

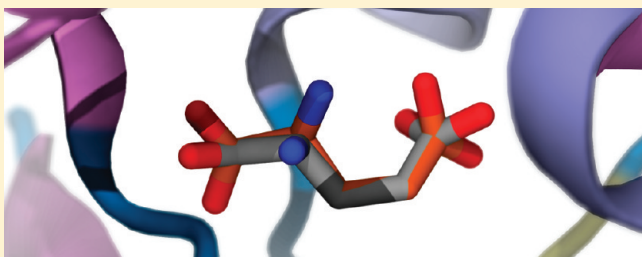
A Robust Force Field Based Method for Calculating Conformational Energies of Charged Drug-Like Molecules

Jacob Poehlsgaard,* Kasper Harpsøe, Flemming Steen Jørgensen, and Lars Olsen

Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

S Supporting Information

ABSTRACT: The binding affinity of a drug-like molecule depends among other things on the availability of the bioactive conformation. If the bioactive conformation has a significantly higher energy than the global minimum energy conformation, then the molecule is unlikely to bind to its target. Determination of the global minimum energy conformation and calculation of conformational penalties of binding is a prerequisite for prediction of reliable binding affinities. Here, we present a simple and computationally efficient procedure to estimate the global energy minimum for a wide variety of structurally diverse molecules, including polar and charged compounds. Identifying global energy minimum conformations of such compounds with force field methods is problematic due to the exaggeration of intramolecular electrostatic interactions. We demonstrate that the global energy minimum conformations of zwitterionic compounds generated by conformational analysis with modified electrostatics are good approximations of the conformational distributions predicted by experimental data and with molecular dynamics performed in explicit solvent. Finally the method is used to calculate conformational penalties for zwitterionic GluA2 agonists and to filter false positives from a docking study.



INTRODUCTION

Identification of the global energy minimum conformation of a small molecule can be approached with a range of computational strategies. Smaller sets of compounds can be studied by molecular dynamics methods in explicit water or by ab initio methods. For large compound libraries, conformational search methods can be used. One such method is Monte Carlo sampling, where thousands of random starting conformations for each compound are generated and subsequently minimized using force field methods. To increase the computational efficiency, this type of calculation is performed in vacuo or using a continuum solvent model. The quality of implicit solvent models is currently such that the behavior of most molecules is well modeled. In the case of polar or charged compounds, some issues still linger. There is a tendency to overestimate the electrostatic interaction between charged groups when these are located close to each other in a given conformation.^{1,2} For some molecules, the opportunity for such interactions during a conformational search leads to a reported global energy minimum conformation, where these groups form an internal hydrogen bond. Such folded conformations may well exist in solution, in equilibrium with their extended counterparts. This is observed in the case of β -alanine, where NMR data and ab initio calculations demonstrate that the two types of conformations, extended and folded, are both represented in water.² It is not a problem that a given method reports the folded conformation, but in the case of β -alanine, the energy difference between the two conformers, as

calculated by force field methods, has been reported as high as 20 kcal/mol.^{2,3} This indicates that the force field methods may overestimate the stability of the folded conformation. This is most likely caused by the incomplete description of solvation effects by the continuum models, in combination with force field specific effects. Moreover, entropic penalties from introducing an internal hydrogen bond are also not included in the energy minimization methods and are difficult to estimate, even with computationally expensive methods due to sampling issues.

In this study, we employ a conformational search based on the Monte Carlo method, in which a force field is modified not to reward intramolecular hydrogen bonding by eliminating electrostatic interactions between potential donors and acceptors in the same molecule.

The conformational search is followed by a constrained energy minimization with full electrostatics turned on. This allows the compounds to adapt to the original force field before energy evaluation without significantly changing the conformations. This method is valid under the assumption that hydrogen-bond interactions are somewhat quenched by solvent effects: If internal hydrogen bonds are possible, we assume that they will not lead to folded conformations which are orders of magnitude more stable than the extended ones. As these folded conformations are ignored, a conformational search using this approach

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will not result in a population that represents the actual conformational ensemble in solvent.

Such a method is potentially useful when working in a virtual screening framework, where reproducing exact population percentages and even geometries may not be critical. Here, the reason for identifying global energy minimum conformations is to calculate the conformational penalty of a given ligand when binding to a receptor. The penalty is defined as the potential energy difference between the ligand in the bioactive conformation and the ligand in its lowest energy conformation in solution. The prime objective is therefore to find a realistic global minimum energy for each compound. Such an energy value enables a decision of whether or not to reject a given compound on the basis of conformational penalty. In this application, a method that does not always give the actual lowest energy is preferable to one that occasionally produces unrealistically low energies, as the latter will lead to erroneous rejection of compounds.

Using the modified force field approach to identify the most stable structure is validated in several ways. By comparison with populations generated by force field based molecular dynamics in the presence of explicit water, we show that for a set of charged model compounds, a Monte Carlo conformational search using native force fields results in overestimation of the stability of the folded conformations for a number of compounds. Repeating the conformational search with the modified force field reliably produces conformations similar to the dominating forms in the simulations using explicit water. We then test the ability of the method on a library of random compounds and show that for the compounds that are not prone to form internal hydrogen bonds, our method gives the same results as a standard conformational search using all four force fields tested. Subsequently, we recalculate the conformational penalties of binding from Boström et al.⁴ and apply the method for calculating conformational penalties for nine charged glutamate receptor ligands. Finally we perform a proof of concept docking study, where the conformational penalty is used to distinguish between binders and nonbinders with similar docking scores.

