds of -1.29 to 1.29 (Å or rad) for 12 conformational variables (3 translation, 3 rotation, and 6 torsion). During analysis of our docked conformations, a value of roughly $\Delta G_{RK} = 12$ kcal/mol was found for the largest clusters, and so the value of 1.29 was chosen to provide a value of 12 kcal/mol for random distributions with many
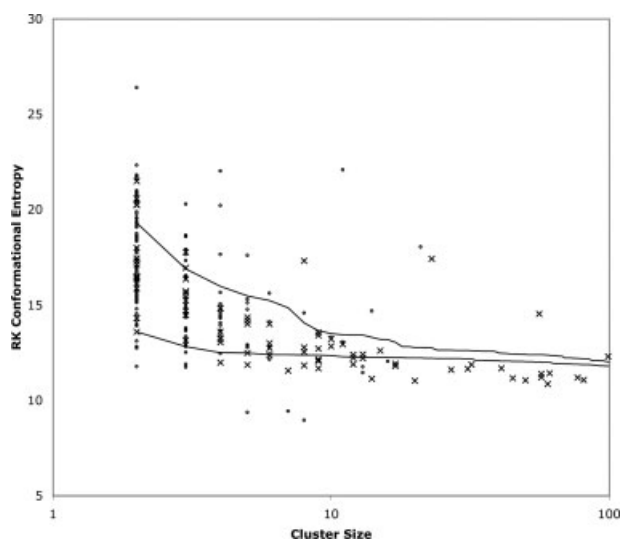
Figure 2. Analysis of RK vibrational entropy results. Each point represents one cluster of docked conformations, with X for clusters within 2 Å RMSD of the expected conformation, and a dot for conformations docked incorrectly. Results for all 22 compounds docked to APS reductase are included. The lines show the upper and lower values obtained for a random sample of points distributed within a small constant volume. The random samples account for much of the range seen in the RK analysis of the docking results.

points that maximally fill the entire available conformational volume.

Colony Method

The "Colony" method[9] uses a RMSD-dependent term to evaluate a conformational free energy. The method was developed for use in protein loop prediction. The authors generated a set of 2000 random backbone conformations, added sidechains using a rotamer approach, performed an energy minimization, and then retained the 1000 lowest energy conformations for use in the free energy estimation. Here, we modify the approach for use with ligand–protein complexes, using 100 conformations from reiterated AutoDock simulations. The energy is based on the RMSD from each conformation to all other conformations obtained in the simulation:

$$\Delta G_i = -RT \ln \left[\sum_j \exp(-\Delta E_j / RT - d_{ij}^3 / 6L) \right] + C \qquad (4)$$

where ΔE values are the predicted energies of each conformation j, d_{ij} is the RMSD between the two conformations, L is number of amino acids in the peptide loops used in the original study and $6L$ Å^3 is an empirical factor defining the approximate volume of conformational space represented by each loop conformation, and C is a constant term that defines the magnitude of the entire conformational space. For use in rescoring multiple conformations of a single ligand, the constant term may be

neglected. For this work, we used a value $L = 2$ because these compounds have a similar number of torsional degrees of freedom as a dipeptide linker.

Cluster Size Method

We have tested two methods of using the cluster size as an estimate of the configurational integral. In these methods, we hypothesize that the probability of finding a conformation in a given cluster is capturing information on the local energy landscape. As mentioned earlier, this hypothesis relies on the properties of Lamarkian genetic algorithm used in AutoDock for searching of conformations, which is a stochastic and heuristic method designed to find extreme minimum values of the complex energy landscape. Our hypothesis is that the docking method is more successful for wide energetic wells, and thus the success of finding a given conformation is proportional to the vibrational entropy.

