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Superimposing the 27 crystal protein/inhibitor complexes of β-secretase to calculate the binding affinities by the linear interaction energy method

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ABSTRACT

BACE-1 is an important target for designing therapeutic agents for the treatment of Alzheimer's disease. An improved linear interaction energy (LIE) model has been developed to calculate the binding free energies of β-secretase (BACE-1) by superimposing the 27 crystal BACE-1/inhibitor complexes to put a diverse set of 27 co-crystallized ligands into the binding pocket. These co-crystallized conformations of ligands were set as the initial binding conformations for LIE simulation. The effects of two protein conformations (i.e., 1W51 and 1FKN), two sampling methods (i.e., energy minimization and hybrid Monte Carlo [HMC]), and energy terms were studied. Using 1W51 crystal structure and HMC sampling technique, the best binding affinity model for the full set of ligands was found to have a root-mean-square error of 0.996 kcal/mol.

It is generally accepted that the accumulation of Aβ in the brain is toxic and supposed to induce pathologies of Alzheimer's disease (AD). Since BACE-1 is the rate-limiting enzyme in the production of Aβ, it has been implicated as a promising therapeutic target for the development of a disease-modifying therapy for AD.[1–3] To date, a number of potent BACE inhibitors have already been reported.[4–8] Younge and Reynolds proposed a new linear binding affinity model of BACE-1 inhibitors based on the generalized Born approximation of electrostatic salvation, they validated on a set of 13 hydroxyethylene-based BACE-1 inhibitors, and observed a root-mean-square error (RMSE) of 1.101 kcal/mol using a three-parameter model.[9] Huang and Caflisch also calculated the binding free energy of the same set of 13 BACE-1 inhibitors by a simple energy minimization using CHARMM, they observed an RMSE of 1.0 kcal/mol using a two-parameter model.[10] Although both Reynolds et al. and Caflisch et al. achieved a good agreement between simulation and experimentation, the following issues still have to be resolved. First, in both studies, the co-crystallized structures of the ligands, except OM99-2 and OM00-3, were not available at that time, therefore, the initial binding conformations were modeled manually according to the binding mode of OM99-2 or OM00-3. It is known that different initial ligand binding poses may lead to different LIE results, docking ligands manually might not be accurate, thus, it would be worth carrying out a systematic study based on the variant initial ligand bindings. Second, many researches using the LIE method for drug design have a small training set nowadays.[11–13] A previous study suggested that a larger data set containing at least 27 data points is required to obtain a good estimate on three variables in the LIE method.[14] Using a set of only 13 BACE-1 inhibitors to develop a three-parameter model, as performed in previous studies, might over-fit the data. Third, the main advantage of the LIE method is to calculate the free energy for ligands with diverse chemical structures. The 13 BACE-1 inhibitors used in both Reynolds et al. and Caflisch's et al. have a similar backbone structure based on hydroxyethylene. In order to develop a more effective and accurate LIE model, we replaced the docking method by superimposing 27 crystal BACE-1/inhibitor complexes to put a diverse set of 27 co-crystallized ligands into the binding pocket. These co-crystallized ligand conformations were set as the initial binding conformations for LIE simulation. As a follow-up, we examined the effect of two different protein conformations (i.e., 1W51[15] and 1FKN[16]), two sampling methods (i.e., HMC and minimization) and energy terms on the LIE results.

So far, 100 X-ray structures of BACE-1/inhibitor complexes have already been released.[17] Complied by the diverse selection of the co-crystallized ligands, we chose a set of 27 crystal structures of BACE-1/inhibitor complexes (i.e., 1W51, 1FKN, 1M4H, 1TQF, 1XS7, 1YM2, 1YM4, 2B8L, 2B8V, 2F3F, 2FDP, 2IQG, 2IS0, 2OAH, 2OHL, 2OHM, 2OHP, 2OHQ, 2OHR, 2OHS, 2OHT, 2OHU, 2P4J, 2QK5, 2QZL, 2VNM, and 2VNN), and downloaded them from the PDB database.[17] The experimental binding affinities in the forms of K_i and IC_{50} were taken from the Binding DB database.[18] The experimental binding free energies were calculated using the following equation:

$$\Delta G_{bind} = RT \ln K_{dissociated} = RT \ln(IC_{50} + 0.5C_{enzyme}) \approx RT \ln IC_{50} \quad (1)$$

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where R is the ideal gas constant; T is the temperature in K; and C_{enzyme} is the concentration of the enzyme, which was a very small value after equilibration and can be omitted in most cases.

