SAMPL7 TrimerTrip host-guest binding affinities from extensive alchemical and end-point free energy calculations

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Abstract

The prediction of host-guest binding affinities with computational modelling is still a challenging task. In the 7th statistical assessment of the modeling of proteins and ligands (SAMPL) challenge, a new host named TrimerTrip is synthesized and the thermodynamic parameters of 16 structurally diverse guests binding to the host are characterized. The challenge provides only structures of the host and the guests, which indicates that the predictions of both the binding poses and the binding affinities are under assessment. In this work, starting from the binding poses obtained from our previous enhanced sampling simulations in the configurational space, we perform extensive alchemical and end-point free energy calculations to calculate the host-guest binding affinities. The alchemical predictions with two widely accepted charge schemes (i.e. AM1-BCC and RESP) are in good agreement with the experimental reference, while the end-point estimates show significant deviations. Surprisingly, the end-point MM/PBSA method seems very powerful in reproducing the experimental rank of binding affinities. Although the length of our simulations is already very long and the intermediate spacing is very dense, the convergence behavior is not very good, which may arise from the flexibility of the host molecule. Enhanced sampling techniques in the configurational space may be required to obtain fully converged sampling. Further, as the length of sampling in alchemical free energy calculations already achieves several hundred ns, performing direct simulations of the binding/unbinding event in the physical space could be more useful and insightful. More details about the binding pathway and mechanism could be obtained in this way. The nonequilibrium method could also be a nice choice if one insists to use the alchemical method, as the intermediate sampling is avoided to some extent.

Introduction

Understanding the interactions in the host-guest systems is at the center of drug discovery. Protein-ligand binding is a special case of host-guest interactions. The model host-guest systems are smaller and simpler than the protein-ligand complexes. Specifically, the interaction network and thus the binding/unbinding pathway in the host-guest complexes are simpler. Further, the host-guest binding affinities are comparable to the protein-ligand ones. Therefore, they serve as nice candidates to validate the experimental and computational protocols.[1-4] The statistical assessment of the modeling (SAMPL) challenges provide a series of challenging systems to assess the accuracy and efficiency of the computational modeling in various cases, e.g. solvation free energies, pKa, and protein-ligand binding, host-guest binding, and partition coefficients.[3, 5-9] The SAMPL challenges often include macrocyclic and rigid hosts and drug-like guests. In the past SAMPL challenges, for the binding affinities of host-guest systems, the computational modelling methods generally provide predictions with about 2 kcal/mol mean deviations from the experimental references.[3, 8]

The thermodynamic determinant of the host-guest binding is the free energy difference between different states.[10-13] Various methods are developed to calculate the free energy difference between the bound and unbound states. The alchemical method enables feasible calculations of the free energy difference between different states.[14-19] The procedure is quite simple to apply and the computational cost is affordable, as only one collective variable (CV) or order parameter is used to describe the alchemical transformation. The correct calculation of the free energy difference requires the accurate estimation of the partition functions or their ratio. The class of estimators relying on this relation includes free energy perturbation (FEP)[20] and its variants, including the Gaussian approximated FEP[21, 22] and acceptance ratio methods of BAR,[23, 24] UBAR,[25] RBAR,[25] and MBAR.[26, 27] Among these methods, BAR and MBAR are statistically optimal and thus are of the highest efficiency.[12, 13, 24, 28-32] Even with the same dataset, they converge better than the other methods. Alternatively, under reasonable approximations, other easier calculation methods are proposed. Integration methods integrate the ensemble averages of the derivatives of the Hamiltonians numerically. Although they theoretically rigorous when the integration step is infinitely small, in practical applications approximations are introduced due to the finite size of the integration step. The methods are often called thermodynamic integration (TI)[33-35] and the variants of TI are named according to their integration methods. The trapezoid rule is the most popular method for numerical integration. Other approximated methods include semi-empirical extensions of linear interaction energy[36-39] and quasi-equilibrium linear response approximation.[39, 40] If the transformation is performed reversibly, FEP and TI

reduce to the slow growth TI, where the reversible work in the transformation is used to estimate the free energy difference.[41-43] The nonequilibrium scenarios of FEP and BAR are Jarzynski's Identity (JI)[30, 44] and Crooks' Equation (CE).[45] The difference between them is that the instantaneous work is used in FEP and BAR while in JI and CE the nonequilibrium work accumulated in nonequilibrium pulling is used. Further extending the approximation leads to coarser methods such as the end-point free energy simulation methods of MM/PBSA[46] and MM/GBSA.[47] These methods are very efficient in computing the free energy difference but are of less accuracy.[48-52] Their most efficient and widely used scheme is the single-trajectory regime, which avoids the need for converging the fluctuation of the intra-molecular energies. Practically, in free energy calculations in drug discovery, these less detailed methods are often employed to screen the probably useful candidates, after which comprehensive free energy calculations with the alchemical method are applied to refine the dataset and find the most useful ones. The alchemical and end-point free energy calculations are efficient when the binding affinity is the only thermodynamic quantity of interest. These methods avoid the simulation of intermediate states in the binding/unbinding process and thus enhance the convergence. However, this is where the weakness lies. The details about the binding/unbinding pathway are unknown. Further, the simulation only explores the neighborhood of the starting configurations, which could lead to systematic bias that may not be eliminated in finite-time simulations.[16, 53-56] Biasing the sampling in the configurational space could partially eliminate this initial-configuration-related bias, but the selection of efficient CV is also a challenging task.[54, 57-59]