■ COMPUTATIONAL METHODS

All calculations and compound preparations were performed in the Schrödinger 2010U1 suite.⁵

Protonation states for the external Boström test set were modeled as in Boström et al.,⁴ and GluA2 ligands, β -alanine, and S-aspartate were modeled as zwitterions. The 98 compound test set is a subset of the “drugs now” compound library from the Zinc database.⁶ We have previously determined protonation states between pH of 5 and 9 and realistic tautomers for the entire set (data not shown) using the Schrödinger module ligprep.⁷ The 98 compounds used were randomly extracted from this prepared database (see Supporting Information).

Conformational Searches. Conformational searches were performed using the torsional sampling method (MCM) in the MacroModel module with automatic setup options.⁵ The maximum number of steps was set to 5000 for the zwitterionic compounds and the random test set and to 10 000 for the external test set. All calculations used 100 steps per rotatable bond and an energy cutoff of 21 kJ/mol above the global energy minimum. Unless otherwise noted, only the lowest energy conformation for each compound was saved. The searches were done using the water continuum model, with standard settings, force field supplied charges, and the following cut-offs: van der

Waals, 8.0 Å; electrostatic, 20.0 Å; and hydrogen bond, 4.0 Å. The keyword CHYD was set to enable searches without energy contributions from internal hydrogen bonds. Addition of this keyword sets the electrostatic interaction between putative intramolecular hydrogen-bonding partners to zero. Constrained minimizations in water were performed using the same force field settings as the conformational searches. A flat-bottomed potential with half-widths of 0.2, 0.3, and 0.4 Å and a force constant of 500 kJ/mol·Å² were applied to all nonhydrogen atoms. Vacuum energies were calculated using the structures generated in water, with the dielectric constant 1.0, force field supplied charges, and the following cut-offs: van der Waals, 7.0 Å; electrostatic, 12.0 Å; and hydrogen bond, 4.0 Å.

Molecular Dynamics Simulations. All molecular dynamics simulations were performed in Desmond version 2.4,⁸ using the OPLS2005 force field. Compounds were solvated in TIP3 water molecules, using a truncated octahedron box, with a size determined by a 12 Å buffer from the molecule. All simulations were run in the NPT ensemble at either 300 K or a scheme of 100 rounds of 100 ps heating to 1000 K, 300 ps cooling to 300 K and 600 ps simulation at 300 K. Simulations were run at 1.01325 bar. The systems were relaxed before simulation by using the default Desmond protocol.

Docking. Docking was performed using Glide 5.7,⁹ with the glide XP scoring function.¹⁰ The receptor was imported and preprocessed in the Schrodinger Suite using the protein preparation wizard: Hydrogens and a sulphur bridge were added, and the hydrogen-bonding network was optimized, followed by a constrained minimization of the entire complex. This system was used to prepare a docking grid, using default settings with the following changes: Extended sampling, root-mean-square deviation (RMSD) for clustering poses: 0.3 Å, max displacement 0.5 Å, no post docking minimization, and 10 poses per ligand reported.

■ RESULTS AND DISCUSSION

Force Field and Parameter Selection. All energies are calculated using native force field parameters in order to evaluate the performance of the force fields in an application-like scenario, such as screening, where it is not feasible to tailor the force field parameters to a large set of compounds. Modifying the force field parameters for this study could also introduce an element of bias into the results. We used the AMBER*, MM3*, MMFFs, and OPLS2005 force fields, as supplied with the Schrödinger molecular modeling suite.⁵ Putative hydrogen bonds between 1–4 connected donors and acceptors are not counted as potentially problematic, as these are assumed to be well described by the bonded force field parameters.

Selection of Zwitterionic Test Set. As our own work is focused on elucidating binding determinants of the ionotropic glutamate receptor agonists, we use the nine GluA2 agonists R-TDPA, S-2-me-tet-AMPA, S-ACPA, S-AMPA, S-des-me-AMPA, S-Br-Hibo, S-ATPA, S-glutamate, and R-TDPA as part of our test set of charged ligands. Crystal structures of these compounds in complex with the GluA2 receptor are available,^{11–15} showing absence of internal hydrogen bonds in the bioactive conformations. Previous NMR studies show the related compound kainate to exist primarily in the extended conformation,¹⁶ while a force field based conformational search predicts a strong preference for the folded conformation.¹ NMR data from β and γ methylated glutamic acid analogues show extended and folded conformations to be present in roughly equal amounts.¹⁷ Apart from the GluA2 ligands, we also