1W51 and 1FKN were used as the reference structures, they were prepared using the protein preparation tools in Maestro.[19] We adjusted the active site aspartates so that Asp32 was protonated, while Asp228 was ionized. Using the program Accelrys DS viewer (version 1.7, Accelrys Inc.), we superimposed the A chain of the other 26 X-ray structures with the reference structures 1W51 and 1FKN, respectively. The 27 co-crystallized ligands of BACE-1 were automatically put into the active pocket of 1W51 or 1FKN (Fig. 1). Thus, the crystal conformations of all the ligands were retained, and they were used as the initial binding conformations in the LIE simulation.

The energy calculations were carried out using the Liaison package from Schrodinger Inc.[20] This implementation of LIE made use of a surface generalized Born (SGB) continuum solvation model. All charges were treated using the OPLS-2001 force field. We found that compounds **1**–**4**, **7**–**14**, **16**–**21**, **22** and **24**–27 all have groups that might be ionized at neutral pH. To obtain the average LIE energies, two sampling methods, energy minimization and HMC were employed. The HMC step employed 20 ps of heating, 300 K of sample target temperature, and 30 ps of sampling for the LIE energies (total simulation time = 50 ps). For this set of HMC simulations, the time step was 0.002 ps.

The two-parameter (Eq. (2)) and three-parameter (Eq. (3)) models for fitting the calculated energy terms to the experimental free energies of binding are shown in the following:

$$\Delta G = \alpha(\langle E^{vdW}\rangle_{bound} - \langle E^{vdW}\rangle_{free}) + (\beta(\langle E^{elec}\rangle_{bound} - \langle E^{elec}\rangle_{free}) \quad (2)$$

$$\Delta G = \alpha(\langle E^{vdW}\rangle_{bound} - \langle E^{vdW}\rangle_{free}) + (\beta\langle E^{elec}\rangle_{bound} - \langle E^{elec}\rangle_{free}) + \gamma(\langle E^{cav}\rangle_{bound} - \langle E^{cav}\rangle_{free}) \quad (3)$$

The parameters obtained by partial-least-square fitting and energy values to Eqs. (2) and (3) is given in Table 2. The correlation between LIE binding energies and experimental values is shown in Figures 2–4.

In this study, an improved LIE model that produces a high q^2 and a low RMSE between the calculated and observed binding affinities has been developed. This is the three-parameter model

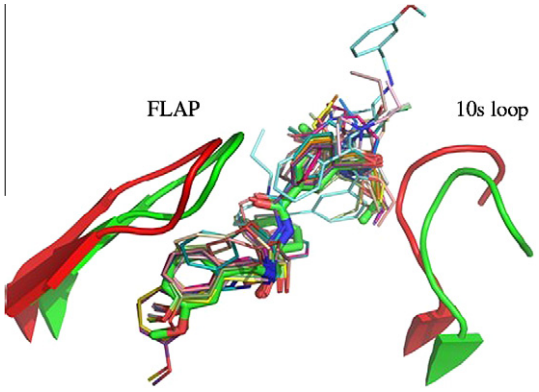


Figure 1. The 27 co-crystallized BACE-1 inhibitors superimposed with flap (residues 68–74, left) and 10s (residues 9–14, right). 1FKN, green; 1W51, red.

