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Linear interaction energy models for β-secretase (BACE) inhibitors: Role of van der Waals, electrostatic, and continuum-solvation terms

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Abstract

Computing the binding affinity of a protein–ligand complex is one of the most fundamental and difficult tasks in computer-aided drug design. Many approaches for computing binding affinities can be classified as linear interaction energy (LIE) models as they rely on some type of linear fit of computed interaction energies between ligand and protein. We have examined the computed interaction energies of a series of β-secretase (BACE) inhibitors in terms of van der Waals, coulombic, and continuum-solvation contributions to ligand binding. We have also systematically examined the effect of different protonation states of the protein and ligands. We find that the binding affinities are relatively insensitive to the protonation state of the protein when neutral ligands are considered. Inclusion of charged ligands leads to large deviations in the coulomb, solvation, and even van der Waals terms. The latter is due to increased repulsive van der Waals interactions in the complex due to the strong coulomb attraction found between oppositely charged functional groups in the protein and ligand. In general, we find that the best models are obtained when the protein is judiciously charged (e.g. $Asp32^{-}$, $Arg235^{+}$) and the potentially charged ligands are treated as neutral.
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Keywords: β-Secretase; BACE; LIE; Structure-based; Modeling; Binding; Affinity

1. Introduction

One of the primary goals of computer-aided drug design is to develop methods for assessing the binding affinities of prospective ligands in a target enzyme or receptor. This is a daunting task given the complex interactions involved, and the level of accuracy necessary. Many approaches have been proposed for tackling this problem [1]. They can generally be classified as: empirical models or scoring functions, theoretically rigorous estimations of the free energy change [2], or models based on a linear relationship between binding and computed interaction energy terms. The latter approaches include the linear response method pioneered by Aqvist, extensions of the linear response approach developed in the Jorgensen laboratory, or the direct fitting of molecular mechanics interaction energies [3–6]. We would classify all of the later methods broadly as linear interaction energy (LIE) models, since they all make use of a fitted model of computed interaction energy terms.

In an earlier contribution, we reported an LIE model for a series of β-secretase (BACE) inhibitors reported by Ghosh and co-workers [7]. This model was derived using a variant of the linear response approach as implemented in the computer program Liaison. While we were able to apply this model successfully outside the training set [8], several questions still remain in regards to the underlying methodology. Huang and Caflisch have also reported an LIE model for BACE using a similar approach except that CHARMM and Poisson–Boltzman were used in place of OPLS and GB/SA [9]. As a follow-up to our work we have evaluated a number of approaches for modeling BACE using variants of the LIE method. Some of these models show promise for modeling BACE, and provide valuable insights into the application of LIE in its many flavors.

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2. Linear interaction energy approach

One can broadly define the linear interaction energy approach as any method that builds an empirical linear model from computed interaction energy terms. The linear response procedure introduced by Aqvist and co-workers represents an attempt to compute free energy changes by only examining the end states rather than carrying out very computationally expensive free energy perturbation calculations [3,10–12]. In the original implementation of the method, molecular dynamics (MD) simulations were performed for the ligand in a bound and free state to obtain the average (denoted by $\langle \ldots \rangle$) intermolecular van der Waals (vdw) and electrostatic (ele) interactions (U). The binding free energy (ΔG_{bind}) was then derived using the following formula:

$$\Delta G_{\text{bind}} = \alpha \langle \Delta U_{\text{vdw}} \rangle + \beta \langle \Delta U_{\text{ele}} \rangle \quad (1)$$

where the Δ term indicates the change in energy from the ligand free and bound states ($U_{\text{bound}} - U_{\text{free}}$), β was fixed at 0.5, and α was derived using a linear regression fit to a set of known binding energies. Jorgensen and co-workers have extended the linear response approach by adding a solvent accessible surface area term (SASA) or using additional descriptors based on recognition of hydrogen bond acceptors or donors [13–16].

In an early study of HIV protease, Holloway and coworkers used a linear fit of computed interaction energies to predict the pIC50's of 32 HIV protease ligands [4]. This approach is perhaps the most straightforward example of an LIE model. The pIC50 values were modeled by relating them directly to the computed interaction energies for the bound ligands with no extensive sampling or inclusion of solvation effects. Nevertheless, the resulting model exhibited good statistics and was used very effectively in the design of Merck's HIV protease drug, indinavir. Many variations have been reported that combine different sampling schemes, computed interaction energy terms, and solvation models.

3. BACE

Sequential cleavage of amyloid precursor protein (APP) by enzymes β- and γ-secretase results in the formation of 40–42 residue Aβ peptides. Accumulation and deposition of these peptides in the brain is linked to the progress of Alzheimer's disease [17,18]. The initial cleavage of APP by β-secretase has been identified as a critical step in the process of Aβ production [19]. This finding has generated considerable interest in β-secretase as a therapeutic target [20–25]. In recent years, multiple crystal structures have been solved for BACE, including some with bound inhibitors [26,27]. In addition,

