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Host–Guest Relative Binding Affinities at Density-functional Theory Level from Semiempirical Molecular Dynamics Simulations

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Abstract

Relative free energies for the binding of nine cyclic carboxylate ligands to the octa-acid deep-cavity host were calculated at the combined density-functional theory and molecular mechanics (DFT/MM) level of theory. The DFT calculations employed the BLYP functional and the 6-31G* basis set for the ligand. We employed free-energy perturbations (FEP) with the reference-potential approach and used molecular dynamics (MD) simulations with the semiempirical quantum mechanical (SQM) PM6-DH+ method for the ligand as an intermediate level between MM and DFT/MM to improve the convergence. Thus, the relative binding free energy of two ligands was first calculated at the MM level by an alchemical transformation from one ligand to another in both the bound and unbound states. Then, for each ligand the free-energy correction for going from the MM to the SQM/MM potentials was calculated using explicit SQM/MM MD simulations. Finally, the free-energy correction for going from the SQM/MM to the DFT/MM potentials was estimated with FEP without running any DFT/MM simulations. Instead, the free energy was calculated by single-step exponential averaging (ssEA) or employing the cumulant approximation to the second order (CA). The results show that CA converges much better than ssEA and with 500–4500 DFT/MM single-point energy calculations, converged free energies with a precision of 0.3 kJ/mol can be obtained. These free energies reproduce the experimental binding free energy differences with a mean absolute deviation of 3.4 kJ/mol, a correlation (R^2) of 0.97 and correct signs for all of the eight free-energy differences. This is appreciably better than the results obtained at the SQM/MM level of theory and also slightly better than those obtained with MM. We show that the convergence of the SQM/MM→DFT/MM perturbations can be monitored by the use of Wu and Kofke’s bias metric Π and by the standard deviation of the difference between the SQM/MM and DFT/MM energies. Finally, we show that the use of the intermediate SQM/MM MD simulations improves the convergence of the free energies by a factor of at least two, compared to doing direct MM→DFT/MM perturbations.

Introduction

Despite its ever-increasing important role in drug design, accurate calculations of ligand-binding free energies is still one of the largest challenges in computational chemistry.¹⁻³ Numerous methods have been proposed, ranging from inexpensive scoring approaches to rigorous methods based on alchemical free-energy perturbation (FEP) calculations.⁴⁻⁸ In FEP, a ligand is changed to another through an unphysical pathway that is sampled via molecular dynamics (MD) or Monte Carlo simulations, typically at molecular-mechanics (MM) level of theory. The associated free-energy difference between the two ligands can be calculated with several different methods, such as exponential averaging,⁹ thermodynamic integration,¹⁰ Bennett acceptance ratio (BAR)¹¹ or its multistate version (MBAR),¹² of which the latter two have the best statistical properties.¹³ However, these calculations converge only if the difference between the two ligands is small. If this requirement is not satisfied, stratification is normally employed, in which the perturbation is divided into many small steps. Several groups have used such methods to calculate relative binding free energies for ligands to various proteins, often with impressive results (mean absolute deviations compared to experimental data below 4 kJ/mol).^{7,14,15} However, for some proteins the results are appreciably worse with errors over 20 kJ/mol.

FEP methods are in principle exact, provided that the sampling is exhaustive and the potential energy function is exact. Therefore, there has been quite some interest recently in employing more accurate quantum-mechanical (QM) methods to improve the results.¹⁶ Such calculations can be performed in several different ways. The simplest way is to run full alchemical FEP calculations at the combined QM/MM level of theory, for which the sampling needs to be done at the QM/MM level, which is extremely expensive. Therefore, such calculations have so far been performed only for small QM regions, treated with a semiempirical QM approach (SQM).¹⁷⁻²⁰

There has been growing interest in employing the reference-potential approach, developed by Gao, Warshel and co-workers.^{21,22} In this approach, the relative binding affinity is first

calculated at a low-level of theory, typically MM. Then, FEP calculations are performed at the end states of the alchemical transformation to convert the potential-energy function from MM to QM/MM. Since free energies are state functions, the sum of the corresponding free energies changes is the relative QM/MM binding free energy of the two ligands, as is illustrated by the thermodynamic cycle in Figure 1a.

The challenge with the reference-potential approach is to converge the MM→QM/MM FEP in a single step, so that no sampling at the QM/MM level is needed. Initial attempts to calculate relative binding affinities with density-functional theory (DFT) methods, large QM systems involving both the ligand and the immediate surroundings, as well as a detailed polarisable multipole model for the remaining were total failures, because the MM→QM/MM perturbations did not converge with a few hundred QM/MM calculations.^{23,24} However, by using a SQM level of theory as the target Hamiltonian, it was shown that the MM→SQM/MM perturbation could be converged to 1 kJ/mol by employing 720 000 SQM calculation for a host–guest system, treating both the host and the guest molecules by SQM.²³ In these calculations, the cumulant approximation to second order (CA) gave appreciably better convergence than the single-step exponential averaging (ssEA) and the non-Boltzmann BAR approach (NBB).