In the newest SAMPL7 challenge, the newly added TrimerTrip host is included and 16 structurally diverse guest molecules are synthesized. The detailed thermodynamic parameters are measured with isothermal titration calorimetry experiments.[1] This challenge includes only the structures of the host and the guest,[60] which indicates that the computational study should initiate from the prediction of possible binding poses. In our previous work, starting from randomly selected configurations, we employ the three-dimensional (3D) spherical-coordinate-(ρ,θ,φ) CV set to perform direct sampling of the binding/unbinding event in the host-guest systems.[61] The spherical coordinates enable a scan of the relative position of the two components of the complex (i.e. the host and the guest).[62, 63] In principle, the binding poses of the host-guest systems obtained from previous spherical-coordinates-biased simulations are more reliable than docking, as all-atom free-energy-weighted simulations are involved. Therefore, in the current work, these binding poses are used as the starting point of the end-point and alchemical free energy calculations. Two widely accepted charge schemes for small molecules are employed to construct the atomic charges of the host and the guests.

Methodology and Computational Details

System preparation. A central glycoluril trimer and two triptycene caps form the host TrimerTrip. The 16 guests targeting the host in the SAMPL7 challenge are simulated. In Fig. 1, we present an illustration of the host-guest binding and the chemical structures of the 16 guest molecules. The protonation states of the guest molecules are adjusted according to the experimental reference.[1] The experimental observation shows that there is no significant self-association for this host.[1] Therefore, in our simulation, the simulated systems are constructed according to the one-to-one binding protocol. Two widely accepted charge schemes including AM1-BCC[64] and RESP are used to parameterize the host and the guests. The general Amber force field (GAFF)[65] force field is used to obtain the other missing force field parameters. The starting configuration in the alchemical free energy calculation is obtained from the previous enhanced sampling simulations[61] at https://github.com/proszxppp/SAMPL7_TTP. The host-g8 complex is used as the reference complex in the relative free energy calculation, and its 3D structure is shown in Fig. 1. This structure of the host molecule is used in the parameterization of the host. As this structure differs from the one used for parameterization of the host in the previous spherical-coordinates-biased simulations,[61] the binding thermodynamics would differ from the previous work. The end-point free energy calculation starts from the first binding pose of each complex provided at the online depository https://github.com/proszxppp/SAMPL7_TTP. Each complex is solvated with TIP3P[66, 67] water molecules with the minimum distance between the box edge and the surface of the complex to be 12 Å. Periodicity is treated with periodic boundary conditions. Non-polarizable spherical counter ions[68, 69] of Na^+ or Cl^- are added for neutralization.

Alchemical free energy calculation. We perform the widely used relative free energy calculations to estimate the relative binding affinities of different guest molecules. The reference guest is set to g8. The difference between binding affinities could be estimated with the difference between the free energy change for mutating g8 to another guest in the solvated complex and that in solvent. The staging technique is used to enhance the convergence of the simulation. 21 equally spaced alchemical windows are used in each transformation. Namely, $\Delta\lambda = 0.05$, $\lambda_{\min} = 0.00$, and $\lambda_{\max} = 1.00$. The charge and vdW transformations are performed in a single-step fashion with the nonlinear separation-shifted softcore potential[42, 70-73] and the linear mixing rule. The simulation in each alchemical intermediate starts from 4000 cycles minimization with 5 kcal/mol/A^2 restraints on non-hydrogen atoms of solute, 6000 cycles minimization without any restraint, 1 ns NVT heating from 0 K to 298 K and 2 ns NPT equilibration. Later, 20 ns sampling with a sampling interval

of 2 ps is performed. The resulting overall simulation time for each alchemical mutation is about 483 ns, and the production run is 420 ns. Thus, the overall computational cost for each relative free energy calculation includes 483 ns sampling for solvated complex and 483 ns sampling for solvated ligands. To extract the results from the alchemical simulations, we used the statistically efficient perturbation estimator MBAR[26, 27] and the integration method TI. The autocorrelation time of the partial derivative of alchemical Hamiltonian $\left.\frac{\partial H}{\partial \lambda}\right|_{\lambda=\lambda_i}$ is calculated to extract independent samples for rigorous uncertainty estimation.[12, 13, 29, 74, 75]

End-point free energy calculation. The end-point methods of MM/PBSA and MM/GBSA[48] are also used to calculate the binding affinities. The starting structures are the first binding poses provided in the online depository in our previous work. For each host-guest complex, we performed 4000 cycles minimization with 5 kcal/mol/A^2 restraints on non-hydrogen atoms of solute, 6000 cycles minimization without any restraint, 1 ns NVT heating and 2 ns NPT equilibration, after which 100 ns production run with a sampling interval of 100 ps is performed. This sampling interval is much longer than the normally used ones. Thus, we take the obtained data points as uncorrelated samples. These independent configurations are then post-processed to calculate the gas-phase enthalpic changes and the changes of solvation free energies upon the host-guest binding. We use the MM/PBSA and MM/GBSA protocols in the end-point calculations. The GB model used in the GBSA calculation is the GB^{OBC} model.[76, 77]

In all MD simulations, the SHAKE[78] algorithm is used to constrain bonds involving hydrogen atoms,[79] and the time step for integrating the equations of motion is set to 2 fs. Langevin dynamics[80] with the collision frequency of 5 ps^{-1} are implemented for temperature regulation. The cutoff for non-bonded interactions in the real space is set to 10 Å, and the PME method is used to treat the long-range electrostatics.[81, 82] The AMBER[83] suite is used to perform MD simulations.