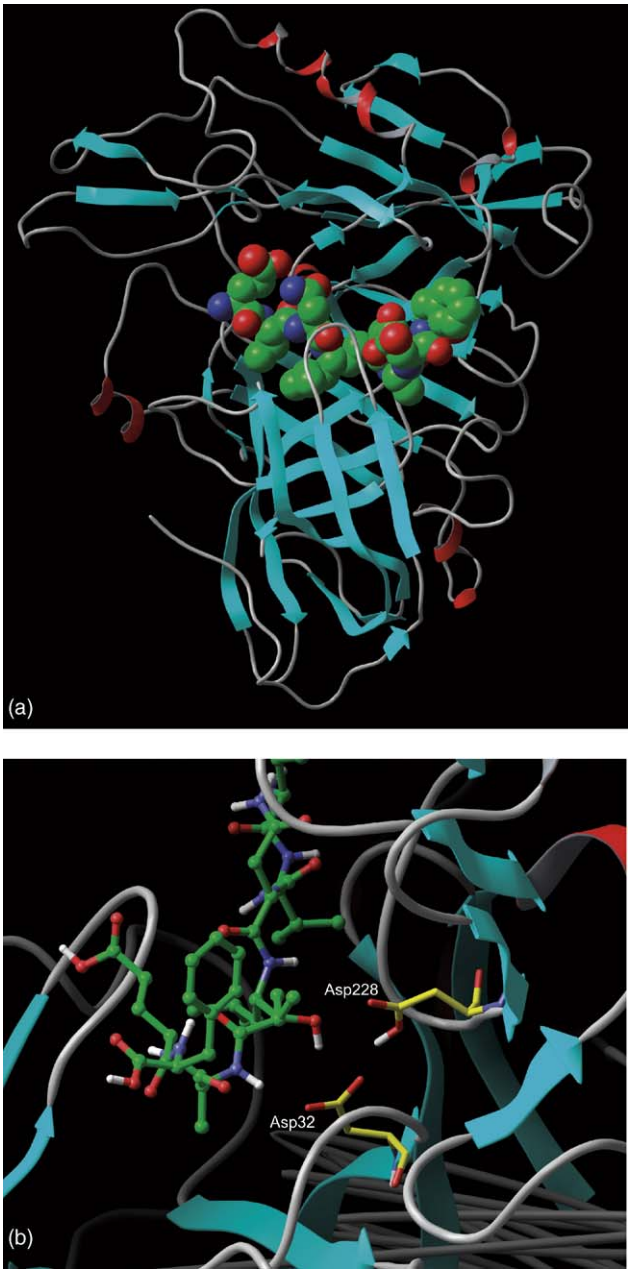


Fig. 1. (a) Crystal structure of OM99-2 bound to BACE; (b) the two catalytic aspartic acids interact with the hydroxyl group of OM99-2.

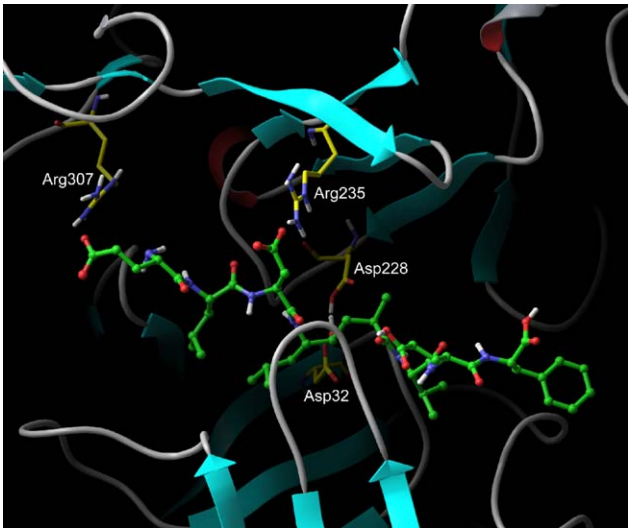


Fig. 2. Binding pocket of BACE with OM00-3. Both Arg307 and Arg235 are close enough to OM00-3 to form salt bridge interactions.

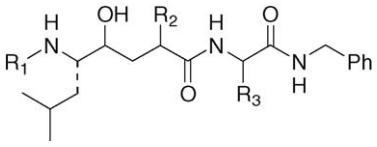
potent inhibitors of this enzyme have been reported. These have typically been based on transition state isosteres, such as hydroxyethylene (HE), statine, and hydroxymethylcarbonyl that bind to the catalytic aspartates. One of the earliest set of ligands reported along with their binding affinities was a collection of 12 inhibitors from the Ghosh laboratory [28]. Since that time new inhibitors have been reported along with their experimental binding affinities [29]. Given access to numerous crystal structures for BACE many groups in industry and academia have been actively applying modeling and structure-based design to the development of improved BACE inhibitors [30–58].