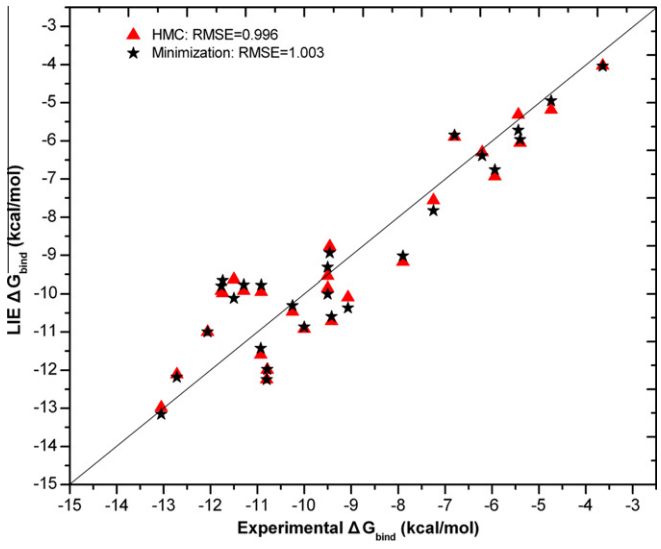


Figure 2. Comparison of the calculated versus experimental binding free energies for 27 BACE-1 inhibitors with two different sampling methods (minimization and HMC), using protein conformation 1W51.

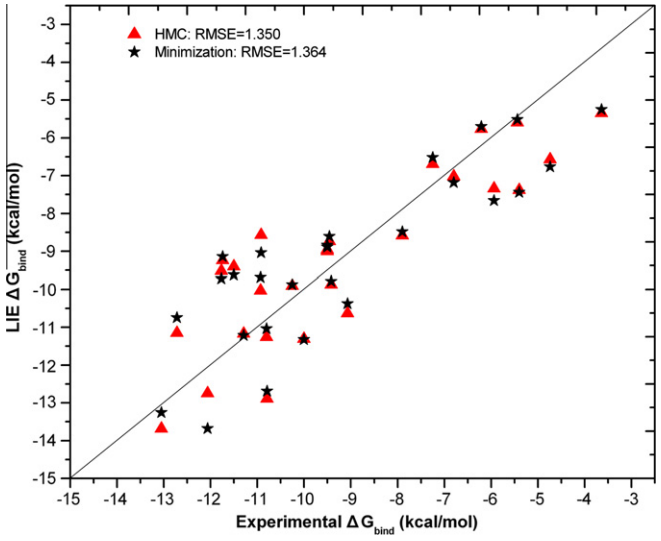


Figure 3. Comparison of the calculated versus experimental binding free energies for 27 BACE-1 inhibitors with two different sampling methods (minimization and HMC), using protein conformation 1FKN.