Still, there were indications that the calculations were not fully converged and the accuracy of the CA and the use of interaction energies (rather than total energies) was unclear. Therefore, we also developed the reference-potential approach with QM/MM sampling (RPQS), in which the MM→QM/MM FEP calculation is divided into four steps (therefore requiring QM/MM MD simulations).²⁰ It was shown that such an approach gave identical results to full QM/MM-FEP (within statistical uncertainty), but with only one fifth of the computational cost for the same host–guest system. This RPQS approach has recently been used in the SAMPL6 blind-test challenge, giving results that were comparable or slightly better than MM FEP.²⁵ We have also developed two approaches to speed up the RPQS approach using either multiple short equilibrium or non-equilibrium MD simulations.^{26,27}

It is well-known that SQM methods are less reliable than DFT methods for intermolecular interactions and binding affinities.^{28,29} Therefore, it is of great interest to develop methods to obtain relative binding free energies at the DFT level of theory. For solvation free energies, it has been shown that the convergence of the free energy associated with the MM→QM/MM transformation can sometimes be improved by an indirect approach via an intermediate SQM/MM state, rather than a direct FEP from the MM Hamiltonian to the QM/MM Hamiltonian.³⁰ Here, we try a similar approach for the calculation of relative binding free energies of nine cyclic carboxylate ligands to the octa-acid deep cavitand from the SAMPL4 competition and employ our RPQS simulations at the SQM/MM level as the reference for SQM/MM→DFT/MM FEP calculations using the ssEA and CA approaches. We show that converged results can be obtained and the convergence is appreciably improved compared to using MM as the reference potential. We also discuss how the convergence can be monitored.

Methods

Theory

In this study, relative binding free energies at the DFT/MM level of several guest molecules targeting a host molecule are calculated, but without performing MD simulations at this level of theory. Therefore, we applied the reference-potential scheme, as is shown in Figure 1b. Previous studies have indicated that it is difficult to converge a MM→DFT/MM energy correction.^{23,24} Therefore, we use simulations at the SQM/MM level as an intermediate step between the MM and DFT/MM potentials. Consequently, we first calculate the relative binding free energies with the MM potential. These calculations are taken from our previous study.²⁴ Next, we calculate a MM→SQM/MM correction for each ligand using the RPQS approach, dividing the perturbation into four steps, thus requiring SQM/MM MD simulations. Again, the results are taken from a previous study.²⁰ Finally, we employ the SQM/MM MD simulations to perform a FEP from SQM/MM to DFT/MM. To avoid run-

ning DFT/MM MD simulations, this perturbation needs to converge in a single step. The ssEA and CA approaches are employed to calculate this free-energy correction.

Figure 1: The reference potential approach, (a) with direct FEP calculations from MM to the DFT Hamiltonian (b) employing an intermediate SQM/MM Hamiltonian between the MM and DFT Hamiltonian.

Using the thermodynamic cycle by Tembre and McCammon,³¹ the difference in the binding affinity of two ligands, L_0 and L_1 , can be calculated at the DFT/MM level from

$$\Delta G_{L_0 \rightarrow L_1}^{\text{DFT/MM}} = \Delta G_{L_0 \rightarrow L_1, b}^{\text{DFT/MM}} - \Delta G_{L_0 \rightarrow L_1, u}^{\text{DFT/MM}}, \quad (1)$$

i.e. from the free-energy differences between these two ligands in the bound state ($\Delta G_{L_0 \rightarrow L_1, b}^{\text{DFT/MM}}$) and in the unbound state ($\Delta G_{L_0 \rightarrow L_1, u}^{\text{DFT/MM}}$). Each of these two terms ($G_{L_0 \rightarrow L_1, s}^{\text{DFT/MM}}$, $s = b$ or u) can be calculated with reference-potential approach, using SQM/MM calculations as the reference (cf. Figure 1):^{20,26,32}

$$\Delta G_{L_0 \rightarrow L_1, s}^{\text{DFT/MM}} = \Delta G_{L_0 \rightarrow L_1, s}^{\text{SQM/MM}} - \Delta G_{L_0, s}^{\text{SQM/MM} \rightarrow \text{DFT/MM}} + \Delta G_{L_1, s}^{\text{SQM/MM} \rightarrow \text{DFT/MM}} \quad (2)$$

Here, the difference in binding affinity between the two ligands is first calculated at the SQM/MM level ($\Delta G_{L_0 \rightarrow L_1, s}^{\text{SQM/MM}}$). Then, for each ligand, the free-energy difference between the SQM/MM and the DFT/MM potentials is estimated in both the bound and unbound states ($\Delta G_{L_i, s}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$, for $i = 0$ and 1). Combining Eqs. 1 and 2, the relative binding affinity can be obtained as:

$$\begin{aligned} \Delta G_{L_0 \rightarrow L_1, s}^{\text{DFT/MM}} &= \Delta G_{L_0 \rightarrow L_1}^{\text{SQM/MM}} \\ &+ \left(\Delta G_{L_1, b}^{\text{SQM/MM} \rightarrow \text{DFT/MM}} - \Delta G_{L_1, u}^{\text{SQM/MM} \rightarrow \text{DFT/MM}} \right) \\ &- \left(\Delta G_{L_0, b}^{\text{SQM/MM} \rightarrow \text{DFT/MM}} - \Delta G_{L_0, u}^{\text{SQM/MM} \rightarrow \text{DFT/MM}} \right) \end{aligned} \quad (3)$$

The terms in the parentheses in Eq. 3 will be denoted as $\Delta G_{L_i}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ for $i = 0, 1$.