4. Computational methodology

4.1. Protein structure

The crystal structure coordinates of OM99-2 complexed with BACE (PDB ID: 1FKN) was used as the reference structure [26,59] (Fig. 1). The ligands were assembled using the

Table 1
Experimental binding energies



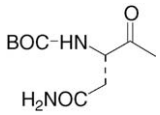
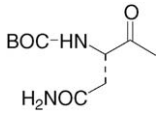
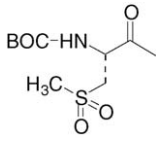
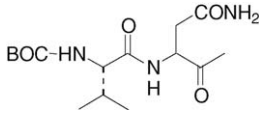
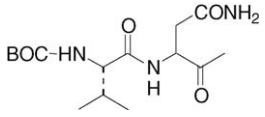
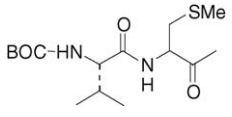
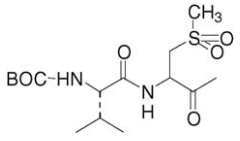
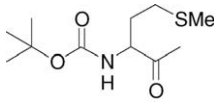
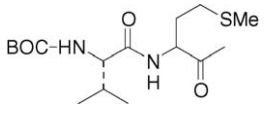
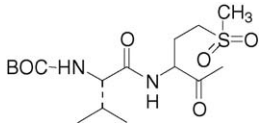
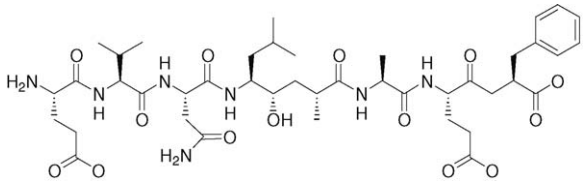
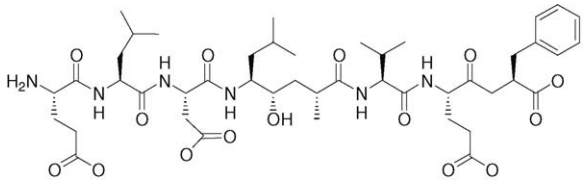
	R_1	R_2	R_3	K_i (nM)	ΔG^{expt} (kcal/mol)
1		Me	Me	22423.0	−6.38
2		Me	$CHMe_2$	3134.0	−7.55
3		Me	$CHMe_2$	1129.0	−8.16
4		Me	Me	61.4	−9.90
5		Me	$CHMe_2$	5.9	−11.30
6		Me	$CHMe_2$	50.1	−10.02
7		Me	$CHMe_2$	9.4	−11.02
8		Me	$CHMe_2$	5808.0	−7.19
9		Me	$CHMe_2$	2.5	−11.81

Table 1 (*Continued*)

	R_1	R_2	R_3	K_i (nM)	ΔG^{expt} (kcal/mol)
10		Me	$CHMe_2$	8.0	−11.11
OM99-2				1.6	−12.06
OM00-3				0.32	−13.05

backbone coordinates of OM99-2 as a guide. The side chains were manually modified to generate the set of inhibitors listed in Table 1. As noted in our previous publication, compound 24 from the set of Ghosh inhibitors tends to be an outlier. For this reason, we have chosen not to included it in our analysis. The protein was prepared using the protein preparation tools in Maestro [60]. Preparation consisted of adding hydrogens, assigning protonation states, and carrying out very limited optimization to reduce bad contacts and the overall strain energy in the protein structure. The active site catalytic aspartates received special attention. Based on a previous study of the protonation state and proton location for BACE [43], the net charge of the catalytic Asps was set to −1 with Asp228 being protonated on the inner oxygen except in one case described below where the all residues were neutralized including Asp32. There is justification for the state where Asp32 and Asp228 are both protonated since the enzyme is most catalytic at relatively low pH (i.e. 3.5–5) and the diprotonated state is computed to be only 3.5 kcal/mol higher in energy than the monoprotonated state. A variety of protonation states for the protein and ligands were considered, and will be discussed later.

4.2. Protein and ligand protonation states

The protein protonation states are, State I where all of the titratable residues are neutral including both Asp32 and 228, State II where only Asp32 and Arg235 are charged, State III where all salt bridges are charged and State IV where all titratable residues in the protein except Asp228 are charged. State II was chosen because Arg235 is know to form a salt bridge with the aspartic acid of OM00-3 (Fig. 2). State III, the salt bridge state, requires a little extra care. Using the default distance-based criteria in Maestro leads to some asymmetric salt bridges where two aspartates interact with a single lysine, for example. In these cases, we manually selected one pair so that the overall charge of each salt bridge, and the protein as a whole, is neutral.

4.3. Modeling binding affinity

The resulting ligand protein complexes were subjected to minimization using the OPLS-AA force field [61]. The protein atoms were held fixed except for the sidechains of residues within 6 Å of the ligand (OM00-3 was used as the reference ligand). A constant dielectric (1.0) with no cut-off was used for both ligand–protein and free ligand states. Solvation effects were included using the GB/SA continuum-solvation model with water as the solvent [62]. The ligand geometries were extracted from the complex and subjected to minimization in GB/SA water with the same cut-off and convergence criteria.

In our original model for BACE, the linear response calculations were carried out using the Liaison package from Schrödinger Inc. [63]. This implementation makes use of a surface generalized Born (SGB) continuum-solvation model and the solvent contribution is summed into the ligands coulombic interaction energy contribution to represent electrostatics as shown in Eq. (2)

$$\Delta U_{ele} = (E_{CoulB} + 2 \times RXNB) - (E_{CoulF} + 2 \times RXNF) \quad (2)$$

where E_{CoulB} and E_{CoulF} are the coulombic interaction energies for the ligand in the protein bound and free state, respectively. The solvation terms are represented by the RXNB (bound ligand) and RXNF (free ligand) reaction field terms. The van der Waals term as implemented in Liaison represents ligand protein intermolecular terms only.