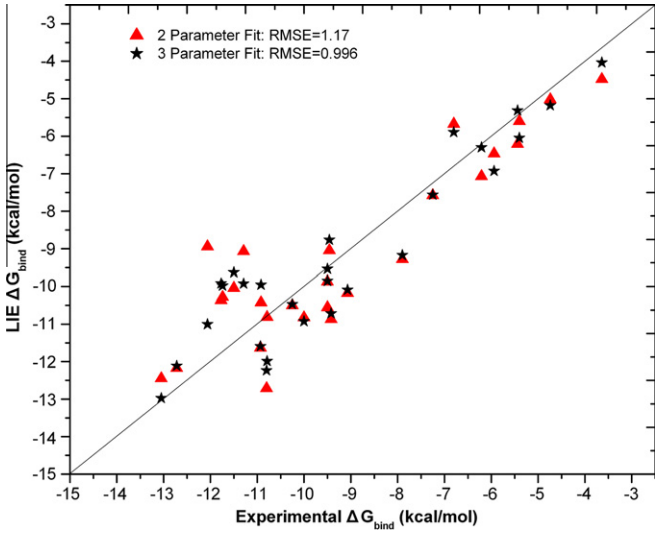
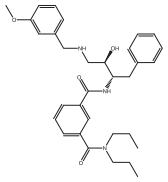
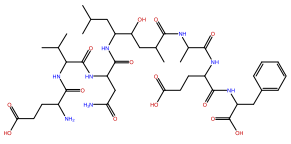
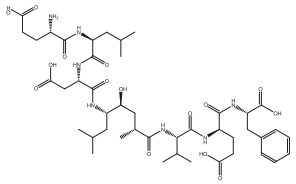
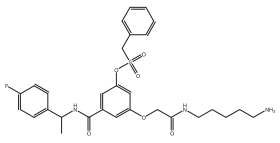
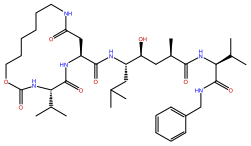
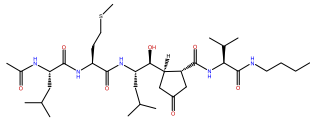
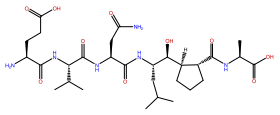
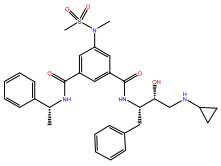
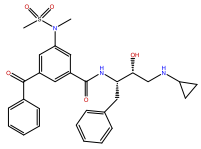
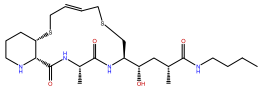
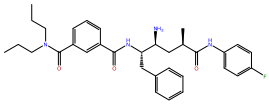
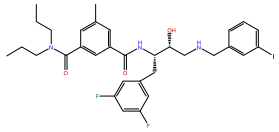
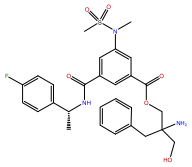
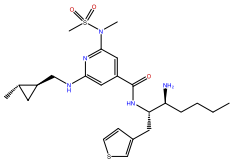
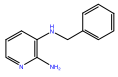
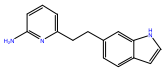
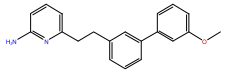
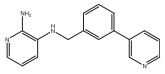
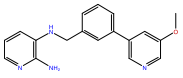
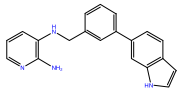
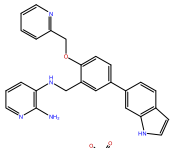
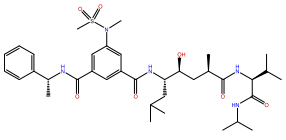
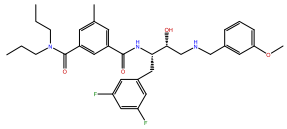
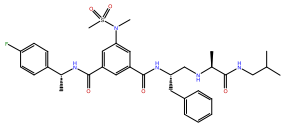
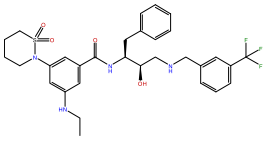
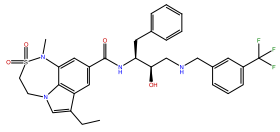


Figure 4. Comparison of the calculated versus experimental binding free energies for two variants of the LIE equation. The three-parameter fit uses Eq. (3), whereas two-parameter fit uses only the van der Waals and electrostatic terms. Protein conformation: 1W51; sampling method: HMC.

Table 1
Data set of 27 co-crystallized ligands of BACE-1 employed in the LIE studies