The first is a probability based on a simple conformation-centered RMSD, which we will refer to as the "RMSD" method. For each conformation i, RMSD values $d_{i,j}$ is calculated over all conformations j not equal to i, and the fraction less than a given threshold d_{max} is evaluated. In this work, we used a threshold of $d_{max} = 2$ Å RMSD.

$$P_i^{RMSD} = \frac{N_{d_{j \neq i} \leq d_{max}}}{N} \qquad (5)$$

where the numerator is the number of conformations with RMSD less than the threshold and N is the total number of conformations. The second is a probability based on a distance-weighted RMSD, which we will refer to as the "wRMSD" method:

$$P_i^{wRMSD} = \frac{\sum_{j \neq i} \exp(-d_{i,j}^2 / 2\sigma^2)}{N} \qquad (6)$$

where the constant $\sigma = 2$ Å. If we assume that the favorable region of conformational space is proportional to these probabilities, then the vibrational contribution to the free energy may be estimated as

$$\Delta G_i = -W^{RMSD} RT \ln(P_i^{RMSD}) \qquad (7)$$

where W^{RMSD} is an empirically determined weight.

Random Sampling Method

We also estimated a value of the configurational integral based on a random sampling of the local energy landscape around each docked conformation. As noted by one reviewer, this method has much in common with the MINTA[12] and Mining Minima[13] methods. One hundred thousand conformations were generated with small random displacements from the docked conformation. Translational displacements were chosen from a random distribution with bounds −0.5 to 0.5 Å, rotational dis-

placements were generated by picking a random axis and rotating by a random angular displacement with bounds −0.5 to 0.5 rad, and torsional displacements were generated with a random angular displacement with bounds of −0.5 to 0.5 rad.

The configurational integral was calculated as

$$\tilde{V}_{\mathrm{B}}(r_i, \Omega_i) = \frac{\sum_j \exp\big((\Delta E(r_j, \Omega_j) - \Delta E(r_i, \Omega_i))/RT\big)}{N} \quad (8)$$

where the ΔE values are predicted energies from AutoDock and the summation is performed over the $N = 100{,}000$ samples j around the conformation of minimum energy i. The vibrational contribution to the free energy is then calculated as in eq. (1).

Binding Constants for Ligands with APS Reductase

Binding constants are available for 22 nucleotide analogues bound to APS reductase (Table 1). Values of K_i were determined under single turnover conditions from the dependence of the observed rate constant (k_{obs}) at a given inhibitor concentration under conditions of subsaturating APS, such that K_i is equal to the K_{d}.[10,14] Kinetic data were nonlinear-least squares fit to a model of competitive inhibition. Each K_{d} reflects the average of at least two independent experiments, and the standard deviation was less than 10% of the value of the mean. The synthesis, characterization, and biochemical analysis of the analogues used in this computational study will be reported elsewhere (Carroll, K. S. Manuscript in preparation).

Docking with AutoDock4

Docked conformations and predicted free energies of association were obtained for 22 nucleotide analogues using AutoDock4 (http://autodock.scripps.edu). Coordinates for APS reductase were obtained from the study by Stout and coworkers prior to release—they are identical with subunit B in entry 2goy at the Protein Data Bank.[11] Coordinates for the enzyme were processed in AutoDockTools by adding all hydrogen atoms, assigning charges with the Gasteiger method,[2,15] and merging nonpolar hydrogen atoms. Coordinates for the nucleotides were constructed in InsightII starting with the conformation of the APS nucleotide bound at subunit B in the crystallographic structure. Charges were assigned in ADT and nonpolar hydrogen atoms merged. Docking experiments were then performed in AutoDock4 using the default docking parameters, with 2,500,000 energy evaluations for each docking experiment and finding 100 separate docked conformations for each nucleotide.

A test of the role of sugar conformation in the nucleotide was performed using 5′-ADP conformations from entries 1e19, 1m7g, 1o0h, and 1rdq from the Protein Data Bank (http://www.pdb.org), which were judged to have different sugar conformations based on the distance between C5′ and N9, and the torsion angle through atoms C5′—C4′—C1′—N9. These ADP coordinates were prepared and docked similarly to the other nucleotides.

Because crystallographic results are only available for the ligand APS, RMSD values were calculated based on the distance between the nucleotide atoms and the modeled nucleotide, which was created to overlap the analogous atoms in the crystallographic conformation of APS. Thus, the RMSD values in this

Table 1. Results of Docking.