In subsequent work, we abandoned the Liaison formalism and simply computed all of the molecular mechanics energies directly for the bound and unbound states using OPLS-AA and GB/SA water. We constructed a series of binding affinity models using different combinations of the computed van der Waals, coulombic, strain, and continuum-solvation energies. The linear models were constructed by carrying out a least squares fit between the selected ΔE terms (i.e. Δvdw,

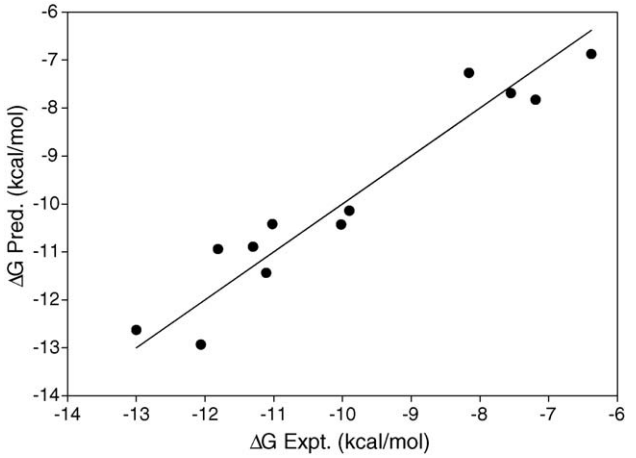


Fig. 3. Plot of experimental and LIE computed ΔG of binding based on Eq. (3).

Δcoulomb, Δsolv) and the experimental free energies of binding. The latter were estimated from experimental K_i values reported in the literature. In both these and the Liaison simulations there are significant differences in the reference states relative to the pure linear response approach due to inclusion of different interaction terms, and substitution of continuum for explicit solvent.

5. Results and discussion

5.1. LIE model

As a follow-up to our original Liaison model, we revisited the Ghosh ligands using the procedure outlined above. First we computed the full molecular mechanics energy for the protein–ligand complex and the unbound ligand. We then examined combinations of terms to determine which computed energies are most predictive. As was obvious from the Liaison model, the van der Waals term is dominant. In the present work, our baseline case was to build a model where the protein is neutral except for Asp32 and Arg235 (State II), and the ligands with potentially charged functional groups (e.g. OM99-2 and OM00-3) are neutral, Eq. (3) (Fig. 3).

$$\Delta G_{\text{bind}} = 0.2228 \times \Delta U_{\text{vdw}} + 0.0577 \times \Delta U_{\text{ele}} + 12.7464 \quad (3)$$

Eq. (3) gives an RMSD of 0.58 kcal/mol and r^2 of 0.92, which represents a slight improvement on the Liaison model reported previously. Some of that improvement is a result of allowing an intercept.

5.2. Coulomb and solvation terms

The original Liaison model for BACE included an electronic term that was the sum of the coulomb energy and a reaction field contribution (solvent contribution). These terms have been separated here (coulombic (coul) and solvation (sol)), at least initially, in order to assess their independent impact on the LIE model. Two ligands in the training set (OM99-2 and OM00-3)

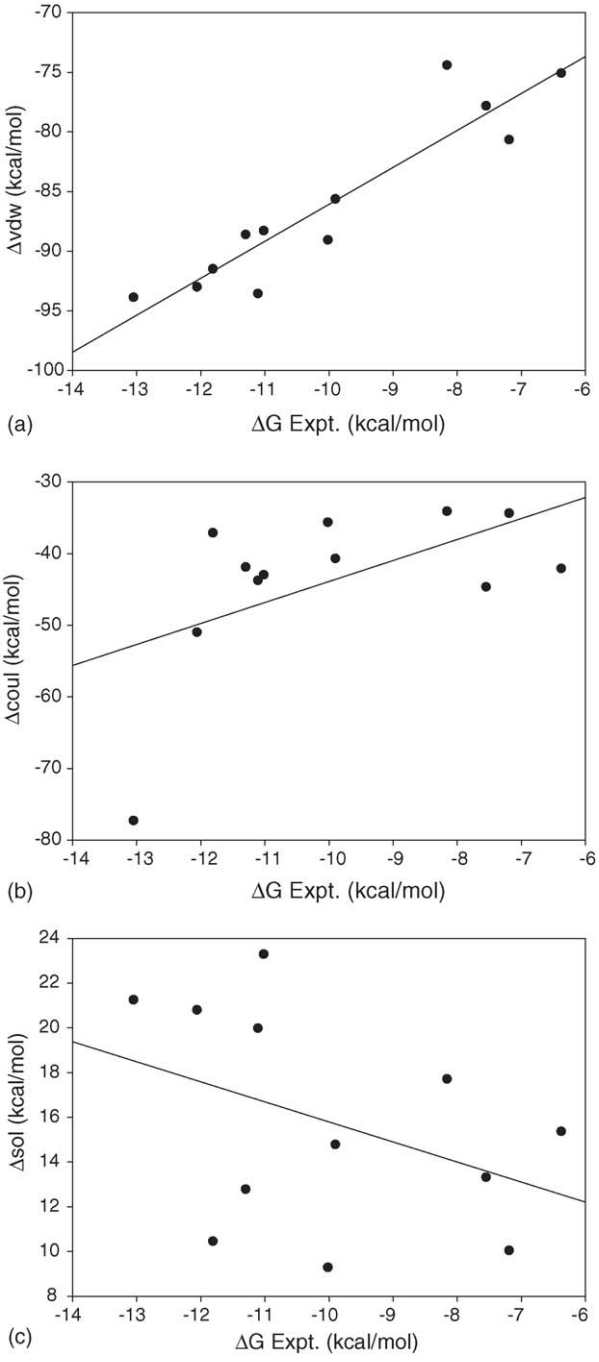


Fig. 4. Plot of delta (a) van der Waals, (b) coulombic, and (c) solvation energies vs. experimental binding affinity for protein State I (all neutral).