Compound number (PDB code, name and ΔG^{Expt} (kcal/mol))	Chemical structure	Compound number (PDB code, name and ΔG^{Expt} (kcal/mol))	Chemical structure	Compound number (PDB code, name and ΔG^{Expt} (kcal/mol))	Chemical structure
1 (1W51, L011506, −**9.5**)		**2** (1FKN, OM99-2, −**12.6**)		**3** (1M4H, OM00-3, −**13.05**)	
4 (1TQF, L124,671, −**7.9**)		**5** (1XS7, amide-urethane, −**10.79**)		**6** (1YM2, NVP-AUR200, −**10.8**)	
7 (1YM4, NVP-AMK640, −**10**)		**8** (2B8L, L-000384950, −**9.42**)		**9** (2B8V, L-000430469, −**9.46**)	
10 (2F3F, BDF488, −**9.07**)		**11** (2FDP, compound **7**, −**10.25**)		**12** (2IQG, inhibitor 6b, −**11.77**)	
13 (2IS0, L-01151442, −**9.5**)		**14** (2OAH, isonicotinamide, 19, −**11.29**)		**15** (2OHL, fragment 19, −**3.64**)	
16 (2OHM, -substituted-2-aminopyridine **5**, −**4.74**)		**17** (2OHP, 6-substituted 2-aminopyridine **3**, −**5.44**)		**18** (2OHQ, 6-substituted 2-aminopyridine **4**, −**6.21**)	
19 (2OHR, 3-substituted 2-aminopyridine **6a**, −**5.4**)		**20** (2OHS, 3-substituted 2-aminopyridine **6b**, −**5.94**)		**21** (2OHT, 3-substituted 2-aminopyridine **7**, −**6.8**)	
22 (2OHU, 3-substituted 2-aminopyridine **8b**, −**7.25**)		**23** (2P4J, GRL-7234, −**12.72**)		**24** (2QK5, inhibitor **6c**, −**10.92**)	
25 (2QZL, psi[CH_2NH] reduced amide isostere, **4b**, −**10.93**)		**26** (2VNM, derivative, **1**, −**11.5**)		**27** (2VNN, derivative, **8a**, −**11.74**)	

using 1W51 as reference structure with HMC sampling, which yielded an RMSE of 0.996 kcal/mol, compared to 1.101 kcal/mol from a previous study that used a training set of 13 peptidic inhibitors of BACE-1.[9] This study was supported by an excellent diversity of data, both in terms of the range of experimental binding energies and the chemical structures of the inhibitors (Table 1). Although the experimental data came from different laboratories, they were reliable and did not affect the LIE results that much. Using the present LIE model, most of the predicted binding energies of the ligands, including the charged ligands, were in line with the experimental binding energies. Some of the BACE-1 inhibitors, such as the co-crystallized ligands in the crystal structure of 1YM2, 2B8L, 2IQG, 2VNM, and 2VNN, might be considered outliers (Figs. 2–4), which RMSEs of more than 1 kcal/mol between the calculated and observed binding affinities. There are several possible reasons for errors by the models, not least the assumption that such a simple form of Eq. (3) may be used to approximate the binding process. There may be errors in the experimental estimates of

ΔG_{bind}: standard errors of 1 kcal/mol would be quite common and this limits the accuracy of the LIE fit. Because the HMC sampling method was used, it was not necessary to perform molecular dynamics before the LIE simulation. During the LIE simulation, both the ligands and the side chains of all protein residues within a radius of 12 Å around the ligands were allowed to relax.

In this study, we found that using the same training set with different protein conformations resulted in different LIE models. Using 1W51 as the reference structure with HMC sampling produced a better fit to the experimental binding energies than 1FKN, (i.e., 0.996 kcal/mol of 1W51 versus 1.350 kcal/mol of 1FKN, Tables 2 and Fig. 3). The 1W51 structure complexed with a small-molecule inhibitor, while the 1FKN complexed with peptidomimetic inhibitors. Most of the 27 co-crystallized ligands in this study were small molecules. Fitting 1W51–1FKN (with an overall RMSD of 1.41 Å) revealed that the protein conformations were a little different, the 10 s loop was opened in 1W51 but closed in 1FKN. This observation indicates that when the same ligand training set is used, there is a possibility for the LIE model to change if the reference crystal structures are different. Therefore, researchers who are trying to discover drugs targeted at BACE-1 or some other proteins should choose carefully the complex crystal structure as reference structure.

In the present study, two different sampling methods, HMC and simple energy minimization, were used to determine the LIE average energies. Statistical analysis showed that the conformation space search provided by HMC produced a little better fit to the experimental binding energies as compared to simple energy minimization for this set of 27 ligands (Table 2 and Figs. 2 and 3). For example, using 1W51 as reference structure, an RMSE of 0.996 kcal/mol was obtained compared to the 1.003 kcal/mol for minimization. In any case, the energy minimization method is easier and more efficient to use than obtaining an average structure by MD or HMC sampling. The required computational effort (about 5–10 min per compound) allows the energy minimization method to be used for post-processing of large libraries of automatically docked compounds.