		Best energy			Largest cluster*		
	ΔGobs	N	ΔGAD4	RMSD	N	ΔGAD4	RMSD	ntor
5′AMP	−8.07	2	−8.73	4.04	61	−7.96	0.81	6
7deazaAMP	−7.51	1	−8.36	3.46	77	−8.14	0.81	6
5′ADP	−7.29	3	−10.07	3.03	41	−9.98	0.78	8
3′deoxyAMP	−7.21	3	−8.29	3.13	81	−8.29	0.81	5
5′PMP	−6.30	1	−8.37	3.33	60	−7.73	0.90	6
NmethylAMP	−5.97	45	−8.24	0.82				6
8aminoAMP	−4.95	50	−8.29	1.63				6
2aminoAMP	−4.76	2	−9.28	3.99	20	−8.19	0.96	6
3′phosphoAMP	−4.76	4	−9.07	3.21				7
2methoxyAMP	−4.57	2	−8.51	3.56	17	−8.13	1.31	6
bmethAPS	−4.22	57	−9.34	0.81				8
2′deoxyAMP	−4.13	1	−8.75	4.10	31	−7.24	1.10	5
adenosine	−3.93	5	−5.58	3.64	27	−4.44	0.69	5
dimethylAMP	−3.90	13	−8.15	3.03				6
5′IMP	−3.44	2	−8.60	3.12	23	−7.26	1.48	7
3′deoxyadenosine	−3.17	1	−5.48	4.74	99	−5.27	0.61	6
5′phosphoribose	−2.73	2	−6.93	3.62	7	−6.06	1.93	5
3′AMP	−2.27	8	−9.25	3.87				6
2′deoxyadenosine	−2.00	4	−5.90	4.78	13	−4.90	3.17	4
ribose	−1.77	2	−3.65	9.99	56	−4.29	1.73	4
adenine	−1.76	21	−4.13	2.81	23	−3.81	1.49	0
5′IDP	−1.54	1	−9.90	3.90	13	−9.00	0.90	9

ΔGobs, the experimental free energy of binding; N, the number of docked conformations in the cluster of best energy; ΔGAD4, the predicted free energy of binding from AutoDock; RMSD, the root mean square difference in coordinates between docked conformation and analogous atoms in the crystallographic structure; ntor, the number of torsional degrees of freedom in the molecule; and N, ΔGAD4, and RMSD are provided for the cluster of best energy and the largest cluster.

Full names of the compounds, in the order presented here, are: 5′-adenosine monophosphate, 7-deaza-5′-adenosine monophosphate, 5′-adenosine diphosphate, 3′-deoxy-5′-adenosine monophosphate, 5′-purine monophosphate, N6-methyl-5′-adenosine monophosphate, 8-amino-5′-adenosine monophosphate, 2-amino-5′-adenosine monophosphate, 3′-phospho-5′-adenosine monophosphate, 2′-methoxy-5′-adenosine monophosphate, β-methylene adenosine 5′-phosphosulfate, 2′-deoxy-5′-adenosine monophosphate, adenosine, N6,N6-dimethyl-5′-adenosine monophosphate, 5′-inosine monophosphate, 3′-deoxyadenosine, 5′-phosphoribose, 3′-adenosine monophosphate, ribose, adenine, 5′-inosine diphosphate.

*Blank entries are cases where the best energy is the largest cluster.

paper refer to the similarity of the binding modes to the observed mode of APS.

Calibration of Empirical Terms

Linear regressions and statistical analysis were performed using the free software R (http://www.r-project.org), forcing the regression to include the origin in all cases.

Results

Role of Sugar Conformation

The APS nucleotide conformation found in the crystallographic structure has an unusual sugar conformation, which orients the

Table 2. Results of Regression.