In this study, we calculate these terms with two different methods. In the first scheme, which is termed ssEA,²³ the free energy difference between the SQM/MM and DFT/MM potentials is calculated with standard exponential averaging using single-point energy calculations on snapshots harvested from MD simulations at the SQM/MM level (i.e. without doing expensive DFT/MM simulation):

$$\Delta G_{L_i, s}^{\text{SQM/MM} \rightarrow \text{DFT/MM}} = -RT \left\langle \exp \left(-\frac{\Delta U}{RT} \right) \right\rangle_{\text{SQM/MM}}, \quad (4)$$

where $\Delta U = U^{\text{DFT/MM}} - U^{\text{SQM/MM}}$ is the difference in the potential energy between SQM/MM and DFT/MM Hamiltonians, R is the gas constant, T is the absolute temperature and the

angle brackets ($\langle \dots \rangle_{\text{SQM/MM}}$) denote an average over the snapshots from a simulation using the SQM/MM potential. The corresponding variance of $\Delta G_{L_i,s}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ can be estimated from

$$\sigma_{\text{ssEA}}^2 = \frac{(RT)^2}{N} \left(\frac{\sigma_x}{\langle x \rangle} \right)^2, \quad \text{with } x \equiv \exp \left[\frac{\Delta U}{RT} \right], \quad (5)$$

where $\langle x \rangle$ and σ_x are the average and standard deviation of x , and N is the number of snapshots employed in the calculations.

Strictly, ΔU in Eq. 4 should be calculated from the difference in the total energy of all atoms in the system. However, the convergence of such energies is rather slow and previous studies have shown that it can be improved by using interaction energy differences instead,^{23,33–35} i.e. $\Delta U_{\text{int}} = U_{\text{RL}} - U_{\text{R}} - U_{\text{L}}$, where the three terms on the right-hand side are the total energies of the complex, the receptor and the ligand, respectively. Thereby, differences in the internal energies of the receptor and the ligand are ignored.

Our QM/MM calculations employ electrostatic embedding, i.e. the QM calculation involve a point-charge model for the surroundings, which polarises the QM region. Thus, the QM/MM energy can be written as

$$U^{\text{QM/MM}} = U_{1,q_2}^{\text{QM}} + U_{2 \setminus 1}^{\text{MM}}, \quad (6)$$

where U_{1,q_2}^{QM} is the QM energy of the QM region (called system 1) and $U_{2 \setminus 1}^{\text{MM}}$ is the MM energy of the surroundings, excluding the QM region. Therefore, we can calculate the interaction energy at the QM/MM level for our receptor–ligand system as:

$$\begin{aligned} \Delta U_{\text{int}}^{\text{QM/MM}} &= U_{\text{RL}}^{\text{QM/MM}} - U_{\text{R}}^{\text{QM/MM}} - U_{\text{L}}^{\text{QM/MM}} \\ &= U_{\text{RL}}^{\text{QM/MM}} - U_{\text{R}}^{\text{MM}} - U_{\text{L}}^{\text{QM}} \\ &= U_{\text{L},q_{\text{R}}}^{\text{QM}} + U_{\text{R} \setminus \text{L}}^{\text{MM}} - U_{\text{R}}^{\text{MM}} - U_{\text{L}}^{\text{QM}}, \end{aligned} \quad (7)$$

where the second equality follows from the fact that only the ligand is in the QM system and

the receptor (and the solvent) is in the MM system. In the third equality, we have used Eq. 6 for the first term, again recognizing that only the ligand belongs to the QM system. Finally, we can calculate the difference in interaction energy between DFT/MM and SQM/MM as:

$$\begin{aligned}
\Delta\Delta U_{\text{int}}^{\text{SQM/MM} \rightarrow \text{DFT/MM}} &= \Delta U_{\text{int}}^{\text{DFT/MM}} - \Delta U_{\text{int}}^{\text{SQM/MM}} \\
&= U_{\text{L},q\text{R}}^{\text{DFT}} + U_{\text{R}\backslash\text{L}}^{\text{MM}} - U_{\text{R}}^{\text{MM}} - U_{\text{L}}^{\text{DFT}} - \left(U_{\text{L},q\text{R}}^{\text{SQM}} + U_{\text{R}\backslash\text{L}}^{\text{MM}} - U_{\text{R}}^{\text{MM}} - U_{\text{L}}^{\text{SQM}} \right) \\
&= U_{\text{L},q\text{R}}^{\text{DFT}} + U_{\text{L}}^{\text{DFT}} - \left(U_{\text{L},q\text{R}}^{\text{SQM}} - U_{\text{L}}^{\text{SQM}} \right). \tag{8}
\end{aligned}$$

Thus, we obtain $\Delta\Delta U_{\text{int}}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ from four QM calculations, two at the DFT and two at the SQM levels of theory, one in gas-phase and one with the point-charge model. This term is used as ΔU in Eqs. 4 and 5.