To maximize the predictive ability of any model, it is necessary to reduce the dimensionality of the data by identifying the most important variables from which to construct orthogonalized components.[14] As a rule, parsimonious models are likely to be more predictive since a model with low-dimensional space is more

Table 2
Coefficients and root-mean-square errors

	α	β	γ	Q^2	RMSE
1W51					
$\alpha\Delta U\text{vdw}^{HMC} + \Delta\beta U\text{ele}^{HMC}$	0.1374	0.0307		0.795	1.173
$\alpha\Delta U\text{vdw}^{HMC} + \Delta\beta U\text{ele}^{HMC} + \gamma\Delta U\text{cav}^{HMC}$	0.1136	0.0001	−0.2619	0.852	0.996
$\Delta\Delta U\text{vdw}^{MIN} + \Delta\beta U\text{ele}^{MIN}$	0.1258	0.0327		0.824	1.089
$\Delta\Delta U\text{vdw}^{MIN} + \Delta\beta U\text{ele}^{MIN} + \gamma\Delta U\text{cav}^{MIN}$	0.1115	0.0176	−0.1649	0.850	1.003
1FKN					
$\alpha\Delta U\text{vdw}^{HMC} + \Delta\beta U\text{ele}^{HMC}$	0.0068	0.2281		0.368	2.060
$\alpha\Delta U\text{vdw}^{HMC} + \Delta\beta U\text{ele}^{HMC} + \gamma\Delta U\text{cav}^{HMC}$	0.0023	−0.0233	−1.0144	0.729	1.350
$\Delta\Delta U\text{vdw}^{MIN} + \Delta\beta U\text{ele}^{MIN}$	0.1171	0.1746		0.380	2.040
$\Delta\Delta U\text{vdw}^{MIN} + \Delta\beta U\text{ele}^{MIN} + \gamma\Delta U\text{cav}^{MIN}$	0.0022	−0.0455	−1.0886	0.723	1.364

Table 3
LIE terms for the two different sampling methods[a], 1W51 as the protein conformation. The energies are in kcal/mol

Ligand number	PDB ID	$\Delta U\text{vdw}^{HMC}$	$\Delta U\text{cav}^{HMC}$	$\Delta U\text{ele}^{HMC}$	$\Delta U\text{vdw}^{MIN}$	$\Delta U\text{cav}^{MIN}$	$\Delta U\text{ele}^{MIN}$
1	1W51	−68.403	7.481	−37.3166	−75.977	7.2483	−33.2533
2	1FKN	−55.418	17.7531	−27.69	−62.854	17.6984	−44.13
3	1M4H	−72.559	17.365	−70.15	−65.91	17.2102	−139.11
4	1TQF	−66.627	6.9539	−13.3022	−66.407	6.8603	−24.9712
5	1XS7	−79.806	14.3723	−20.8812	−85.451	14.4906	−33.1792
6	1YM2	−77.576	12.6956	−66.1392	−83.799	12.9519	−64.6608
7	1YM4	−59.035	13.5468	−61.242	−68.566	13.1671	−53.482
8	2B8L	−75.766	9.38816	−29.929	−79.955	9.31869	−29.7134
9	2B8V	−58.398	7.09479	−24.1024	−65.184	7.53763	−20.0184
10	2F3F	−62.185	10.3378	−41.0686	−71.783	10.3195	−40.8686
11	2FDP	−73.058	9.4299	−27.2324	−78.379	8.67514	−27.2095
12	2IQG	−68.554	8.0706	−32.9268	−71.439	7.9009	−34.595
13	2IS0	−69.011	7.12622	−21.4242	−68.223	7.28307	−28.4858
14	2OAH	−58.311	12.2069	−24.8292	−63.495	12.0717	−29.0947
15	2OHL	−17.168	1.56996	−5.3908	−17.674	1.75988	−9.557
16	2OHM	−27.902	3.70826	3.6806	−27.373	3.60417	−2.2074
17	2OHP	−37.192	0.562	−4.0104	−39.031	0.88869	−17.8499
18	2OHQ	−48.968	1.24294	0.2052	−49.698	1.49635	−5.3136
19	2OHR	−35.656	5.12464	6.1352	−40.424	4.38256	4.19462
20	2OHS	−46.381	5.56198	8.2784	−47.598	5.60675	3.0816
21	2OHT	−34.431	4.55017	2.2458	−37.881	3.99833	−2.9206
22	2OHU	−53.114	5.22775	−2.7096	−58.205	5.39502	−10.582
23	2P4J	−82.831	12.0662	−44.3116	−89.564	12.4613	−44.1688
24	2QK5	−73.535	7.1688	−24.6044	−75.689	7.3024	−23.136
25	2QZL	−88.104	9.65802	−22.4728	−90.098	9.48797	−30.6916
26	2VNM	−63.554	8.27286	−35.5704	−72.233	7.74313	−48.1494
27	2VNN	−73.972	7.4585	−20.5864	−72.056	7.361	−30.0216