	SdtErr	MultRsq	ΔGAD4 term	Cluster term
			Coeff (*t* value)	
ΔGAD4	1.806	0.864	0.577 (11.6)	n/a
ΔGAD4 + RMSD	1.737	0.881	0.658 (9.6)	1.148 (1.6)
ΔGAD4 + wRMSD	1.709	0.884	0.658 (10.2)	1.098 (1.9)
ΔGAD4 + RK	1.841	0.866	0.380 (2.2)	0.084 (0.6)
Colony	1.703	0.880	n/a	0.483 (12.4)
ΔGAD4 + Vb	1.691	0.887	1.381 (3.4)	1.030 (2.0)

adenine base and phosphate-sulfur group unusually close to one another. To test the importance of this conformation, we performed docking experiments with four different conformations of 5′-ADP. All four of these conformations did not yield docked conformations within 2 Å RMSD of the observed nucleotide conformation. So for the entire work, we used exclusively the sugar conformation found in the APS reductase structure.

Docking of Nucleotides to APS Reductase

For each of the 22 nucleotides, we performed 100 docking experiments, and clustered the resulting conformations using a 2 Å threshold. The results, shown in Table 1, are typical of results of AutoDock docking experiments. In 3/22 compounds, the conformation with best energy was in the proper position,

but in the remaining 19, they were greater than 2 Å RMSD different than the crystallographic position. If, however, we look at the best conformation in the largest cluster, 18/22 conformations are within 2 Å of the expected location.

These types of results, which are commonly obtained for AutoDock experiments, are the motivation for this work. Tight binding ligands, such as 5′-AMP (Fig. 1a), show excellent clustering and weakly binding ligands, such as 3′-AMP (Fig. 1b), show poor clustering, although both show similar predicted binding energies. The docking protocol, as revealed in the clustering, is capturing some aspect of the binding energetics that is missing from the current empirical free energy force field.

Vibrational Entropies from Cluster Size

Table 2 includes the results from regression analysis. Observed binding energies were fit with models that included the predicted AutoDock4 energy and one of the two clustering models: the 2 Å threshold model RMSD or the distance-weighted model wRMSD. In both cases, modest improvement was seen. The standard error of the predicted binding energy was reduced slightly, and the multiple *R*-squared increased.

Table 3 shows the effectiveness of the cluster size models in rescoring. The first column shows the poor predictive ability of the basic AutoDock4 method: when looking at only the conformation of best energy, only 3/22 identifies the proper conformation (these results are also shown in Table 1). The second and third columns show the results when the cluster size measure is included. Both methods show excellent predictive ability, rank-

Table 3. Results of Rescoring.

	AD4	wRMSD	RMSD	Best RK	Fit RK	Best colony	Fit Vb	Lowest RMSD
5′AMP	**4.04**	0.83	0.81	0.81	**4.04**	0.93	0.95	0.78
7deazaAMP	**3.46**	0.80	0.81	0.81	0.87	0.80	0.85	0.77
5′ADP	**3.03**	0.78	0.78	0.78	0.80	0.77	**2.72**	0.69
3′deoxyAMP	**3.13**	0.90	0.90	0.81	**3.47**	**3.13**	0.81	0.77
5′PMP	**3.33**	0.89	0.89	0.90	**3.34**	0.89	0.93	0.81
NmethylAMP	0.82	0.84	0.82	0.82	**2.37**	0.87	**2.42**	0.78
8aminoAMP	1.63	1.63	1.63	1.63	1.78	1.58	0.80	1.52
2aminoAMP	**3.99**	0.96	0.97	0.96	**3.99**	1.79	**3.87**	0.81
3′phosphoAMP	**3.21**	**3.21**	**3.20**	**3.48**	**3.50**	**3.80**	**3.08**	**2.76**
2methoxyAMP	**3.56**	1.31	1.31	1.31	**3.18**	**2.42**	**3.89**	0.81
bmethAPS	0.81	0.81	0.81	0.81	0.78	0.73	**2.61**	0.63
2′deoxyAMP	**4.10**	1.10	1.39	**3.45**	**3.89**	**3.89**	**2.72**	0.94
adenosine	**3.64**	0.68	0.69	**3.64**	**2.60**	**3.37**	**4.81**	0.59
dimethylAMP	**3.03**	**2.69**	**3.22**	**3.03**	**4.41**	**3.84**	**2.71**	1.35
5′IMP	**3.12**	**2.83**	1.48	0.83	**4.08**	0.93	0.88	0.99
3′deoxyadenosine	**4.74**	0.59	0.61	0.61	0.61	0.58	**5.16**	0.56
5′phosphoribose	**3.62**	**3.78**	**3.78**	**3.89**	**2.83**	**3.77**	0.93	1.75
3′AMP	**3.87**	**3.82**	**3.87**	**3.87**	**3.95**	**5.16**	**3.96**	**3.02**
2′deoxyadenosine	**4.78**	**4.78**	**4.78**	**4.69**	**2.39**	**2.79**	1.79	1.12
ribose	**10.62**	1.72	1.73	1.62	**10.62**	1.71	1.55	1.55
adenine	**2.81**	**2.81**	**2.81**	**6.44**	**2.97**	1.49	1.58	1.48
5′IDP	**3.90**	0.98	0.98	0.80	**2.26**	0.74	**2.56**	0.81
	3	15	16	14	5	13	10	20/22