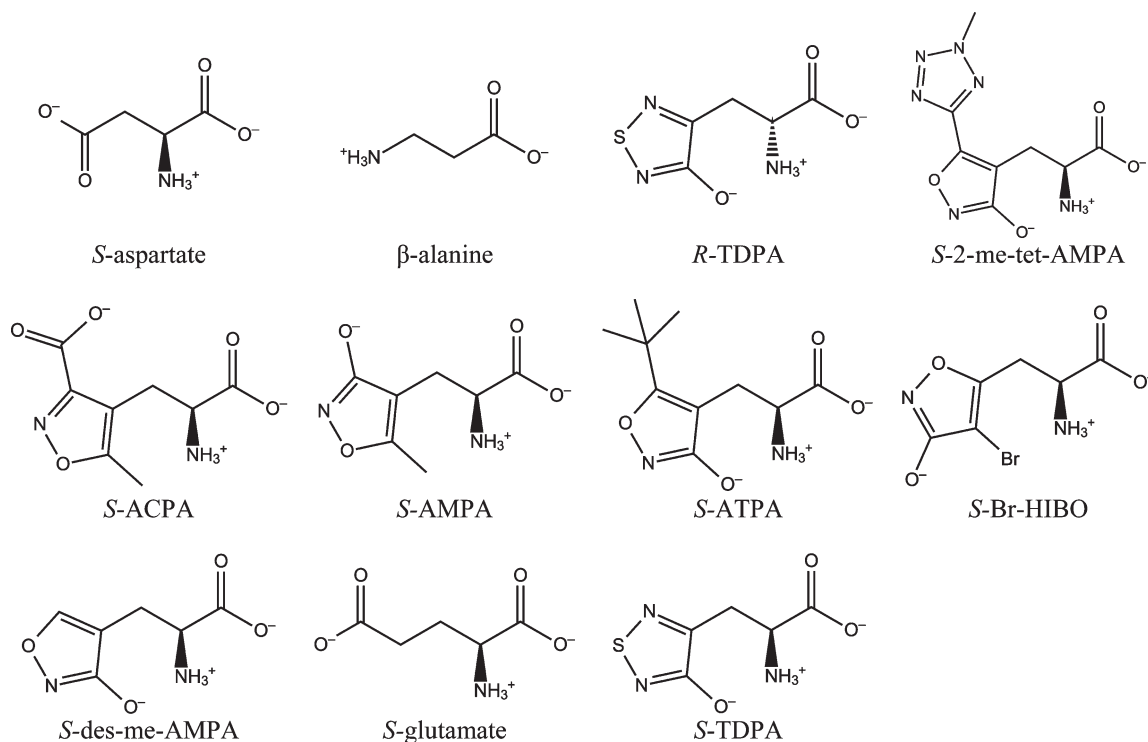


Figure 1. Chemical structures of S-aspartate, β -alanine, and the nine GluA2 ligands from the zwitterionic test set.

Table 1. Calculated Energy Differences between Extended and Folded Conformations ($E_{\text{extended}} - E_{\text{folded}}$) of β -alanine, S-aspartate, S-glutamate and S-ACPA, using Four Different Force Fields^a

compound	β -alanine	S-aspartate	S-glutamate	S-ACPA
ΔE -AMBER	3	ND	17	18
ΔE -MM3	6	22	17	ND
ΔE -MMFFs	7	2	23	28
ΔE -OPLS2005	8	36	13	7

^a ND: Only folded conformation found. All energies in kJ/mol.

include β -alanine and S-aspartate as the conformational flexibility of these small zwitterionic compounds previously has been studied by both NMR and ab initio methods.^{2,3,18} β -Alanine has been shown to have a weak preference for a folded conformation, roughly 3:1, while S-aspartate predominately exist in a folded conformation, with a ratio of roughly 10:1.

Monte Carlo Conformational Search on Zwitterionic Test Set. In order to verify that the energy calculations involving internal hydrogen bonds are a significant problem using current versions of force fields and parameters, we first performed a Monte Carlo conformational search with the native OPLS2005 force field on β -alanine, S-aspartate, and the GluA2 agonists (cf. Figure 1).

An internal hydrogen bond was observed in the lowest energy conformation of β -alanine, S-aspartate, S-ACPA, and S-glutamate (cf. Table 1 and Figure 2)

In most cases, repeating the conformational search for these compounds with the MMFFs and AMBER force fields gave rise to similar or larger energy differences between the folded and first extended minima (cf. Table 1). For S-aspartate, the conformational search using the AMBER force field failed to find any

extended minima, even with the search energy window enlarged to several hundred kJ/mol. Conversely, using the MMFFs force field identified an extended minimum just 2 kJ/mol higher in energy than the folded global energy minimum.

As a rule of thumb, 4 kJ/mol in energy difference between two conformations corresponds to an order of magnitude in the relative number of conformations in the ensemble. The high energy difference found between the folded and extended conformations of many of these compounds would result in a distribution where the folded conformation was dominating heavily, in contradiction to what is expected from the available NMR data.^{2,3,18} Based on these results, β -alanine and S-aspartic acid were chosen for further studies with more computationally expensive methods, as experimental data exist for these compounds. S-glutamate and S-ACPA are also included as representatives of the GluA2 agonists. These compounds form internal hydrogen bonds with all three force fields used, which is not consistent with their bioactive conformations in the GluA2 receptor and NMR data for related compounds.

Generation of Conformations by Molecular Dynamics Simulation. As previous studies have suggested that the use of an implicit solvation model is one of the primary causes of the overestimation of the importance of intramolecular hydrogen bonds,² 100 ns molecular dynamics simulations of systems with the compounds surrounded by 12 Å of explicit water were performed. Compared to the previous Monte Carlo conformational searches, the conformational ensembles are closer to those observed in the experimental results, as both folded and extended conformations were present in the trajectories. However, it was also clear, that for these compounds, traditional simulations need to be run for longer than practical in a virtual screening or drug discovery perspective to ensure proper sampling: During some of the simulations, the solute molecules