should be particularly sensitive to electrostatics since they should carry significant charges at neutral pH. Further when they are included the overall charge on the ligands can potentially vary from 0 to −3. It is generally accepted that developing an LIE model for ligands with different overall charges poses a serious challenge [64–66]. In order to probe the role of electrostatics we examined a variety of charged models for both the protein and ligands.

As an initial test of the protein protonation states (I–IV) the van der Waals, coulombic, and solvation energy changes were plotted against the experimental free energies of binding. The

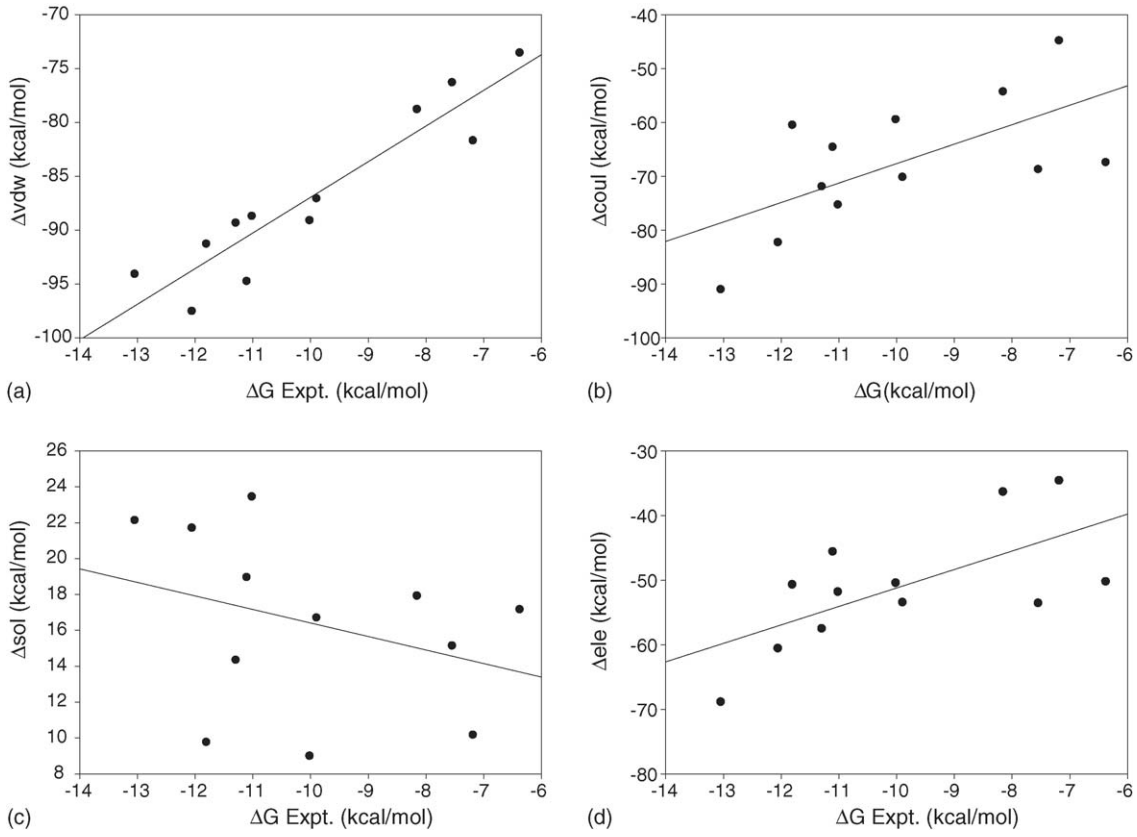


Fig. 5. Plot of delta (a) van der Waals, (b) coulombic, (c) solvation, and (d) electrostatics energies vs. experimental binding affinity for protein State II (Asp32 and Arg235 charged).

ligands with acidic groups, OM00-3 and OM99-2, are shown for their neutral states. This allows us to systematically examine the van der Waals, coulombic, and solvation terms to determine if they correlate with activity. Further, we can directly compare the coulombic and solvation terms for the different charged states of OM00-3. The statistics for these comparisons are summarized in Table 2. Models for all four protein protonation states are given in Table 3 using the van der Waals and coulomb terms. Finally, all of the computed van der Waals, coulombic, solvation, and electrostatic (sum coulombic and solvation terms) energies are reported in Table 4 for the model using protonation State II (corresponds to Eq. (3)).