RMSD values are given for the docked conformation of best energy as determined by each method, with values >2.00 Å in bold. The final line gives the number of conformations in each column with RMSD < 2.00 Å.

ing the expected conformation as the best in 15/22 or 16/22 cases.

The significance of this result may be estimated by comparison with a statistical method based on Bernoulli trials. We calculated the fraction of dockings with RMSD less than 2 Å for each compound, which ranges from 0.00 for 3′-phospho-5′-AMP to 0.99 for 3′-deoxyadenosine. Using these fractions, we can estimate the expected number of correct conformations we would obtain by randomly choosing a conformation for each compound. This analysis estimates that random choice would give a correct answer in 12.35 cases, with a standard deviation of 1.72, out of the 22 compounds.

Vibrational Entropies from the RK Method

We also tested the RK method for evaluating the entropic component for ranking the binding energies. The entropy calculated by this method is typically in the order of tens of kcal/mol, and so if it is simply added to the predicted energy from AutoDock4, the binding energy is predicted to be positive in all cases. However, these energies are effective for reranking, as shown in the fourth column in Table 3 (marked "best RK"), correctly predicting the conformation of 14/22 compounds.

A regression analysis of the RK entropy showed a slightly worse prediction of the binding energy, as shown in Table 2, and a very slight improvement in reranking, correctly predicting 5/22 compounds.

In our work with the RK method, we have noticed one potential limitation: the evaluation of the configurational integral is strongly influenced by the number of observations in each cluster. The method attempts to evaluate the extent of favorable regions of the local energy landscape by evaluating the bounds on each cluster. However, because these clusters are composed of a small number of individual observations, clusters with few individuals will give smaller extents just through simple statistics. Figure 2 shows a scatter plot of cluster size vs. the RK entropy. The two lines show the upper and lower bounds of clusters composed of a set of points randomly distributed within a small constant volume of conformational space, and then used to calculate the RK entropy. The random distribution captures many of the features of the RK data, and so we might expect that the RK method, when applied to clusters generated by AutoDock, is more a reflection of the cluster size than of the extent of the local energy landscape. Notably, in a recent publication,[16] a direct measure of cluster size was used instead of the estimated conformational extent in a similar study to estimate vibrational entropies in the context of several scoring methods.

Conformational Free Energies from the Colony Method

The "Colony" method,[9] originally developed to score candidate loop conformations in protein structure prediction, uses the RMSD values to the constellation of neighboring conformations to evaluate a conformational free energy. In cases where a conformation has many close neighbors, the Colony energy will be more favorable. As shown in Table 3, the Colony energy performs well for the rescoring of docked conformations in APS reductase, predicting the expected conformation in 13/22 cases.

The use of the Colony method for prediction of binding free energies is problematic, because the constant term in the Colony equation is difficult to calculate. However, if we assume that this term in approximately the same across our test set of compounds, a regression analysis of Colony energies with the observed binding energies shows a moderate improvement over the predicted energies from AutoDock4, as shown in Table 2.