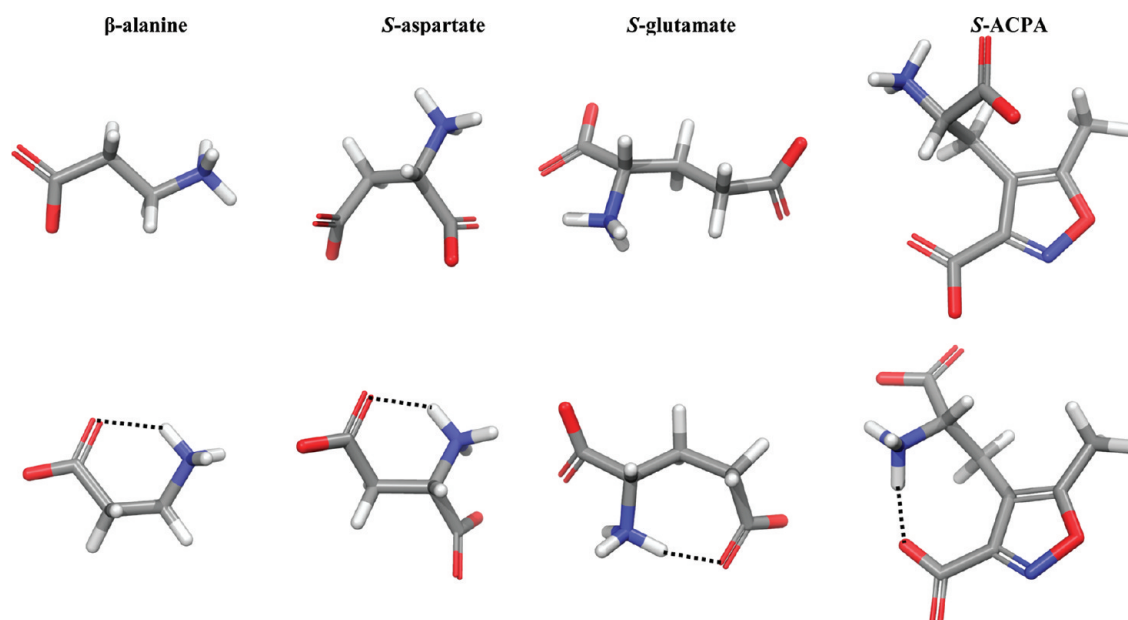


Figure 2. Extended and folded (top and bottom rows) conformations of β -alanine, *S*-aspartate, *S*-glutamate, and *S*-ACPA.

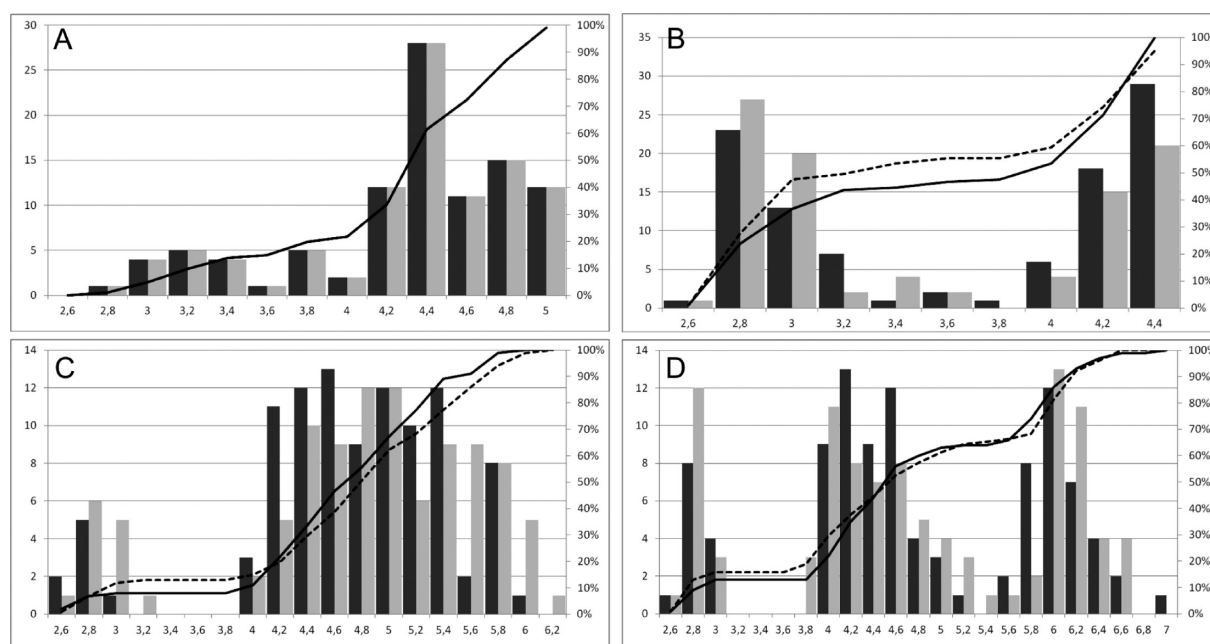


Figure 3. Histograms with frequencies and cumulated percentages of distances between each of the carboxyl oxygens and the primary amines from snapshots from the ends of the 300 K phases of 100 rounds of molecular dynamics. Each of the two relevant oxygen–nitrogen distances is represented by light- or dark-gray bars and the cumulative percentages as dotted or solid lines, respectively. A: β -alanine, B: *S*-aspartate, C: *S*-glutamate, and D: *S*-ACPA.

would be caught in specific conformations for tens of nanoseconds (see Supporting Information). To overcome this, a heating scheme was used. A simulation protocol with 100 rounds of 100 ps heating to 1000 K, 300 ps cooling to 300 K and 600 ps of simulation at 300 K was used. Snapshots were extracted from the ends of the 300 K phases and the conformations of the ligands analyzed by measuring the distances from each oxygen atom of the relevant carboxylate to the nitrogen atom of the amino group. We define a conformation as folded, if one of the oxygens is found within 3 Å of the nitrogen atom. Histograms of the O–N

distances from the molecular dynamics experiments can be seen in Figure 3.