Figs. 4–7 show the van der Waals, coulombic, and solvation terms plotted against the free energies of binding for protein protonation States I–IV, respectively. Examining Fig. 5 for State II, the van der Waals, coulomb, and total electrostatics plots are reasonably linear if one considers the neutral ligands, although the correlations for the coulombic and total electrostatic energies are poorer. The solvation term

Table 2
Correlation coefficients for ΔG experimental and each of the individual energy terms

Protein state	Δvdw	Δele	Δcoul	Δsol	Total ΔE
I	0.93	0.45	0.54	−0.40	0.77
II	0.93	0.65	0.63	−0.33	0.90
III	0.92	0.33	0.44	−0.25	0.83
IV	0.95	0.45	0.48	−0.30	0.84

Table 3
Summary of fits using protein protonation States I–IV

Protein state	Linear regression model	R^2	RMSD (kcal/mol)
I	$13.6320 + 0.2686 \times \Delta vdw + 0.0181 \times \Delta ele$	0.8687	0.7481
II	$12.7464 + 0.2276 \times \Delta vdw + 0.0577 \times \Delta ele$	0.9218	0.5757
III	$10.8050 + 0.2546 \times \Delta vdw - 0.0092 \times \Delta ele$	0.8461	0.8101
IV	$13.8167 + 0.2457 \times \Delta vdw + 0.0427 \times \Delta ele$	0.9377	0.5157

Table 4
The computed van der Waals, coulomb, solvation, and electrostatic energies (kcal/mol) for the model using protonation State II (corresponds to Eq. (3))

Compound	Δvdw	Δcoul	Δsol	Δele	ΔG^{expt}	ΔG^{pred}
1	−73.51	−67.37	17.18	−50.19	−6.38	−6.88
2	−76.25	−68.67	15.15	−53.52	−7.55	−7.69
3	−78.76	−54.23	17.94	−36.29	−8.16	−7.27
4	−87.05	−70.11	16.72	−53.40	−9.90	−10.04
5	−89.31	−71.85	14.36	−57.48	−11.30	−10.89
6	−89.07	−59.42	9.01	−50.41	−10.02	−10.43
7	−88.68	−75.25	23.46	−51.78	−11.02	−10.42
8	−81.66	−44.75	10.19	−34.56	−7.19	−7.83
9	−91.26	−60.43	9.78	−50.66	−11.81	−10.94
10	−94.73	−64.54	18.97	−45.57	−11.11	−11.44
OM99-2-neutral	−97.50	−82.26	21.72	−60.53	−12.06	−12.93
OM00-3-neutral	−94.06	−90.98	22.14	−68.84	−13.05	−12.63

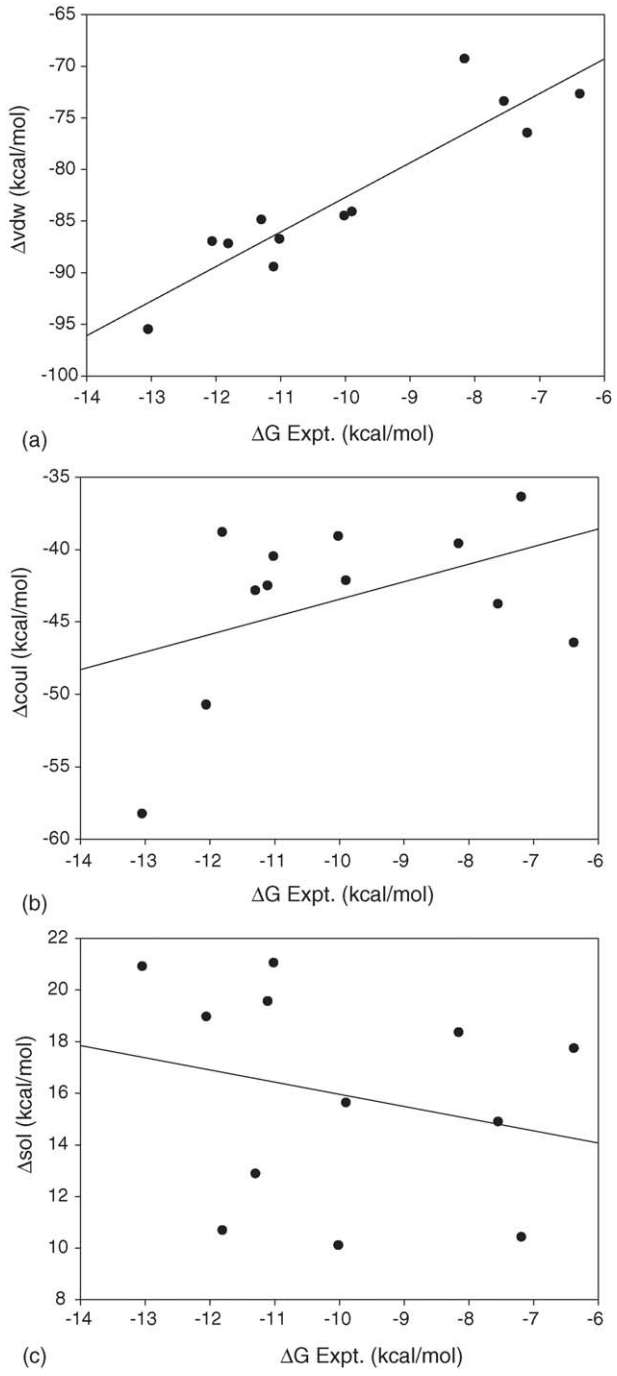


Fig. 6. Plot of delta (a) van der Waals, (b) coulombic, and (c) solvation energies vs. experimental binding affinity for protein State III (salt bridge charged).