Result and discussion

The first thing to check in free energy calculations is the convergence. As the spacing of the alchemical windows in our simulations is already very dense, we then just check the time-dependence of the free energy estimates. In Fig. S1, the time-evolutions of the free energy estimates under two charge schemes are presented. We can see that for some systems the convergence behavior is good, while there are some cases that the free energy estimates seem still un-converged at the end of the simulation. Note that our simulation with 21 windows and 23 ns sampling in each window is already very long in modern alchemical free energy calculations. In previous SAMPL challenges such as the SAMPL6 challenge, the relative free energy

calculations used about 11 alchemical windows and several ns (e.g. 3 ns) sampling in each intermediate.[2] The resulting overall simulation time for each alchemical transformation is about an order of magnitude shorter than the current work. Therefore, our results could be used to approximate the long-time estimates of alchemical free energy calculation with the unbiased sampling technique. The systems with convergence problems may need enhanced sampling methods to aid the sampling in the configurational space and obtain fully converged estimates. We further visualize the trajectories to check the fluctuation of the complexes. In our previous work with enhanced sampling techniques in the configurational space, the fluctuation of the TrimerTrip pocket is observed to be large.[61] The TrimerTrip host in the current unbiased simulation experiences some degrees of fluctuations, but the magnitude is relatively small, which also indicates the poor convergence behavior of the sampling. Further, when the sampling achieves such a time scale (i.e. ~500 ns), performing alchemical free energy calculation is already not a computationally efficient choice. Note that the calculation of each relative binding affinity includes two parts of simulations (i.e. solvated complex and solvated ligands), which further increases the computational cost. Direct simulation of the binding/unbinding event with enhanced sampling techniques could converge within such a time scale, and much more details about the binding/unbinding pathway and mechanism could be obtained. The nonequilibrium technique could be useful in dealing with this convergence problem of the alchemical method, as the sampling in the intermediate states is avoided to some extent. The end-point free energy calculations also employ a long-sampling-time protocol (i.e. 100 ns sampling and 100 ps sampling interval). Therefore, the end-point results could also be used as the long-time estimates.

In Table 1 and 2, the predicted binding affinities from alchemical free energy calculations are presented. We use the mean signed error (MSE), the mean absolute error (MAE), the root-mean-squared error (RMSE) to assess the deviations of the predicted binding affinities from the experimental reference. To compare the consistency of the predicted ranks of binding affinities and the experimental one, we calculate the Kendall τ rank coefficient and the Pearlman's predictive index (PI). Under both charge schemes, the error estimates are about 2.5-3 kcal/mol. Therefore, RMSE and other mean deviations are comparable to the SAMPL6 cases.[3, 8, 62] The ranking coefficients are about 0.2, which indicates that the predicted ranks show some similarity with the experimental reference, but the consistency is not good. The results obtained with RESP charges are better than the AM1-BCC ones, but the improvement is very small. Interestingly, although the error estimates of TI are comparable to the MBAR ones, the ranking coefficients of TI are better than MBAR. Upon convergence, these two methods should provide identical results, which also indicates that the degree of convergence is not very good.

These error estimates are larger than the ones reported in our previous work. One possible reason is that the free energy estimates obtained with the alchemical method may have convergence problems. As a result, the current estimates may have a systematic bias (e.g. initial-configuration-induced bias) and thus are not directly comparable to our previous predictions with enhanced sampling techniques in the configurational space.[61] Another possible reason is the difference between the configurations used in parameterizing the host and the guests. The structure of the host used in the current parameterization is the one in the host-g8 bound structure obtained from our previous work. This difference leads to different atomic charges and thus the outcome of the simulation.

As for the end-point free energy calculations, the detailed results are summarized in Table S1 and S2, and the relative binding affinities are given in Table 1 and 2. For both charge schemes, the MM/PBSA method outperforms the MM/GBSA one. With AM1-BCC charges, RMSE of MM/PBSA is about 1.4 kcal/mol smaller than the MM/GBSA one. The ranking coefficients of MM/PBSA are all larger than zero, which indicates that the predicted rank is in agreement with the experimental one to some extent. By contrast, the ranking coefficients obtained from MM/GBSA are near-zero, which suggests that the predicted rank shows random behavior and is uncorrelated with the experimental reference. When the RESP method is used to parameterize the systems, improvements are observed, especially for the MM/PBSA method. Its RMSE decreases by about 3 kcal/mol, and the ranking coefficients are also improved significantly. The ranking prediction with MM/PBSA and RESP charges is the best one among all protocols employed in the current work. The MM/GBSA estimates improve about 1 kcal/mol for RMSE and about 0.3 for the ranking metric PI with RESP charges.

The prediction-experiment correlation plots are presented in Fig. 2. The alchemical estimates (TI and MBAR) scatter around the y=x line, while the end-point estimates (MM/PBSA and MM/GBSA) show significant deviations. The MM/PBSA estimates with AM1-BCC charges seem to consistently overestimate the binding affinities, and the MM/GBSA ones also have this tendency. However, the end-point estimates with RESP charges do not have this behavior, which indicates that the RESP charges could perform better when dealing with host-guest systems.