Both the extended and folded conformations are observed in varying ratios for the four compounds. The folded conformation of β -alanine, *S*-aspartate, *S*-glutamate, and *S*-ACPA were present in 10, 84, 20, and 28% of the 100 snapshots for each ligand, respectively. The percentages were estimated by summing the cumulated frequencies of distances of 3 Å or below for each molecule. This corresponds to the percentage of conformations in which either of the carboxyl oxygens is interacting with the

Table 2. Relative Potential Energies for Global Energy Minima Using Native and Modified Force Fields^a

compound	AMBER ΔE		MMFFs ΔE		OPLS ΔE		PDB
	manual	modified	manual	modified	manual	modified	
R-TDPA	—	—	3	3	0	1	3BFU ¹¹
S-2-me-tet-AMPA	28	28	0	0	0	2	1MSB ¹²
S-ACPA	18	18	28	28	7	7	1MSE ¹²
S-AMPA	30	30	4	4	0	0	1FTM ¹³
S-ATPA	33	33	7	7	0	0	1NNP ¹⁴
S-Br-HIBO	0	0	0	0	0	0	1MSC ¹²
S-glutamate	17	17	23	23	13	13	1FTJ ¹³
S-des-me-AMPA	32	32	3	3	0	0	1MQD ¹⁵
S-TDPA	—	—	3	3	0	1	3BFT ¹¹
β -alanine	3	3	7	7	8	8	—
S-aspartate	ND	ND	2	2	2	0	—

^a Manual: Difference between first extended structure and global energy minimum found by Monte Carlo conformational search using native force field. Modified: Conformational energy difference between global energy minimum reported with modified and native force field. All energies in kJ/mol.

nitrogen oxygen. The results for S-glutamate and S-ACPA fit the hypothesis that these compounds exist in their extended conformation in solution in relevant amounts as well as NMR data for related compounds. Comparing these numbers and the NMR data with the results from Table 1, it is clear that the standard force field based conformational search is not a suitable method for identifying the global energy minimum for this type of compounds.

Modified Monte Carlo Conformational Search on Zwitterionic Test Set. From the crystal structures, NMR studies of related compounds, and the molecular dynamics studies above, it is likely that the GluA2 agonists in solution are not found exclusively in folded conformations. To avoid the overestimation of the importance of the intermolecular hydrogen bonds, a force field modification where the energy terms for internal hydrogen bonds are turned off can be used. In order to test if a Monte Carlo conformational search using such a modified force field is able to give more realistic global energy minimum conformations for the zwitterionic test set than when using the native force fields, we compared three different methods for identifying the global minimum for each of the four force fields: (1) lowest energy reported by Monte Carlo conformational search, (2) Monte Carlo conformational search followed by manual inspection of the output conformations to identify the extended conformation with the lowest energy, and (3) Monte Carlo conformational search with the modified force field. The results are summarized in Table 2.

In nearly all cases, using the modified force field during the conformational search results in the same energy as found when manually inspecting the results from using the native force field and selecting the extended conformation with the lowest energy. Any lower energy obtained when using the native force fields stems from folded conformations with internal hydrogen bonds. Interestingly, there is a marked difference in the tendency to overestimate the energy of internal hydrogen bonds between the force fields. The OPLS2005 force field gives what is probably the most realistic result, with folded conformations for only two out of nine compounds. This is followed by MMFFs with five out of nine compounds resulting in folded conformations and AMBER with seven out of eight. When using the modified force field with β -alanine, the extended conformation is reported as the global minimum with all three force fields, in line with the molecular

Table 3. Comparison of Standard and Modified Conformational Search on 98 Random Compounds^a

force field	processed	$\Delta E > 1$	folded
AMBER	80	2	2
MM3	49	12	12
MMFFs	97	3	3
OPLS2005	98	8	8

^a Processed indicates how many compounds out of the 98 the system was able to handle with automatic settings. $\Delta E > 1$ indicates the number of compounds for which the energy difference between the global minimum found with the standard and modified force field is larger than 1 kJ/mol. The folded column indicates how many of the compounds with an energy difference larger than 1 kJ/mol has global energy minimum conformations with internal hydrogen bonds.

dynamics results. As in the previous conformational search, S-aspartate stands out. Even with the energy contribution from internal hydrogen bonds turned off, the AMBER force field does not produce any extended conformations. OPLS2005 produce extended conformations, but they are not reported as the minimum energies. However, aspartate is an almost optimal compound for the formation of an internal hydrogen bond, as the two gauche conformations both allow for an internal hydrogen bond, while the anti conformation brings the two carboxyl groups into close proximity. This means that for S-aspartate, the selection of these conformations is driven as much by van der Waals and bonded interactions as electrostatics, which is why folded conformations are found even in the absence of contributing energy terms for internal hydrogen bonds. From these results it can be seen that removing the internal hydrogen-bonding energy terms during a Monte Carlo conformational search on charged compounds eliminates the reporting of folded conformations with overestimated energy contributions from internal hydrogen bonds. Furthermore, conformations reported as having the overall lowest conformational energy are the lowest energy extended conformations.

Validation on 98 Random Compounds. To be generally applicable, the strategy of modifying the force field energetics of internal hydrogen bonds must be able to accurately predict the global energy minimum energies for all types of compounds.

Table 4. List of Compound Numbers and Parent PDB Codes for the External Test Set^a

compound	PDB code	compound	PDB code	compound	PDB code
1	1NHB ¹⁹	10	1TNG ²⁰	20a	2IFB ²¹
2	184L ¹⁹	11	1TNL ²⁰	20b	1LIE ²²
3	186L ¹⁹	12	1TNH ²⁰	21a	1HMT ²³
4a	1PHD ²⁴	13	1TNJ ²⁰	21b	1LIF ²⁵
4b	1PHE ²⁴	14	1TNK ²⁰	22a	1HMS ²³
4c	1PHF ²⁴	15	1TNI ²⁰	22b	1LID ²⁵
5	1PHG ²⁴	16a	1CBS ²⁶	23	1HMR ²³
6	2DHC ²⁷	16b	1EPB ²⁸	24	3CLA ²⁹
7	1ACJ ³⁰	17	1CRB ³¹	25	1ETA ³²
8	1ACL ³⁰	18	1STP ³³	26	1LAG ³⁴
9	1ACK ³⁰	19	1ICM ³⁵	27	1LAH ³⁴

^a See Supporting Information for chemical structures.