It is well-known that ssEA may suffer from numerical instabilities.^{36–39} An alternative to this approach is to employ the cumulant approximation to second order (CA). The cumulant expansion of Eq. 4^{40,41} is

$$\Delta G = \langle \Delta U \rangle - \frac{\sigma_{\Delta U}^2}{2RT} + \dots, \tag{9}$$

where $\langle \Delta U \rangle$ and $\sigma_{\Delta U}$ are the average and the standard deviation of ΔU . If the distribution of ΔU is close to a Gaussian function, the cumulant expansion can be truncated after the second term. The corresponding variance can be calculated from

$$\sigma_{\text{CA}}^2 = \frac{\sigma_{\Delta U}^2}{N} + \frac{\sigma_{\Delta U}^4}{2(N-1)}. \tag{10}$$

The advantage of this approximation is that it depends on all ΔU estimates (through the average and the standard deviation), whereas the exponential average is often strongly dominated by a few values with the most negative ΔU . Several previous studies have illustrated the advantage of the cumulant approximation.^{32,41–44}

Setup of the Calculations

The host molecule and the ligands are shown in Figure 2. Eight ligand transformations were considered, viz. MeBz $\rightarrow$ Bz, EtBz $\rightarrow$ MeBz, pClBz $\rightarrow$ Bz, mClBz $\rightarrow$ Bz, Hx $\rightarrow$ Bz, MeHx $\rightarrow$ Hx, Hx $\rightarrow$ Pen and Hep $\rightarrow$ Hx. The MM calculations²⁴ employed the general Amber

Figure 2: The octa-acid host and the nine guest molecules studied in this work.

force field (GAFF)⁴⁵ for both the host and the guest molecules and the partial charges were obtained by the restrained electrostatic potential (RESP)^{46,47} method, using the electrostatic potential computed at HF/6-31G* level of theory.²⁴ Water molecules were treated by the TIP3P model.⁴⁸

The SQM calculations employed the PM6-DH+ method.^{49,50} Only the ligand was treated by the SQM Hamiltonian, whereas the host and water molecules were treated by the MM potential. We employed the QM/MM implementation in AMBER.^{51,52} The PM6-DH+/MM simulations were started from the last snapshot of our previous study²⁰ and a 10 ns production simulation was carried out. The temperature was regulated to 300 K using Langevin dynamics,⁵³ and the pressure was set to 1 atm using Berendsen’s barostat.⁵⁴ Long-range Coulomb interactions were calculated using the particle mesh Ewald (PME)⁵⁵ method, whereas the Lennard-Jones non-bonded interactions were truncated at 8.0 Å. No bond-

length constraints were used and the time step was set to 1 fs. Altogether, 5 000 snapshots were collected from these simulations at a regular interval of 2 ps. All the MD simulations were carried out with the AMBER14 software.⁵¹

For each snapshot, DFT single point energy calculations were performed with the BLYP functional^{56,57} and the 6-31G* basis set.⁵⁸ Again, only the ligand was included in the QM region. These calculations were performed with the QM/MM module⁵² in AMBER package⁵¹ interfacing with Gaussian 09.⁵⁹

Convergence Criteria and Quality Assessment

The primary purpose of the reference-potential approach is to reduce the computational cost. However, reaching a convergence of $\Delta G_{L_i,s}^{SQM/MM \rightarrow DFT/MM}$ is challenging. To monitor the convergence, two criteria were employed. The first is the standard deviation of the ΔU energy difference distribution, σ , recently recommended by Ryde.⁶⁰ The second is the bias metrics (Π) from Wu and Kofke.^{61,62} As the DFT distribution is not available, we make the assumption that the entropy is the same in the forward (SQM $\rightarrow$ DFT) and backward (DFT $\rightarrow$ SQM) directions. Then, Π can be calculated from

$$\Pi = \sqrt{W_L \left[\frac{1}{2\pi} (N-1)^2 \right]} - \sqrt{\frac{2(\langle \Delta U \rangle - \Delta G)}{RT}}, \quad (11)$$

where $W_L(x)$ is the Lambert W function.⁶¹

The performance of the estimated relative binding affinities as compared to the experimental values⁶³ was quantified by the mean absolute deviation (MAD), the correlation coefficient (R^2) and Kendall’s rank correlation coefficient (τ_r). τ_r is calculated only for the transformations actually studied. It should be noted that R^2 depends on the direction of the perturbation ($L_0 \rightarrow L_1$ or $L_1 \rightarrow L_0$). We solved this problem by considering both directions when calculating R^2 .²⁵

The variance of both the convergence and quality measures was estimated with boot-

strapping,⁶⁴ repeated 1000 times.⁶⁵

Results and Discussion

In this work, we use the reference-potential method to calculate relative binding free energies of nine cyclic carboxylate ligands to the octa-acid deep-cavity host at DFT/MM level, using the BLYP/6-31G* method for the ligand. Similar to all reference-potential approaches, the challenge is to converge the MM→DFT/MM free energy, without running DFT/MM MD simulations. To improve the convergence, we employ intermediate SQM/MM MD simulations at the PM6-DH+ level, as is shown in Figure 1. Moreover, we calculate the free-energy differences from SQM to DFT with two different schemes, single-step exponential averaging (ssEA), based on the Zwanzig’s equation, and the cumulant approximation to the second order (CA).

Convergence of $\Delta G_{L_i,s}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$

Figures 3 and 4 show how $\Delta G_{L_i,u}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ and $\Delta G_{L_i,b}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$, as well as the standard error (SE), vary with the number of energy evaluations at the DFT/MM level, N , calculated using ssEA and CA (the raw data are listed in Tables S1 and S2). It can be seen that in general, the SE is slightly lower for the ligand in the bound state than in the unbound state. Moreover, the SE is appreciably lower for CA than for ssEA. With 5 000 snapshots, the SE is 0.1–0.3 kJ/mol with CA, whereas it is 0.2–0.7 kJ/mol for the bound ligand and 0.3–1.8 kJ/mol for the unbound ligand with ssEA. Using CA, the SE is below 1 kJ/mol (which was used as the convergence criterion in our previous study³²) for all ligands with only 500 snapshots. However, using ssEA, the SE is still larger than 1 kJ/mol for mClBz in the unbound state even with 5 000 snapshots.