Conclusion

In this work, we performed alchemical and end-point free energy calculations on the TrimerTrip-guest complexes. The binding poses obtained from our previous enhanced sampling simulations in the configurational space are used as the starting structures. Two widely accepted charge schemes are used to

describe the host and the guests. The predicted binding affinities with the alchemical method are in agreement with the experimental reference, while the end-point methods provide predictions with significant deviations. The various mean deviations with the alchemical method are about 2.5 kcal/mol and the end-point ones are much larger, which is similar to the SAMPL6 cases. Surprisingly, the end-point MM/PBSA method outperforms the other methods in the prediction of the rank of binding affinities. The performance of the RESP charge scheme is better than the AM1-BCC one. The improvement is small for the alchemical method, but is remarkable when using the MM/PBSA method. Another interesting observation is that the convergence behavior of the TrimerTrip-guest systems is not very good. Although our alchemical free energy simulation employs dense intermediate spacing ($\Delta\lambda = 0.05$) and long simulation times in each intermediate (23 ns), the free energy estimates seem still un-converged. This phenomenon may arise from the flexibility of the host molecule and enhanced sampling techniques in the configurational space may be required to get fully converged results. Further, the computational cost of the current alchemical free energy calculation is already very high (i.e. sampling time ~ 500 ns). Note that the relative free energy calculation includes two parts (i.e. solvated complex and solvated ligands), which further increases the computational cost. With these computational resources, directly simulating the binding/unbinding event in the physical space could be more insightful, as more details about the binding pathway and mechanism could be obtained. Another choice could be the nonequilibrium method. Coupling it with the alchemical method could help the sampling problem to some extent, as the extensive sampling in the intermediate states is avoided.

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Conflicts of interest

There are no conflicts of interest to declare.

Supporting information

The time evolutions of the binding affinities obtained with the alchemical free energy calculation with two charge schemes and the detailed free energy components of MM/PBSA and MM/GBSA estimates with

two charge schemes are given in the supporting information.

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Table 1. The TrimerTrip-guest binding free energies (in kcal/mol) computed via various methods ($\Delta\Delta G_{TI}$, $\Delta\Delta G_{MBAR}$, $\Delta\Delta G_{\mathrm{MM/PBSA}}$, and $\Delta\Delta G_{\mathrm{MM/GBSA}}$) with the AM1-BCC charges and the corresponding experimental values (ΔG_{EXP}, $\Delta\Delta G_{EXP}$). All values are in kcal/mol. MSE, MAE, RMSE, τ and PI serve as quality measurements. The guest g8 is used as reference and thus its values are all zeros.

Guest	ΔG_{EXP}	$\Delta\Delta G_{\mathrm{EXP}}$	$\Delta\Delta G_{\mathrm{TI}}$	SD	$\Delta\Delta G_{\mathrm{MBAR}}$	SD	$\Delta\Delta G_{\mathrm{MM/PBSA}}$	SD	$\Delta\Delta G_{\mathrm{MM/GBSA}}$	SD
g1	-6.1	3.4	2.6	0.3	0.9	0.6	7.6	0.3	1.7	0.3
g2	-8.3	1.1	-1.5	0.3	-3.1	0.8	4.9	0.3	1.8	0.3
g3	-10.1	-0.6	-2.2	0.2	-0.9	0.5	0.3	0.2	-0.5	0.3
g5	-11.1	-1.7	-1.2	0.3	-0.9	0.5	3.0	0.3	1.6	0.2
g6	-9.6	-0.2	-3.0	0.6	-2.6	0.4	7.0	0.2	14.6	0.3
g7	-6.5	3.0	2.0	0.4	2.0	0.8	6.4	0.3	-1.6	0.3
g8	-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g9	-7.6	1.9	3.7	0.5	3.4	0.4	11.5	0.2	14.7	0.3
g10	-8.2	1.3	-2.2	0.4	-2.5	0.7	4.2	0.3	-0.9	0.3
g11	-9.0	0.4	0.3	0.5	0.3	0.4	6.7	0.3	10.4	0.3
g12	-8.3	1.2	-1.0	0.5	-1.0	0.3	9.2	0.2	11.0	0.2
g15	-10.5	-1.1	-4.8	0.3	-5.2	0.4	10.3	0.2	15.8	0.2
g16	-11.5	-2.1	2.5	0.3	2.3	0.4	4.2	0.3	5.0	0.3
g17	-11.8	-2.4	3.6	0.4	4.2	0.5	6.1	0.3	7.3	0.3
g18	-10.6	-1.1	-0.5	0.3	-3.0	0.5	15.3	0.2	8.4	0.2
g19	-11.7	-2.3	-7.7	0.3	-7.2	0.5	-1.9	0.2	4.8	0.2
RMSE			3.0		3.2		7.2		8.6	
MSE			0.6		0.9		-5.9		-5.8	
MAE			2.3		2.5		5.9		6.9	
τ			0.1		0.1		0.3		-0.1	
PI			0.3		0.2		0.4		0.0	

Table 2. The TrimerTrip-guest binding free energies (in kcal/mol) computed via various methods ($\Delta\Delta G_{TI}$, $\Delta\Delta G_{MBAR}$, $\Delta\Delta G_{\mathrm{MM/PBSA}}$, and $\Delta\Delta G_{\mathrm{MM/GBSA}}$) with the RESP charges and the corresponding experimental values (ΔG_{EXP}, $\Delta\Delta G_{EXP}$). All values are in kcal/mol. MSE, MAE, RMSE, τ and PI serve as quality measurements. The guest g8 is used as reference and thus its values are all zeros.