Nonpolar compounds that do not show a propensity for internal hydrogen bonds must be reported as having the same energy as when using the native force field. To test this, we generated a small library of 98 compounds that were randomly selected from the “drugs now” subset of the zinc database and performed Monte Carlo conformational searches with native and modified force fields. The distributions of the relative energies reported by the two methods are shown in Table 3.

The energy differences fall within one kJ/mol for the majority of the tested compounds, while the remainders display a lower energy when using the native force fields. Inspection of these compounds showed all identified global energy minimum conformations to have internal hydrogen bonds. For this set of compounds, there are no observable adverse effects of disabling energy contributions from internal hydrogen bonds, only the intended result is observed.

Taken together, these results demonstrate that performing a Monte Carlo conformational search with a force field modified to disregard internal hydrogen bonds gives the same results as using the native force field for most compounds. Differences are only observed in cases where internal hydrogen bonds can cause an overestimation of the stability of the folded conformation, and in those cases, the force field modification leads to identification of extended conformations with the lowest energy.

Conformational Penalties of Binding. As our goal for developing and validating a modified conformational search procedure is to enable rapid and realistic calculation of conformational energies, we decided to use the set of compounds from a previous study by Boström et al.⁴ as an additional external test set (cf. Table 4).

The thermodynamic cycle of ligand–protein interaction can be decomposed in a number of ways. In the interest of computational simplicity, contrary to Boström et al. we model the relevant step of the ligand changing from the global minimum conformation in solvent to the bioactive conformation as happening before desolvation, rather than introducing an extra step of calculating single point energies in vacuo. The compounds examined by Boström et al. represent a relatively diverse set of small molecule protein binders with known bioactive conformations. As our methodology for calculating the conformational penalties is a simplified version of the one reported in their paper, we found it relevant to attempt to reproduce their findings. For each of the compounds, we performed conformational searches with the

native and modified force fields. The conformations from the conformational search using modified force fields and the bioactive conformations were subsequently subjected to constrained minimizations with flat-bottomed potentials with a half-width of 0.2, 0.3, and 0.4 Å, respectively, and a force constant of 500 kJ/mol·Å² applied to the heavy atoms outside the well. Figure 4A shows the results from the AMBER force field, while Figure 4B shows the results from the MM3 force field.

For both sets of penalties, we used the same well half-widths as the Boström et al. paper: 0.3 Å for most compounds and 0.4 Å for the more flexible compounds (8, 14, 15, 19–24, and 26). The conformational penalties for most compounds are not influenced by eliminating the energy contribution from possible internal hydrogen bonds. Small differences are seen for some of the long chained fatty acids (e.g., compound 19). Inspection of the conformations reveals that this is caused by sampling issues in the conformational searches, not by the modification of the force field (data not shown); for these flexible compounds, even 10 000 iterations during the search may not be enough. A large difference can be seen with compound 27 when using the native AMBER force field, which has an internal hydrogen bond in the most stable conformation, resulting in an artificially high conformational penalty. This compound is the only case, where there is a large impact of switching off the internal hydrogen-bond contributions, again demonstrating that the modification only has the desired effect. When comparing the obtained conformational penalties with the previous work,⁴ it is clear that there is not a complete agreement of the conformational penalties. This was expected, as this study employs a different version of the force fields, and no modification of parameters was used in our work. Interestingly the best match with their in vacuo penalties seems to be from the penalties calculated with the use of implicit solvent. Here, the calculated MM3 penalties are generally slightly higher than found by Boström et al., while the penalties found using AMBER are very similar to their work. To examine the force field dependency of the conformational energies, we compared the penalties calculated by AMBER, MM3, MMFFs, and OPLS2005, using the modified force field and a well half-width of 0.3 Å (cf. Figure 5).

From this it can be seen, that while the overall relative penalties for the different compounds follow a similar pattern, the force fields do not give the same numerical results.

The conformational penalties calculated here show that modifying a force field to disregard internal hydrogen bonds during a Monte Carlo conformational search alleviates the issue of overestimated stability of the folded conformation. Such a modification to the force field does not change the reported conformations of compounds not susceptible to internal hydrogen bonds. In turn, the method corrects the unrealistically high conformational penalties often calculated for this type of compound. The force field and solvation dependence indicate that there may not currently be one correct number for a given conformational penalty when using force field methods. If the goal is to define a cutoff for an acceptable conformational penalty, then force field methods are still relevant, but it is important to evaluate the force field in terms of available parameters, energies reported, and applications for the class of compounds under investigation.

Conformational Penalties for GluA2 Agonists. After validating the use of modified force fields during the conformational search for generating conformational penalties of binding, we calculated the penalties for the GluA2 agonists. As the crystal