It can also be seen that the CA curves in Figure 3 are more smooth than those obtained with ssEA. The latter often show the step-wise jumps, as is frequently seen with exponential

(a)

(b)

Figure 3: Evolution of (a) $\Delta G_{L_i,u}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ and (b) $\Delta G_{L_i,b}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ as a function of the number of the frames N using ssEA (black) and CA (red).

(a)

(b)

Figure 4: Standard error of calculated (a) $\Delta G_{Li,u}^{SQM/MM \rightarrow DFT/MM}$ and (b) $\Delta G_{Li,b}^{SQM/MM \rightarrow DFT/MM}$ using ssEA (black) and CA (red).

averaging. This reflects that a new low value of ΔU has been sampled, which significantly changes the results, showing that the ssEA results often depend on only a few values of ΔU .

If we define convergence as reproducing the result at $N = 5\,000$ within $\pm 1.96\sigma$ (95% confidence), all the ligands in the unbound state reached convergence using CA with 2000 snapshots at the most, whereas for the bound state, MeBz and EtBz encountered convergence difficulties for N up to 3 000 and 4 000, respectively. Using ssEA, all the ligands have convergence problems, with Pen being the only exception. These convergence problems are also reflected in the behaviour of SE with respect to N , shown in Figure 4. For CA, essentially all curves are smooth and show the expected $\frac{1}{\sqrt{N}}$ dependence. However, with ssEA, most of the curves are more jagged and uneven.

For most of the ligands the ssEA and CA results converge to the same value, indicating that the terms beyond the second order in the cumulant expansion do not make a significant contribution. However, at $N = 5\,000$, there is still a significant difference (at 95% confidence) for mClBz, Hx and MeHx in the unbound state, and for Bz, MeHx and Hep in the bound state.

Figure 5 shows the distributions of ΔU for all the ligands. It can be seen that they are in all cases close to a Gaussian distribution. The largest deviation is observed for the bound EtBz ligand, which explains the poorest convergence with CA. However, small deviations can also be observed for most of the ligands showing a significant difference between the results obtained with ssEA and CA.

Finally, $\Delta G_{L_i}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ was obtained from the difference between $\Delta G_{L_i,b}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ and $\Delta G_{L_i,u}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ for each guest molecule and the results are shown in Figure 6 (the corresponding data are given in Tables S3 and S4). It can be seen that using CA, all the calculated $\Delta G_{L_i}^{\text{MM} \rightarrow \text{QM/MM}}$ reached convergence already with 2000 snapshots except for EtBz (using the same convergence criterion as above). The SE decreases quite smoothly for all ligands, from 0.8–1.0 kJ/mol with $N = 500$ to 0.3 kJ/mol for $N = 5\,000$. For ssEA, the convergence is much worse, the curves are more jagged and the SE is 2–7 times larger than

(a)

(b)

Figure 5: Distribution of the differences in the interaction energies between SQM/MM and DFT/MM level (ΔU_{int}), shown in red. The blue curve shows a Gaussian distribution with the same mean and standard deviation.

that from CA.

Figure 6: Calculated $\Delta G_{L_i}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ using ssEA (black) and CA (red).

The net magnitude of the SQM $\rightarrow$ DFT correction ($\Delta G_{L_i}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$) is rather large for all ligands, in a range of 7–12 kJ/mol using CA and $N = 5\,000$. It is largest for the pClBz ligand and smallest for the Hep, Pen and Bz ligands.

Convergence Measures

The results in Figure 3 show that some of the ssEA results have an uncertainty that is larger than what is indicated by the SE estimate (by comparing to the CA results). Such a behaviour is often observed for ssEA and it is of course of great interest to detect such an uncertainty in a quantitative manner. Moreover, we need to know whether the CA results can be trusted. Such questions have been considered in many studies and several convergence criteria have been suggested.^{15,20,23,27,30,60,61,66}

In this investigation, we have considered two convergence criteria, viz. σ and Π (initially, we tested two additional criteria, viz. the reweighting entropy and the weight of the

Table 1: Convergence criteria for $\Delta G_{Li,s}^{MM \rightarrow QM/MM}$ (shown in Figure 3) with 5 000 snapshots. σ has units of RT .

guest	unbound		bound	
	σ	Π	σ	Π
Bz	2.66 ± 0.03	0.60 ± 0.15	2.19 ± 0.02	1.18 ± 0.08
MeBz	3.09 ± 0.03	0.36 ± 0.06	3.03 ± 0.03	0.46 ± 0.09
EtBz	3.09 ± 0.03	0.44 ± 0.08	2.97 ± 0.03	0.44 ± 0.07
pClBz	3.14 ± 0.03	0.41 ± 0.08	2.28 ± 0.02	1.26 ± 0.03
mClBz	2.79 ± 0.02	0.23 ± 0.25	2.30 ± 0.02	1.14 ± 0.08
Hx	2.69 ± 0.03	0.60 ± 0.12	2.30 ± 0.02	1.17 ± 0.05
MeHx	2.61 ± 0.03	0.56 ± 0.10	2.34 ± 0.02	0.93 ± 0.10
Pen	2.84 ± 0.03	0.64 ± 0.05	2.39 ± 0.02	1.14 ± 0.04
Hep	2.70 ± 0.03	0.86 ± 0.04	2.37 ± 0.02	0.93 ± 0.10