Guest	ΔG_{EXP}	$\Delta\Delta G_{\mathrm{EXP}}$	$\Delta\Delta G_{\mathrm{TI}}$	SD	$\Delta\Delta G_{\mathrm{MBAR}}$	SD	$\Delta\Delta G_{\mathrm{MM/PBSA}}$	SD	$\Delta\Delta G_{\mathrm{MM/GBSA}}$	SD
g1	-6.1	3.4	5.9	0.2	4.4	0.7	11.4	0.2	15.7	0.2
g2	-8.3	1.1	0.3	0.2	1.1	0.9	-0.1	0.2	-2.4	0.2
g3	-10.1	-0.6	-2.4	0.2	-1.9	0.9	-4.5	0.2	-5.6	0.2
g5	-11.1	-1.7	-1.4	0.2	-0.8	0.6	-0.7	0.2	-2.5	0.2
g6	-9.6	-0.2	-2.0	0.6	-1.7	0.4	-0.5	0.2	-7.8	0.2
g7	-6.5	3.0	6.7	0.2	7.4	0.5	6.7	0.2	-3.5	0.2
g8	-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g9	-7.6	1.9	1.0	0.5	-0.3	0.4	6.0	0.2	-0.5	0.2
g10	-8.2	1.3	-0.3	0.2	-2.1	0.6	1.6	0.3	-13.6	0.3
g11	-9.0	0.4	0.1	0.5	-1.2	0.6	1.3	0.2	-2.2	0.2
g12	-8.3	1.2	-2.5	0.4	-2.7	0.3	1.7	0.2	-3.3	0.2
g15	-10.5	-1.1	-1.4	0.3	-1.8	0.4	0.3	0.2	-6.2	0.2
g16	-11.5	-2.1	1.5	0.4	1.8	0.7	1.7	0.2	1.6	0.2
g17	-11.8	-2.4	2.9	0.3	2.9	0.5	1.8	0.2	2.3	0.2
g18	-10.6	-1.1	3.9	0.4	1.5	0.5	6.7	0.2	-1.4	0.2
g19	-11.7	-2.3	-5.0	0.5	-5.6	0.5	-11.3	0.2	-21.0	0.2
RMSE			2.7		2.7		4.3		7.7	
MSE			-0.4		0.0		-1.3		3.2	
MAE			2.1		2.3		3.1		5.8	
τ			0.2		0.0		0.4		-0.1	
PI			0.4		0.3		0.7		0.3	

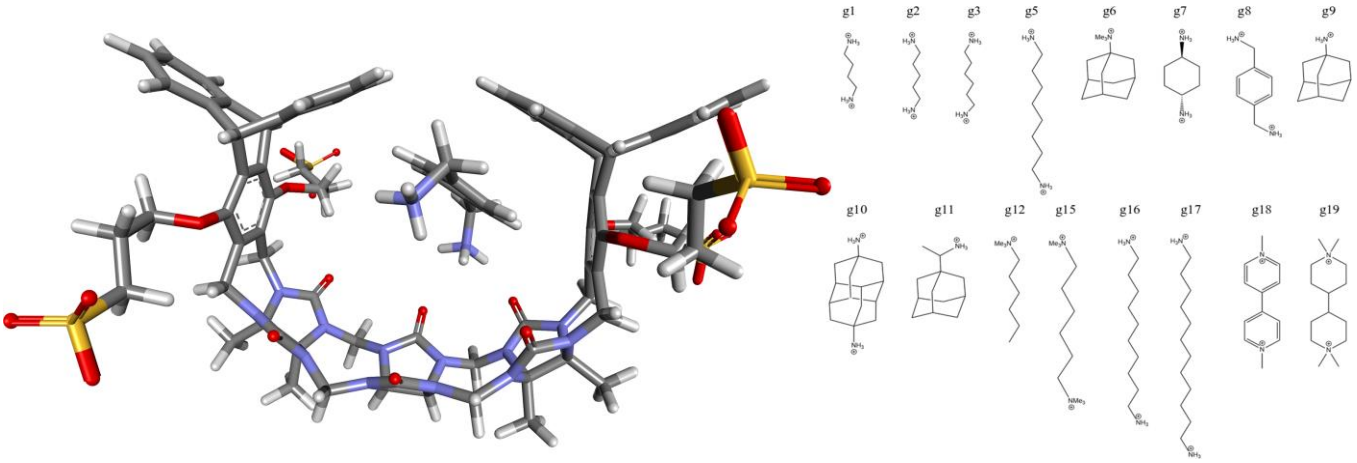

Fig. 1. An illustration of the host-guest binding (left) and the 16 guests (right) of the host TrimerTrip. The host-g8 complex is used as the reference structure in the alchemical free energy calculation and thus is used to generate the picture.