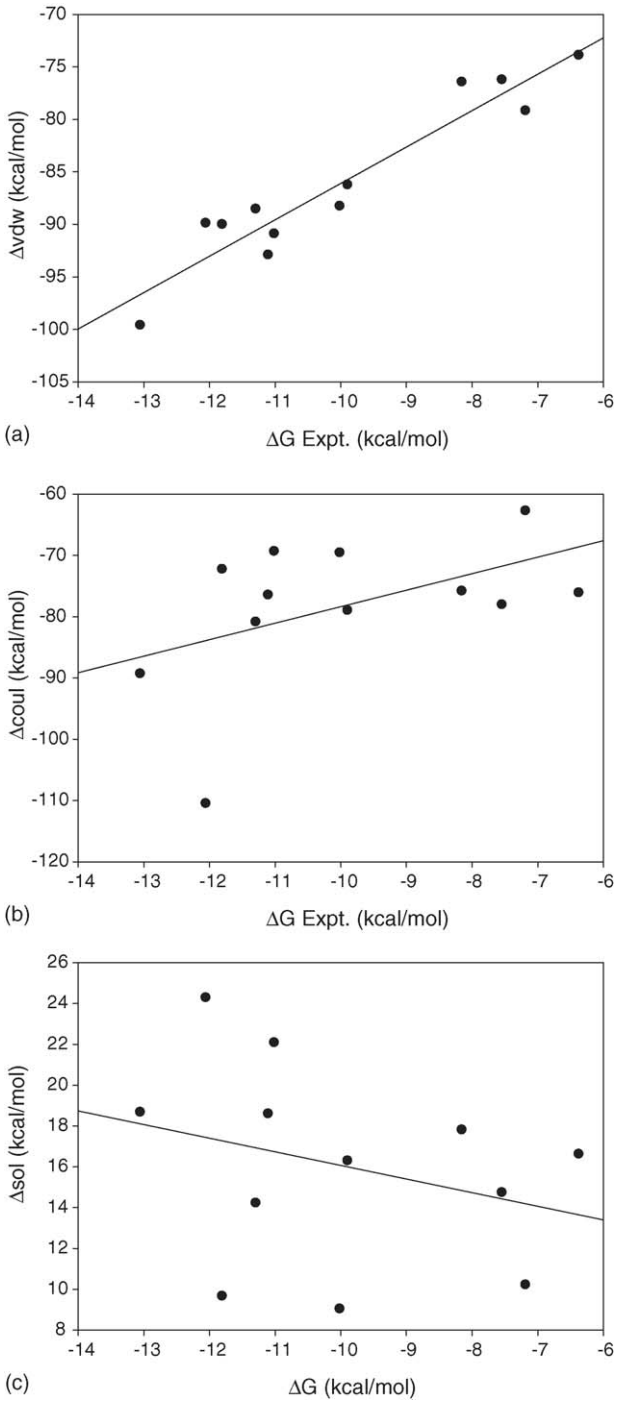


Fig. 7. Plot of delta (a) van der Waals, (b) coulombic, and (c) solvation energies vs. experimental binding affinity for protein State IV (all charged).

(Fig. 5c) is really just a scatter of points. Comparison of Fig. 5b and c shows that the coulomb and solvation terms tend to oppose each other, as one would expect. Placing one or more charges on OM00-3 causes dramatic swings in the electrostatic energy (Fig. 8b). This is consistent with earlier observations from the Liaison calculations.

Plots for the van der Waals, coulombic, and solvation energies for protein protonation States I, III, and IV give similar trends. In all cases the van der Waals terms are most correlated and have the greatest slope. The coulombic and solvation terms are less correlated and, not surprisingly, are more sensitive to the protonation state of the protein and ligand. These plots show that the computed results are actually remarkably robust with regard to the protonation state of the protein, at least if only neutral ligands are considered. Fig. 8 shows that the same cannot be said for the ligands. Including a charged group in OM00-3 causes huge swings in the coulomb and solvation energies. Even using GB/SA, or presumably other continuum models, free charges with no corresponding counter ion create what would appear to be unrealistically large electrostatic