maximum term in the exponential average;^{15,30,38} however, in agreement with our previous studies,^{27,60} we found it hard to find any consistent and useful limits for these criteria). The σ and Π convergence criteria are listed in Table 1 for $N = 5\,000$. It can be seen that $2.2 \leq \sigma \leq 3.1\,RT$ and $0.2 \leq \Pi \leq 1.3$ and that the results are in general better (i.e. larger Π and smaller σ) for the ligands in the bound state than in the unbound state. However, the question is what criteria to use to decide whether the results are converged or not. For σ , values between 1.5 and 4 RT have been suggested.^{60,67} Π should be positive for reliable results and it has been suggested that $\Pi > 0.5$ should be used to include a safety margin.⁶¹ Consequently, six simulations are in the safety region for Π and all values of σ are between the suggested convergence limits.

However, we have recently suggested a simple approach to decide whether both ssEA and CA estimates are converged⁶⁰ (based on an idea from ref.⁶⁶). If the distribution of ΔU is approximately Gaussian, a simple simulation approach can be used to estimate how many calculations are needed before reliable results can be obtained. Once σ is known, you only have to specify the desired confidence of the results. This approach which be called CGS in the following, convergence estimates from a Gaussian-distribution simulation. If we want to get a result that is within 1 kJ/mol of the correct results with 95% confidence, CGS suggests

that N needs to be 400 for CA and 1600 for ssEA when $\sigma = 2.2RT$. On the other hand, when $\sigma = 3.1RT$, 1400 and 44 000 snapshots are needed, respectively (CGS results for some different values of σ are given in Table S5). Conversely, with $N = 5\,000$, the convergence CGS criterion for ssEA is $\sigma \leq 2.6RT$, whereas it is $\sigma \leq 4.3RT$ for CA.

Using these CGS criteria, we can see from Table 1 that all CA results should be converged (to 1 kJ/mol) already with $N = 1\,500$. In practice, this seems to be somewhat too optimistic, most likely because the ΔU distribution is not exactly Gaussian: At $N = 1\,500$, three of the individual calculations differ by more than 1 kJ/mol from the results at $N = 5\,000$. When N increases to 2 000, there is only one such deviation (EtBz in the bound state).

On the other hand, CGS indicates that none of the ssEA calculations for the unbound ligand and only two of the ssEA calculations of the bound state are converged (although many are approaching the limit). Judging from Figure 3, such a conclusion may seem too restrictive, as several of the ssEA curves seem to be converged. However, it should be kept in mind that the ssEA results are strongly dominated by rare events (especially when σ is large), which may not be observed when N is small. In fact, $\Pi \leq 0.5$ for four of the ligands, and for four additional ligands $\Pi = 0.6$ is just outside the safety margin with an overlap if the uncertainty in Π is considered to 95% confidence. It is only for the unbound Hep ligand that $\Pi = 0.86 \pm 0.04$ and $\sigma = 2.70 \pm 0.03$ give significantly different conclusions.

Therefore, we recommend the use of both σ and Π as convergence criteria. σ should be used together with the CGS approach, which also takes into consideration the desired accuracy and confidence. For an accuracy of 1 kJ/mol and 95% confidence, recommended N values are given in Table S4. σ is the only criterion that can be used for CA and here we recommend to use a significant safety margin, owing to the dependence on the Gaussian approximation. For, Π , the limit of 0.5, suggested by Kofke and Wu,⁶¹ seems to be appropriate.

Relative Binding Affinities

Finally, we combined $\Delta G_{L_i}^{\text{SQM/MM} \rightarrow \text{DFT/MM}}$ estimated by the CA scheme (listed in Table S3) with the relative binding affinities at the SQM/MM level calculated in our previous work²⁰ ($\Delta G_{L_0 \rightarrow L_1}^{\text{SQM/MM}}$ in Eq. 3). In this way, the final relative affinities at DFT/MM level can be obtained, which are listed in Table 2. The SQM $\rightarrow$ DFT correction for the relative binding

Table 2: Relative binding affinities (kJ/mol). The DFT/MM results were obtained using CA with $N = 5\,000$. The SQM/MM results were obtained with four Λ values.²⁰

	DFT/MM	SQM/MM ²⁰	MM ²⁴	Exp. ⁶³
MeBz $\rightarrow$ Bz	12.7 $\pm$ 0.7	16.3 $\pm$ 0.5	15.9 $\pm$ 0.1	8.9 $\pm$ 0.5
EtBz $\rightarrow$ MeBz	2.3 $\pm$ 0.7	-1.2 $\pm$ 0.5	1.0 $\pm$ 0.1	1.7 $\pm$ 0.5
pClBz $\rightarrow$ Bz	19.4 $\pm$ 0.6	23.8 $\pm$ 0.5	19.1 $\pm$ 0.1	12.5 $\pm$ 0.2
mClBz $\rightarrow$ Bz	7.7 $\pm$ 0.7	10.3 $\pm$ 0.6	7.1 $\pm$ 0.1	6.4 $\pm$ 0.3
Hx $\rightarrow$ Bz	10.1 $\pm$ 0.7	9.5 $\pm$ 0.6	13.1 $\pm$ 0.3	7.9 $\pm$ 0.4
MeHx $\rightarrow$ Hx	16.6 $\pm$ 0.7	18.1 $\pm$ 0.6	15.4 $\pm$ 0.2	8.3 $\pm$ 0.4
Hx $\rightarrow$ Pen	8.5 $\pm$ 1.0	7.9 $\pm$ 0.9	7.5 $\pm$ 0.7	7.9 $\pm$ 0.4
Hep $\rightarrow$ Hx	6.5 $\pm$ 0.9	7.0 $\pm$ 0.8	5.5 $\pm$ 0.7	4.1 $\pm$ 0.3
MAD	3.3 $\pm$ 0.3	5.0 $\pm$ 0.2	3.6 $\pm$ 0.2	
R^2	0.97 $\pm$ 0.01	0.94 $\pm$ 0.01	0.96 $\pm$ 0.01	
τ_r	1.00 $\pm$ 0.00	0.75 $\pm$ 0.03	1.00 $\pm$ 0.00	