[a] The terms from the hybrid Monte Carlo and minimization runs are indicated with HMC and MIN, respectively.

efficient. From a detailed statistical analysis, to obtain good estimates on three variables, a larger data set that contains at least 27 data points is required to reduce the likelihood of over-fitting.[14] In this study, the fit using just the van der Waals and electrostatic term sampling with HMC yielded an RMSE of 1.173 kcal/mol (Table 2 and Fig. 4). This predictive power is slightly worse than the RMSE of 0.996 kcal/mol found for the fit to three terms. For the 27 inhibitors, the correlation coefficient between the cavity and electrostatic terms was 0.75. The results indicate that cavity term does not make significant contributions to the change in free energy on binding. In the previous work, ΔU_{elec} and ΔU_{vdw} have been found to be the most important variables for making predictions of ΔG with an RMSE of 1.202 kcal/mol.[9]

The β value is very low in either the two-parameter model or the three-parameter model, the reason for this result is that compounds **1**–**4**, **7**–**14**, **16**–**22** and **24**–27 all have groups that might be ionized at neutral pH. We can see the large electrostatic energy difference between the protein and water simulations in Table 3. The statistics for the model are more or less identical to those for ΔU_{vdw} only, the van der Waals energy alone can be used for fitting the training sets. However, it is not sufficient to simply omit the electrostatics term from the final model, because charged ligands also has a large effect on the computed van der Waals terms.[21] In addition, the relative importance of van der Waals and electrostatics might be significantly different for ranking only known inhibitors as opposed to a large database of (mainly) inactive compounds. In fact, the electrostatic term are necessary to reduce false positives in high-throughput docking.[22]

In summary, we used a diverse set of 27 co-crystallized inhibitors of BACE-1 to develop LIE models by superimposing crystal protein/inhibitor complexes. From a series of fits using different sampling techniques and reference structures, the best binding affinity model is the three-parameter model, which had an RMSE of 0.996 kcal/mol using 1W51 as the reference structure with HMC sampling. The initial binding conformation has an important influence on LIE simulation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.050.