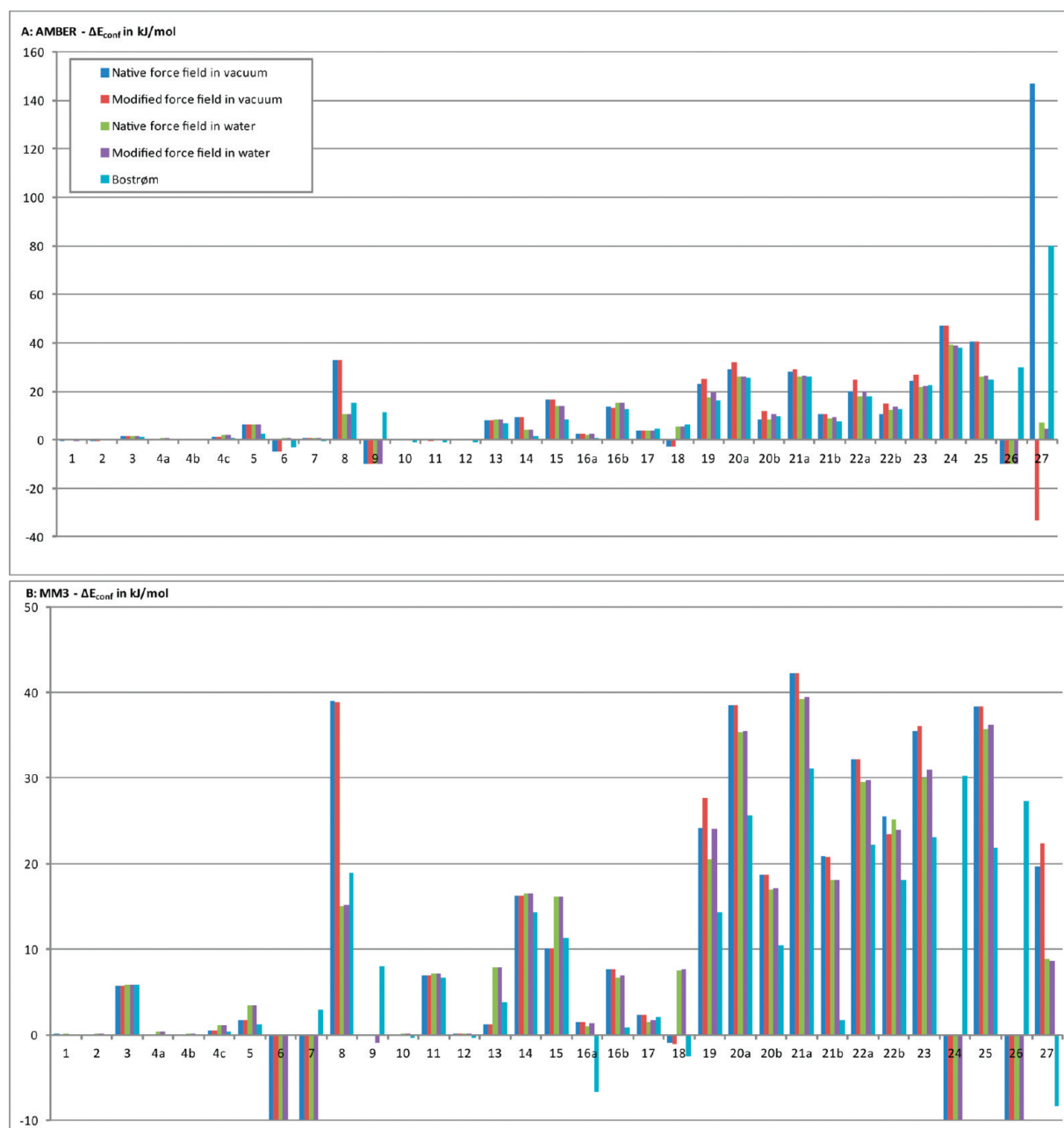


Figure 4. Conformational penalties (ΔE_{conf}) of the external test set from two different force fields in water and vacuum, using the standard and modified method for identifying the global energy minimum. (A) AMBER and (B) MM3. For clarity, the conformational energy for compound 6, 7, 24, and 26 has been set to -10 kJ/mol for those force fields that lacked parameters for these compounds. Boström refers to calculations performed as in Boström et al.⁴

structures of the GluA2 receptor complexes contain up to four subtly different chains in the asymmetric unit, this gave us the opportunity to test the sensitivity of the method to small differences in geometry. For each of the OPLS2005, MMFFs, and AMBER force fields, the ligands were extracted from all chains, and constrained minimizations with well half-widths of 0.2, 0.3, and 0.4 Å were performed in implicit solvent. The MM3 force field in the Schrödinger implementation was unable to parse the heterocyclic rings of 8 of the compounds and is thus not used for this test set. The resulting energies were subtracted from the previously found global energy minima to yield the conformational penalties. As an example, the results from the AMBER force field can be seen in Figure 6.

As would be expected from the previous conformational search results, there are large differences between the conformational penalties, depending on whether the conformational search was performed with the native or modified force field. Where internal hydrogen bonds are found in the global energy minimum structure, a prohibitively high conformational energy is observed. For each of the compounds, calculations of the conformational penalty for the individual chains in the crystal structure give rise to only minor variances in the energy. Even for highly charged systems, such as the GluA2 complexes, there seem to be no critical dependence on the small differences between the geometries in the different chains. Variation of the minimization well half-width affects the energies as expected. The larger the