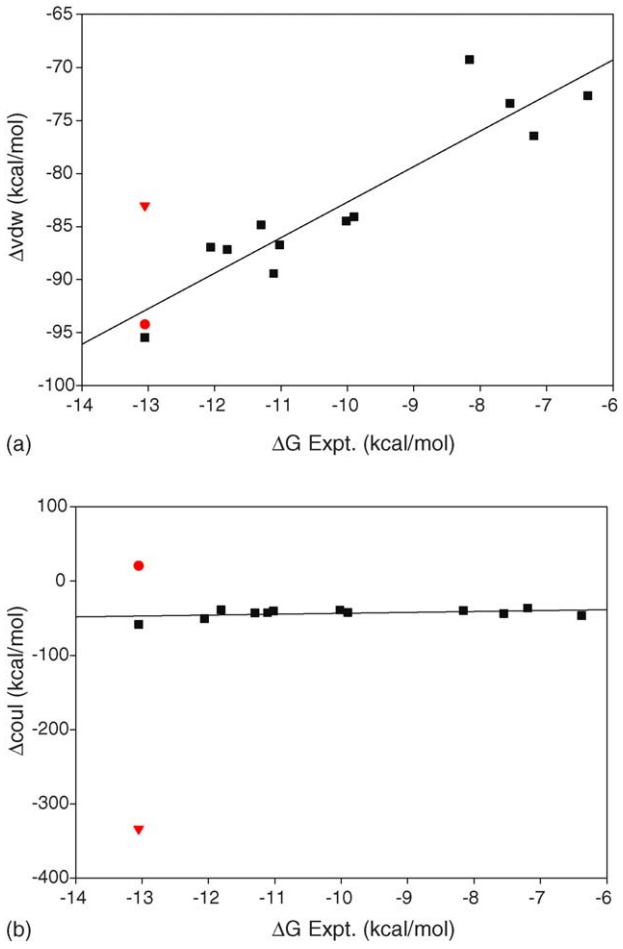


Fig. 8. Plot of delta (a) van der Waals and (b) coulombic energies vs. experimental binding affinity for protein State III. The energies for OM00-3 charged so as to form a salt bridge with Arg235 and Arg307 are shown in red. The red triangles are for OM00-3 without using a counter ion in the free state. The red circles are the energies for OM00-3 using a counter ion in the free state.

interactions. The use of continuum-solvation leads to total electrostatic terms that are large relative to explicit solvent simulations reported by Aqvist, and correspondingly smaller coefficients for β. In general, the results are best in all cases when the ligands are treated as neutral.

In an effort to improve the reference state for the electrostatic and solvation energies, we examined a charge model where titratable functional groups on the ligand were only treated as charged if they can form an internal salt bridge, or interact with the protein to form a salt bridge. The only ligand residues that fit the salt bridge criterion are a Glu and Asp in OM00-3 that can form a salt bridge with Arg307 Arg235, respectively. This leaves OM00-3 with a net charge of −2. The interaction energy terms computed for all ligands including the interacting pairs where OM00-3 has a net charge of −2 and Arg307 and Arg235 are protonated are shown in Fig. 8. For comparison the reference state was computed in two ways: first, simply computing values for the charged ligand in GB/SA water or, second, including counter ions in solution (energies in Table 5). In the second case two monovalent Na^+ atoms from OPLS were used as counter ions. This is also an imperfect

Table 5
Comparison of Δvdw and Δcoul terms (kcal/mol) for different charged treatments of OM00-3 in protein State III

Compound	Charge	Counter ion	Δvdw	Δcoul
OM00-3	Neutral	None	−95.48	−58.23
OM00-3	−2	None	−83.02	−333.61
OM00-3	−2	2 Na^+	−94.23	20.58

reference state, but provides some insight into the factors that modulate the electrostatics of binding. For example, the electrostatic term in Fig. 8 is hugely negative unless the counter ions are included in the reference state. Further, the unshielded charges lead to a van der Waals energy that is much too positive (Fig. 8a). Comparison with the reference state including counter ions shows that part of this is due to an increase in the repulsive van der Waals interaction in the complex due to the strong attractive coulombic interactions between the anions in the ligand and the positively charged arginines in the protein. Adding the counter ion creates a compensating repulsive van der Waals term for the ligand free state. This balances the repulsive van der Waals term seen in the protein–ligand complex and brings the Δvdw energy back onto the line (Fig. 8a). A key observation here is that it is not sufficient to simply omit the electrostatics terms from the final model. Including charged ligands also has a large effect on the computed van der Waals terms. The large attractive coulomb energy drives the two oppositely charged functional groups together at considerable cost in repulsive van der Waals energy. This must be accounted for either by eliminating the very large coulomb attractive term during minimization or providing a compensating van der Waals term in the reference state (i.e. using a counter ion).

5.3. Electrostatics in LIE

Our experience with modeling BACE has shown that it is difficult to obtain the correct balance of coulomb and solvation terms using a continuum method, particularly where the charge on the ligands varies. This is consistent with other work in the area where charged ligands have often been avoided altogether, large distance-dependent dielectrics have sometimes been employed to dampen electrostatics, or direct evaluation of electrostatic terms is replaced with more heuristic terms such as counts of unsatisfied hydrogen bonds. In general, it may be that the best strategy is to only introduce charged groups in both the protein and ligand that are directly relevant to ligand binding and carefully consider the reference state. A conservative approach to protein–ligand electrostatics is likely to be reasonable given that electrostatics do not drive ligand binding in most cases.

6. Conclusion

We have carried out a systematic assessment of the van der Waals, coulombic, and continuum-solvation terms used to build LIE models of β-secretase. Consistent with previous work on BACE and other targets, we find that the van der Waals term dominates ligand binding. Comparison of a variety of protein

protonation states shows that the models are relatively insensitive to the protein protonation state when neutral ligands are employed. This is partly due to the fact that the electrostatic terms are not highly correlated with binding affinity. Inclusion of charged ligands leads to large deviations in the coulomb and solvation terms that are difficult to balance effectively. This may be complicated by the difficulties associated with defining a valid reference state for the charged ligands when using continuum-solvation models. In general, we find that the best models are obtained when the protein is minimally charged (i.e. only selected relevant residues are charged and an overall neutral state is maintained) and potentially charged ligands are treated as neutral. This work underscores the need for improved electrostatic and solvation models for proteins, ligands, and their complexes.

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