free energies (at $N = 5\,000$) amounts to 0.5–4.4 kJ/mol. It is larger for the aromatic ligands (2.6–4.4 kJ/mol) than for the other ligands (0.5–1.6 kJ/mol), including the Hx $\rightarrow$ Bz transformation. The precision of this correction is uniformly 0.4–0.5 kJ/mol.

In Figure 7, we compare the final relative binding free energies to the experimental results.⁶⁸ The performance of the estimated relative binding affinities in reproducing the experimental values were quantified by the mean absolute deviation (MAD), the correlation coefficient (R^2 , calculated with both directions of the perturbations) and Kendall’s rank correlation coefficient (τ_r , calculated only for the studied transformations; all differences are statistically significant at the 90% level). These quality criteria are also included in Table 2. It can be seen that the DFT relative free energies reproduce the experimental data quite well. The error is in a range of 0.6–8.3 kJ/mol, with the smallest error for EtBz $\rightarrow$ MeBz and the largest error for the MeHx $\rightarrow$ Hx transformation. On average, the MAD is 3.3 ± 0.3

kJ/mol, whereas the correlation is (R^2) is 0.97 ± 0.01 and τ_r equals to 1.0, indicating that all the eight transformations have the correct sign.

Figure 7: Comparison between the experimental and calculated relative free energies obtained with different methods (kJ/mol). The full line shows the perfect correlation.

Our previous results obtained at the SQM/MM and MM levels of theory are also presented in Figure 7 and Table 2. It can be seen that the DFT results are appreciably better than the PM6-DH+/MM results, which gave larger maximum error (11 kJ/mol for the pClBz→Bz transformation) and MAD (4.7 ± 0.2 kJ/mol), and gave an incorrect sign for one of the eight transformation. This shows that the SQM/MM→DFT/MM correction has worked properly. In addition, the DFT results are also slightly better than the MM results (MAD = 3.6 ± 0.2 and $R^2 = 0.96 \pm 0.01$), although the differences are not statistically significant. The DFT calculations improved the results for two transformations with poor MM results (MeBz→Bz and Hx→Bz), whereas the result is unchanged for one transformation with poor MM performance (pClBz→Bz) and slightly deteriorated for the MeHx→Hx transformation.

Direct MM→DFT/MM Transformations

As mentioned in the introduction, our previous attempts to converge a direct MM→DFT/MM perturbation with $N = 100$ failed completely.^{23,24} It is therefore interesting to also look at the convergence of a direct MM→DFT/MM perturbation in the present study with appreciably more frames. We chose the ligands with the poorest convergence in the SQM/MM→DFT/MM perturbation, i.e. MeBz and EtBz.

It can be seen from Figure 8 that the results of the MM→DFT/MM perturbation are quite similar to those of the SQM/MM→DFT/MM perturbation, e.g. with poorer convergence for ssEA than for CA. The SE (cf. Figure S1) is only slightly worse than for the SQM/MM→DFT/MM perturbation, decreasing from 0.4–0.9 to 0.2 kJ/mol when going from $N = 500$ to 10 000 for CA, showing rather smooth curves with the expected $1/\sqrt{N}$ dependence. However, for ssEA, the curves are much more jagged and the SE actually increases for EtBz in the bound state up to 1.0 kJ/mol with $N = 10\,000$.

Figure 8: $\Delta G_{Li,s}^{MM \rightarrow DFT/MM}$ for MeBz and EtBz in the unbound and bound states using ssEA (black) and CA (red).

This is also reflected for the $\Delta G_{Li,s}^{MM \rightarrow DFT/MM}$ results, which for the bound states show a variation of 14–19 kJ/mol depending on N . In particular, the ssEA results for EtBz drops by 9 kJ/mol between $N = 4\,500$ and $5\,000$, because a snapshot with a very low ΔU is found. For $\Delta G_{Li,u}^{MM \rightarrow DFT/MM}$, the variation is much smaller, 2–5 kJ/mol. Consequently, it converges to within 1 kJ/mol of the result at $N = 10\,000$ after 3 000–6 000 frames, even if the difference is statistically significant up to $N = 9\,500$ owing to the low SE for the CA calculations. For the bound simulations, the convergence is slower, requiring 7 000–8 500 frames. With $N = 10\,000$, the ssEA and CA results differ by 1.7–3.8 kJ/mol for all the simulations, except for that of EtBz in the unbound state (0.6 kJ/mol).