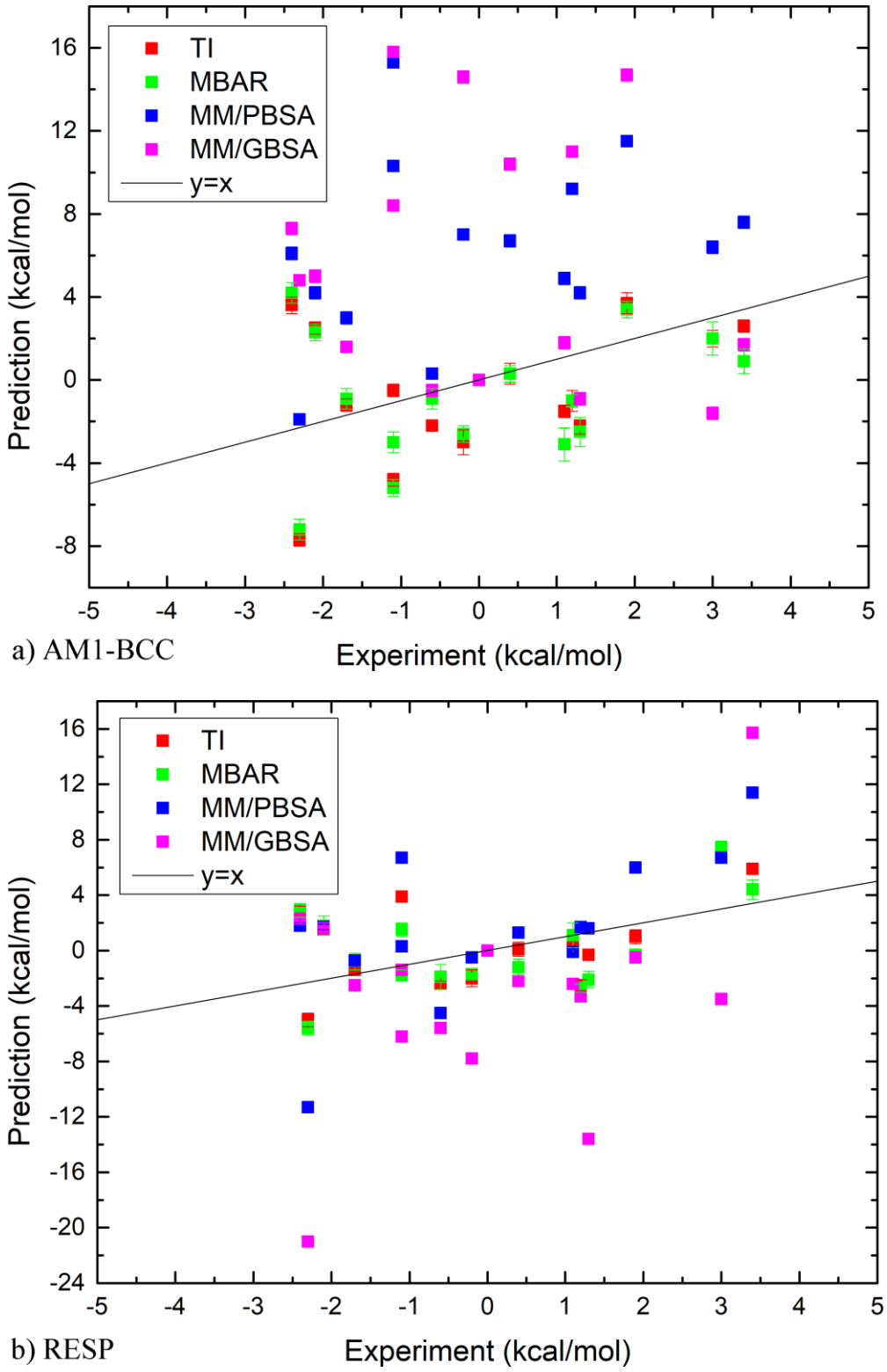

Fig. 2. Correlation between the predicted binding free energies and the experimental references for TrimerTrip-guest systems constructed with a) AM1-BCC charges and b) RESP charges. The exact values of the binding affinities are presented in Table 1 and 2.

Supporting Information: SAMPL7 TrimerTrip host-guest binding affinities from extensive alchemical and end-point free energy calculations