References and notes

[1]. Haniu, M.; Denis, P.; Young, Y.; Mendiaz, E. A.; Fuller, J.; Hui, J. O.; Bennett, B. D.; Kahn, S.; Ross, S.; Burgess, T.; Katta, V.; Rogers, G.; Vassar, R.; Citron, M. *J. Biol. Chem.* **2000**, *275*, 21099.
2. Wangsell, F.; Gustafsson, K.; Kvarnstrom, I.; Borkakoti, N.; Edlund, M.; Jansson, K.; Lindberg, J.; Hallberg, A.; Rosenquist, A.; Samuelsson, B. *Eur. J. Med. Chem.* **2010**, *45*, 870.
3. Bjorklund, C.; Oscarson, S.; Benkestock, K.; Borkakoti, N.; Jansson, K.; Lindberg, J.; Vrang, L.; Hallberg, A.; Rosenquist, A.; Samuelsson, B. *J. Med. Chem.* **2010**, *53*, 1458.
4. De Corte, B. L.; Kinney, W. A.; Liu, L.; Ghosh, S.; Brunner, L.; Hoekstra, W. J.; Santulli, R. J.; Tuman, R. W.; Baker, J.; Burns, C.; Proost, J. C.; Tounge, B. A.; Damiano, B. P.; Maryanoff, B. E.; Johnson, D. L.; Galemmo, R. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5227.
5. Stachel, S. J.; Coburn, C. A.; Steele, T. G.; Jones, K. G.; Loutzenhiser, E. F.; Gregro, A. R.; Rajapakse, H. A.; Lai, M. T.; Crouthamel, M. C.; Xu, M.; Tugusheva, K.; Lineberger, J. E.; Pietrak, B. L.; Espeseth, A. S.; Shi, X. P.; Chen-Dodson, E.; Holloway, M. K.; Munshi, S.; Simon, A. J.; Kuo, L.; Vacca, J. P. *J. Med. Chem.* **2004**, *47*, 6447.
6. Iserloh, U.; Wu, Y.; Cumming, J. N.; Pan, J.; Wang, L. Y.; Stamford, A. W.; Kennedy, M. E.; Kuvelkar, R.; Chen, X.; Parker, E. M.; Strickland, C.; Voigt, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 414.
7. Sealy, J. M.; Truong, A. P.; Tso, L.; Probst, G. D.; Aquino, J.; Hom, R. K.; Jagodzinska, B. M.; Dressen, D.; Wone, D. W.; Brogley, L.; John, V.; Tung, J. S.; Pleiss, M. A.; Tucker, J. A.; Konradi, A. W.; Dappen, M. S.; Toth, G.; Pan, H.; Ruslim, L.; Miller, J.; Bova, M. P.; Sinha, S.; Quinn, K. P.; Sauer, J. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6386.
8. Bjorklund, C.; Adolfsson, H.; Jansson, K.; Lindberg, J.; Vrang, L.; Hallberg, A.; Rosenquist, S.; Samuelsson, B. *Bioorg. Med. Chem.* **2010**, *18*, 1711.
9. Tounge, B. A.; Reynolds, C. H. *J. Med. Chem.* **2003**, *46*, 2074.
10. Huang, D.; Caflisch, A. *J. Med. Chem.* **2004**, *47*, 5791.
11. Rajamani, R.; Reynolds, C. H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4843.
12. Carlsson, J.; Boukharta, L.; Aqvist, J. *J. Med. Chem.* **2008**, *51*, 2648.
13. De Amorim, H. L.; Caceres, R. A.; Netz, P. A. *Curr. Drug Targets* **2008**, *9*, 1100.
14. Wall, I. D.; Leach, A. R.; Salt, D. W.; Ford, M. G.; Essex, J. W. *J. Med. Chem.* **1999**, *42*, 5142.
15. Patel, S.; Vuillard, L.; Cleasby, A.; Murray, C. W.; Yon, J. *J. Mol. Biol.* **2004**, *343*, 407.
16. Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. *J. Sci.* **2000**, *290*, 150.
17. http://www.rcsb.org.
18. Liu, T.; Lin, Y.; Wen, X.; Jorissen, R. N.; Gilson, M. K. *Nucleic Acids Res.* **2007**, *35*, D198.
19. MAESTRO, Schrödinger, LLC, New York, NY, 2006.
20. LIAISON, Schrödinger, LLC, New York, NY, 2005.; STRIKE, version 1.5, Schrödinger, LLC, New York, NY, 2005.
21. Tounge, B. A.; Rajamani, R.; Baxter, E. W.; Reitz, A. B.; Reynolds, C. H. *J. Mol. Graphics Modell.* **2006**, 24475.
22. Kolb, P.; Huang, D.; Dey, F.; Caflisch, A. *J. Med. Chem.* **2008**, *51*, 1179.