The Π convergence measure (shown in Figure S2) indicates that most of the perturbations are problematic, $-0.3 \leq \Pi \leq 0.6$, except at the beginning of the bound simulations, for which $0.7 \leq \Pi \leq 1.0$. This is also confirmed by $\sigma_{\Delta U}$, which is 3.0–3.4 RT , again except at the beginning of the bound simulations (1.9–2.6 RT). Using the CGS approach, requesting

an accuracy of 1 kJ/mol with 95% confidence, 26 000 – 96 000 snapshots are needed for convergence. Again, we see that $\sigma_{\Delta U}$ gives a more pessimistic view than Π (which actually indicates that EtBz in the unbound state is converged for $N \geq 9\,500$, compared to $N = 26\,000$ from $\sigma_{\Delta U}$). In this case, we tend to trust the more conservative $\sigma_{\Delta U}$, as the results show that Π may indicate too early convergence, e.g. for bound MeBz at $6\,500 \leq N \leq 8\,000$.

However, for CA, the CGS approach indicates that only 1 300 – 1 800 frames are needed for convergence. Clearly, this is an underestimate, as $N = 2\,000$ gives a difference of 11 kJ/mol for bound EtBz and 2–3 kJ/mol for the other simulations, compared to the results at $N = 10\,000$. The reason for this is that the distributions of ΔU are less Gaussian than for the SQM/MM→DFT/MM perturbation (cf. Figure S3). For lower ΔU values, most of these distributions have a tail that deviate largely from Gaussian distribution. Clearly, extensive convergence studies are needed to ensure convergence of CA.

Figure 9: Calculated relative binding free energy for the transformation from EtBz to MeBz at DFT/MM level.

Still, as can be seen in Figure 9, the net CA result at $N = 10\,000$ for the EtBz→MeBz

transformation using the direct MM→DFT/MM perturbation agrees the corresponding result employing the intermediate SQM/MM→DFT/MM perturbation to within 0.5 kJ/mol. However, it can also be seen that for $N = 7\,500 - 8500$, the difference is 2 kJ/mol, reflecting that the convergence of direct MM→DFT/MM perturbations is still somewhat questionable.

Conclusions

In a series of publications,^{20,24,26,27,32} we have studied the relative binding affinities of nine cyclic carboxylate ligands to the octa-acid deep-cavity host, taken from the SAMPL4 challenge.^{68,69} In the first work, we completely failed to obtain converged results, treating both the host and guest, as well as a significant number of water molecules at the DFT level.²⁴ These convergence problems were partly solved by using many more QM calculations, employing cumulant expansion and treating a smaller QM systems at the SQM/MM level of theory.³² Next, truly converged results were obtained by actually performing SQM/MM MD simulations with only the ligand in the SQM system,²⁰ and we have proposed two approaches to speed up such calculations.^{26,27}

In this study, we take a first step back towards the more accurate DFT level of theory, by showing that we can converge the SQM/MM→DFT/MM perturbation. We use the BLYP/6-31G* method for only the ligand. Using the cumulant expansion to the second order and interaction energies, 500–4500 DFT calculations per ligand are needed in order to reach a convergence within 1 kJ/mol and a precision of ~ 0.3 kJ/mol. With ssEA, the convergence is much slower and many more DFT calculations are needed for convergence (up to 44 000). Quite satisfying, the DFT results provide a significant improvement over the SQM/MM relative binding affinities, with a MAD of 3.4 kJ/mol, $R^2 = 0.97$ and with correct signs of all transformations, compared to experimental data. In fact, the results are also slightly better than the MM results, although the differences are not fully statistically significant.

We have also compared different criteria to determine whether the SQM/MM→DFT/MM perturbation is converged. We recommend the use of Wu and Kofke’s Π measure, with the criterion $\Pi > 0.5$ for converged results, as well as the standard deviation (σ) of the energy difference between the SQM/MM and DFT/MM potentials (ΔU). Assuming a Gaussian distribution of ΔU , a simple simulation (CGS) can estimate the number of DFT calculations needed to obtain a proper result within a desired accuracy and significance.⁶⁰ This approach can also be used for CA, although the needed number of calculations seems to be significantly underestimated.

The use of SQM/MM simulations as an intermediate step between MM and DFT/MM seems to be important. It significantly improves the convergence of the results and reduces the number of DFT calculations by a factor of two or more. It also speeds up the calculations. Table S6 shows the timing of the various steps of the calculations. The MM→SQM simulations take about 89 h on 20 CPUs, whereas the SQM→DFT corrections take 174 h, giving a total time consumption of 263 h. On the other hand, the direct calculations of the MM→DFT corrections take 361 h for the same system, i.e. $\sim 40\%$ more.

However, it should be noted that the use of a smaller QM system also improves the convergence: The SE of the CA results in Figure 8 are less than 1 kJ/mol already for 500 snapshots, although 60 000 snapshots were required for such a precision in our previous study, which employed also the host and the closest water molecules in the QM system.³² Therefore, we can expect poorer convergence if the QM system is enlarged and converging relative binding free energies at the DFT/MM level with large QM systems will continue to be a computational challenge.

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Supporting Information Available

Detailed results with the ssEA and CA schemes, estimated number of snapshots needed to obtain converged result with CGS method, computational cost for the various approaches, as well as detailed information for direct MM→DFT/MM transformations. The Supporting Information is available free of charge via the internet at <http://pubs.acs.org>.

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Graphical TOC Entry