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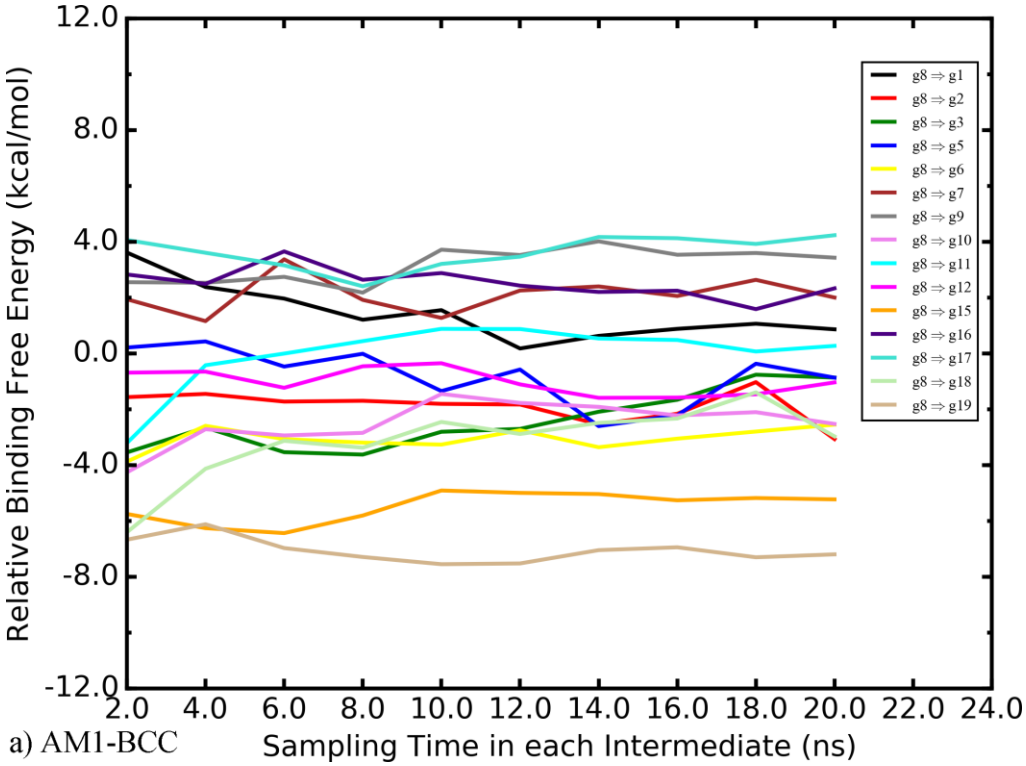
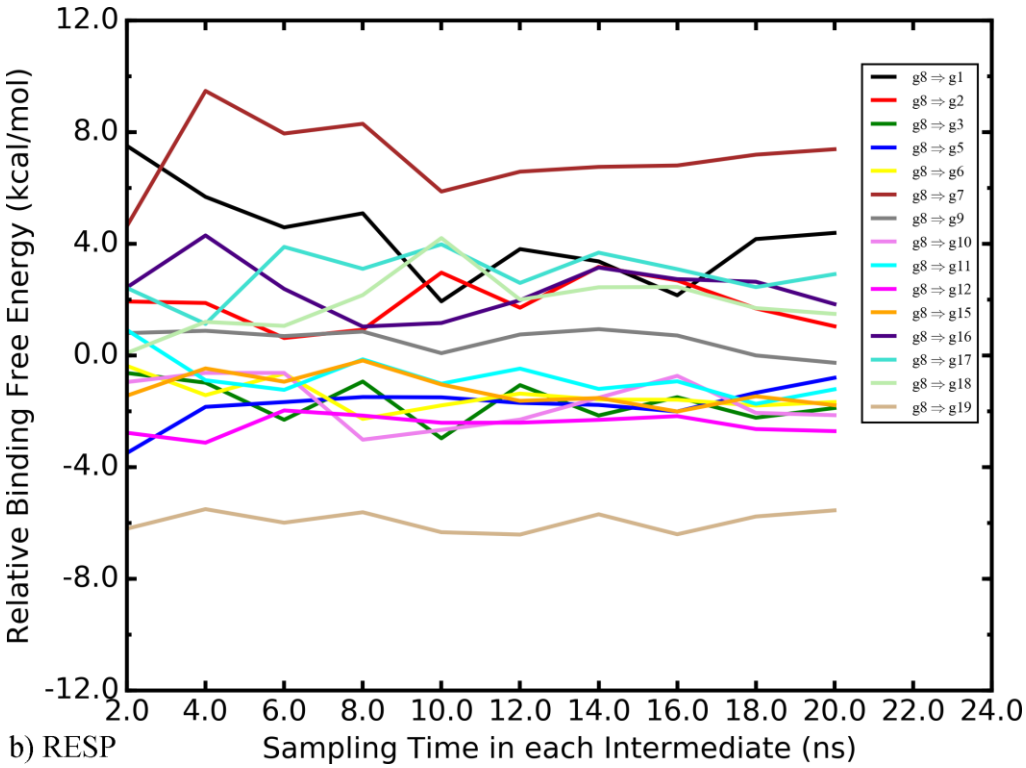
Fig. S1. Time evolution of the relative binding affinities obtained with alchemical free energy calculations different charge schemes: a) AM1-BCC and b) RESP.



a) AM1-BCC



b) RESP

Table S1. Detailed free energy components from MM/PBSA and MM/GBSA with AM1-BCC charges. ΔH_{gas} is the gas-phase enthalpy change upon binding or the protein-ligand interaction energy. ΔG_{sol} is the solvation free energy and the subscripts of PB and GB denote the implicit solvent model used to calculate the solvation free energy. $\Delta\Delta G_{\mathrm{MM/PBSA}}$ and $\Delta\Delta G_{\mathrm{MM/GBSA}}$ are the relative binding affinity with the guest g8 as the reference.