Vibrational Entropies from Local Sampling of the Energy Landscape

Ideally, we like to start with a single docked conformation and, by analyzing the local energy landscape, evaluate this entropic contribution to the binding strength. As a first step toward this goal, we have randomly sampled the conformational space around each docked conformation and calculated a configurational integral based on the energy landscape. This is partially effective for improving the prediction of free energies and in reranking. The regression showed a small improvement in the standard error, and the method was able to rank 10/22 compounds.

Comparing two of the compounds from this study, we can see how these configurational integrals capture the underlying landscape. 5′-AMP and 3′-AMP have the same number and type of atoms and the same number of torsional degrees of freedom, but widely different experimental binding constants. In docking, 5′-AMP gives a tight cluster of 61/100 docked conformations in the expected location, whereas weaker-binding 3′-AMP shows a scatter of different, small cluster conformations.

Looking at the energy landscape around the docked conformation, as shown in Figures 3 and 4, we find that 5′-AMP has a broader energy well than 3′-AMP. Thus, small motions of 3′-AMP will run up against large steric contacts, whereas small motions of 5′-AMP do not encounter bad contacts.

Unfortunately, these types of correlations were difficult to extract for other compounds, where the structural similarity was not as great. Looking at the entire set, the greatest trend was a strong correlation between the value of the configurational integral and the number of torsional degrees of freedom in the molecule. This is not a surprise, because this merely reflects the magnitude of the entropy involved in freezing these torsional degrees of freedom into a confined space of the active site. The more subtle effect of the local shape of that active site, as seen in the 5′-AMP vs. 3′-AMP landscapes, is overshadowed by this larger effect.

Discussion

The ultimate goal of this work is to find a computationally tractable method to evaluate the vibrational entropy contribution of binding, and thus improve our predicted binding energies. This is essential for the future success of docking in computer-aided drug design, where the common presence of false positives and false negatives during virtual screening is a major problem in current studies.

The results presented here suggest that the cluster size is an effective and cheap method for evaluating these vibrational con-
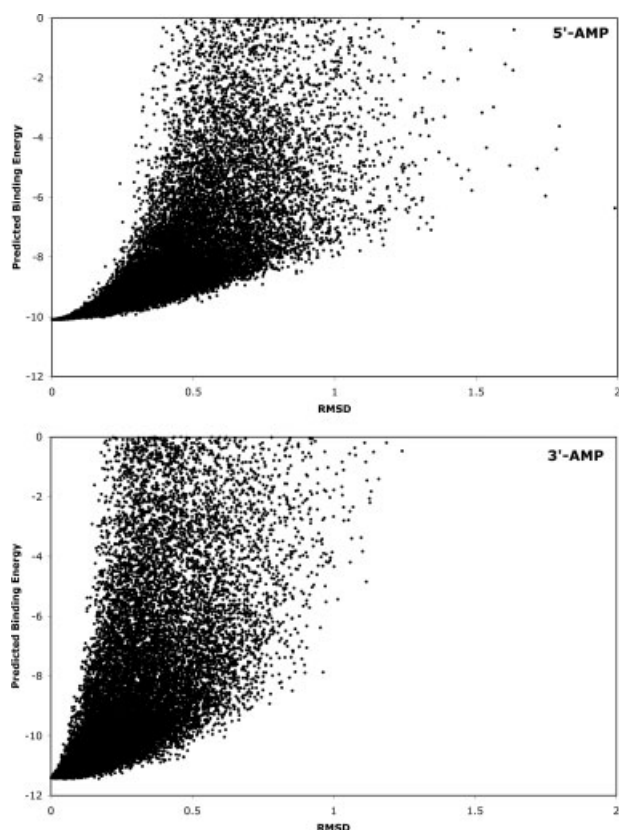
Figure 3. Analysis of the local energy landscape. Each point represents a small random change in conformation away from the most favorable bound conformation. RMSD values are calculated between the perturbed conformation and the starting conformation. 5′-AMP shows a wide basin, with very few unfavorable conformations until they are a distance of about 0.5 Å RMSD from the bound conformation. 3′-AMP shows a narrower basin, with many unfavorable conformations as distances less than 0.25 Å RMSD.

tributions, which significantly improve the identification of proper binding modes within a single complex, and slightly improve the ranking of different compounds. The Colony method shows a similar success, which should come as no surprise because it also uses a measure of cluster size to evaluate the entropic contribution.

These cluster size methods, however, are not satisfying from a conceptual level, because they are relying on some unknown combination of the overall energy landscape and the details of the docking protocol. Ideally, we like to develop a computationally inexpensive method that analyses the energy landscape, both locally and globally, and uses that information to identify the major binding modes and affinities. The RK method was designed with this goal in mind. It uses a simple method to estimate the local conformational area that is available to a given ligand. Unfortunately, the results shown in Figure 2 suggest that the method is primarily a reflection of the cluster size, as opposed to a quantification of the local energy landscape. The RK term is highly dependent on the actual number of observations in each cluster and their orientation relative to one another

in conformational space, not just the overall conformational volume available to the conformations.

Our attempt to characterize the local energy landscape through random sampling has provided some provocative, but not definitive, results. The results presented in Figure 3 show that there are significant differences in the local energy landscape for two forms of AMP, differences that correlate strongly with the large difference in binding constants between these two compounds. However, this principle did not generalize over the entire set. Our current hypothesis is that the docking analysis, and thus the clustering, is capturing information over a larger area of conformational space that we sampled in this work, and that sampling of this larger space will be necessary to develop
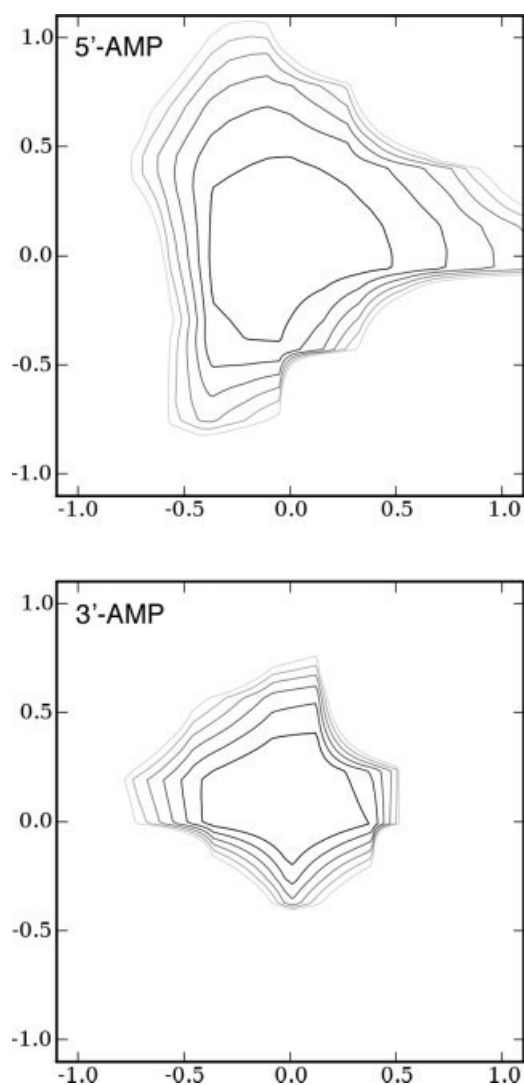

Figure 4. Analysis of the local translational energy landscape. Conformations were sampled in the range of −1 to +1 Å in the *x* and *y* directions around the most favorable bound conformation. The energy of the sampled conformations is shown here, with the outer contour at −1.5 kcal/mol and additional contours at −1.5 kcal/mol increments.

an effective method for directly evaluating the conformational entropy contribution to binding. However, use of the cluster size in multiple docking experiments is a fast and easy way to estimate this contribution, and is a viable method for improving current docking results.

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