Guest	ΔH_{gas}	SD	$\Delta G_{sol,PBSA}$	SD	$\Delta G_{sol,GBSA}$	SD	$\Delta G_{\mathrm{MM/PBSA}}$	SD	$\Delta G_{\mathrm{MM/GBSA}}$	SD	$\Delta\Delta G_{\mathrm{MM/PBSA}}$	SD	$\Delta\Delta G_{\mathrm{MM/GBSA}}$	SD
g1	-469.7	1.1	451.3	1.0	426.2	1.0	-18.4	0.2	-43.5	0.2	7.6	0.3	1.7	0.3
g2	-468.2	1.2	447.1	1.0	424.8	1.0	-21.1	0.2	-43.4	0.2	4.9	0.3	1.8	0.3
g3	-461.8	0.9	436.0	0.8	416.1	0.8	-25.7	0.1	-45.7	0.2	0.3	0.2	-0.5	0.3
g5	-451.3	0.9	428.2	0.8	407.7	0.8	-23.0	0.2	-43.6	0.2	3.0	0.3	1.6	0.3
g6	-227.6	0.3	208.6	0.3	197.1	0.3	-19.0	0.1	-30.6	0.1	7.0	0.2	14.6	0.2
g7	-474.7	1.3	455.1	1.1	428.0	1.1	-19.6	0.3	-46.8	0.2	6.4	0.3	-1.6	0.3
g8	-468.4	1.0	442.4	0.9	423.2	0.8	-26.0	0.2	-45.2	0.2	0.0	0.0	0.0	0.0
g9	-225.6	0.6	211.2	0.5	195.1	0.5	-14.5	0.1	-30.5	0.2	11.5	0.2	14.7	0.3
g10	-450.6	1.3	428.8	1.1	404.5	1.1	-21.8	0.2	-46.1	0.2	4.2	0.3	-0.9	0.3
g11	-241.9	0.7	222.6	0.6	207.1	0.6	-19.3	0.2	-34.8	0.2	6.7	0.3	10.4	0.3
g12	-231.1	0.4	214.3	0.3	196.9	0.4	-16.8	0.1	-34.2	0.1	9.2	0.2	11.0	0.2
g15	-422.8	0.8	407.1	0.7	393.4	0.8	-15.7	0.1	-29.4	0.1	10.3	0.2	15.8	0.2
g16	-423.8	0.8	402.0	0.7	383.6	0.7	-21.8	0.2	-40.2	0.2	4.2	0.3	5.0	0.3
g17	-396.4	0.9	376.5	0.8	358.5	0.8	-19.9	0.2	-37.9	0.2	6.1	0.3	7.3	0.3
g18	-441.7	0.9	431.0	0.8	404.9	0.8	-10.7	0.1	-36.8	0.1	15.3	0.2	8.4	0.2
g19	-466.4	0.6	438.5	0.5	426.0	0.6	-27.9	0.1	-40.4	0.1	-1.9	0.2	4.8	0.2

Table S2. Detailed free energy components from MM/PBSA and MM/GBSA with RESP charges. ΔH_{gas} is the gas-phase enthalpy change upon binding or the protein-ligand interaction energy. ΔG_{sol} is the solvation free energy and the subscripts of PB and GB denote the implicit solvent model used to calculate the solvation free energy. $\Delta\Delta G_{\mathrm{MM/PBSA}}$ and $\Delta\Delta G_{\mathrm{MM/GBSA}}$ are the relative binding affinity with the guest g8 as the reference.

Guest	ΔH_{gas}	SD	$\Delta G_{sol,PBSA}$	SD	$\Delta G_{sol,GBSA}$	SD	$\Delta G_{\mathrm{MM/PBSA}}$	SD	$\Delta G_{\mathrm{MM/GBSA}}$	SD	$\Delta\Delta G_{\mathrm{MM/PBSA}}$	SD	$\Delta\Delta G_{\mathrm{MM/GBSA}}$	SD
g1	-416.1	1.1	408.1	1.0	393.8	1.0	-8.0	0.1	-22.3	0.1	11.4	0.2	15.7	0.2
g2	-455.3	0.8	433.9	0.8	412.9	0.7	-19.5	0.2	-40.5	0.2	-0.1	0.2	-2.4	0.2
g3	-462.5	1.0	438.7	0.9	418.9	0.9	-23.8	0.1	-43.7	0.1	-4.5	0.2	-5.6	0.2
g5	-445.1	0.8	425.1	0.7	404.5	0.7	-20.0	0.1	-40.6	0.1	-0.7	0.2	-2.5	0.2
g6	-229.5	0.3	209.7	0.3	183.7	0.3	-19.9	0.1	-45.8	0.1	-0.5	0.2	-7.8	0.2
g7	-442.8	0.6	430.2	0.6	401.3	0.5	-12.7	0.1	-41.6	0.1	6.7	0.2	-3.5	0.2
g8	-458.8	1.0	439.5	0.9	420.8	0.8	-19.3	0.2	-38.1	0.2	0.0	0.0	0.0	0.0
g9	-222.4	0.4	209.0	0.4	183.8	0.3	-13.4	0.1	-38.6	0.2	6.0	0.2	-0.5	0.2
g10	-451.8	1.0	434.1	0.9	400.2	0.8	-17.7	0.2	-51.6	0.2	1.6	0.3	-13.6	0.3
g11	-235.3	0.6	217.3	0.5	195.1	0.5	-18.0	0.2	-40.2	0.2	1.3	0.2	-2.2	0.2
g12	-235.3	0.3	217.7	0.3	194.0	0.3	-17.6	0.1	-41.4	0.2	1.7	0.2	-3.3	0.2
g15	-435.7	0.8	426.6	0.7	391.4	0.7	-19.1	0.1	-44.3	0.2	0.3	0.2	-6.2	0.2
g16	-417.0	0.9	399.3	0.8	380.5	0.8	-17.6	0.1	-36.5	0.1	1.7	0.2	1.6	0.2
g17	-390.8	0.9	373.2	0.8	355.0	0.8	-17.6	0.1	-35.8	0.1	1.8	0.2	2.3	0.2
g18	-448.7	0.7	436.1	0.6	409.2	0.6	-12.6	0.1	-39.5	0.1	6.7	0.2	-1.4	0.2
g19	-474.8	0.6	444.2	0.5	415.7	0.5	-30.6	0.1	-59.0	0.1	-11.3	0.2	-21.0	0.2