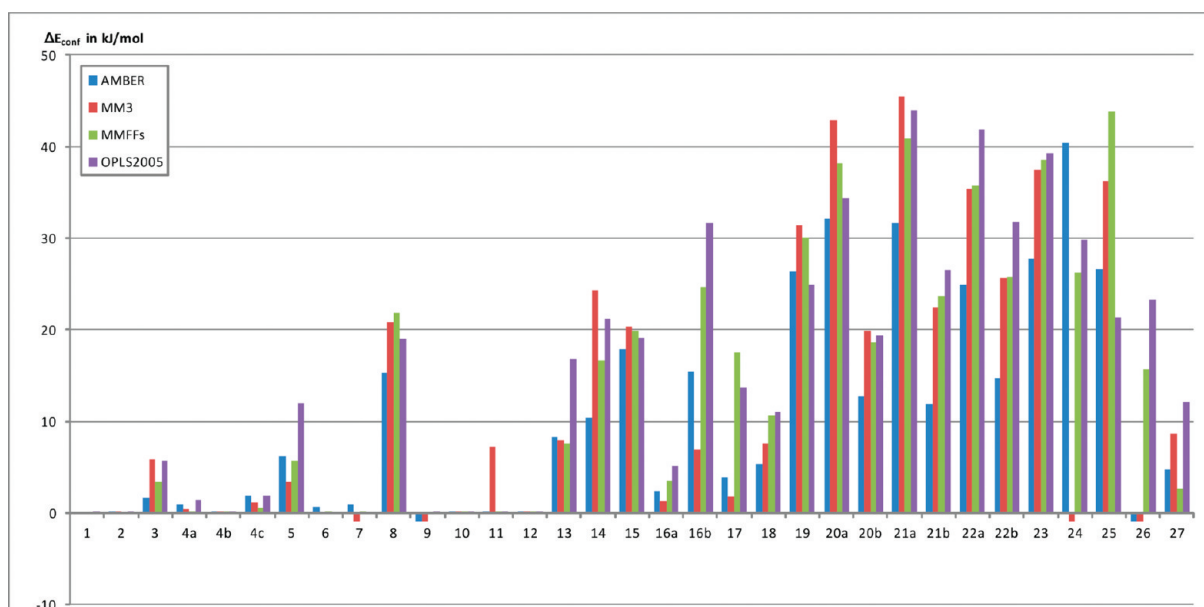


Figure 5. Conformational penalties (ΔE_{conf}) for the external test set calculated in solvent, using four different force fields with the modified method for identifying the global energy minimum and a well half-width of 0.3 Å. For clarity, the conformational energy for compound 7, 9, 24, and 26 has been set to -1 kJ/mol for the force fields that lacked parameters for these compounds.

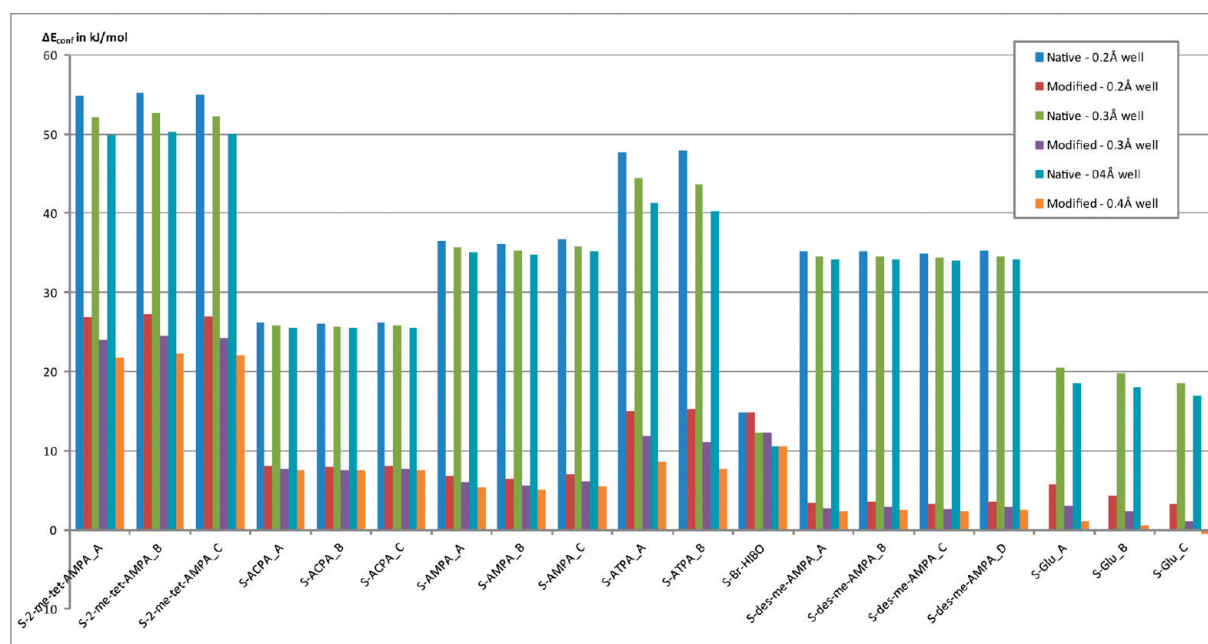


Figure 6. Conformational penalties (ΔE_{conf}) for the GluA2 agonists calculated with the AMBER force field. Conformational penalties are calculated for each chain in the crystal structures, using conformational searches with both the native and modified force field and 0.2, 0.3, and 0.4 Å well half-widths during constrained minimization.

well, the smaller the conformational penalties, as the bioactive conformation is allowed to relax into deeper minima, away from the starting conformation, even though the structural changes were subtle. Maximum average RMSDs for the whole molecules of 0.1, 0.2, and 0.3 Å were observed after minimization with well half-widths of 0.2, 0.3, and 0.4 Å, respectively. The maximum distance for a single atom was 0.3, 0.5, and 0.6 Å, respectively. A critical consequence of the selection of the well width can be seen in the case of S-glutamate chain C, where we observe a negative

conformational penalty when using a 0.4 Å half-width. This is caused by an intramolecular electrostatic interaction, the establishment of which is allowed by the more lax constraints. Further expanding the well to 0.8 Å results in a progressively more folded conformation and in the establishment of an internal hydrogen bond (cf. Figure 7), with a corresponding lowering of the conformational energy by more than 15 kJ/mol.

In this example, the small difference between chains means that the ligand in chain C is the only one with a conformation,

which is susceptible to this effect, when using a 0.4 Å half-width. For this reason, a well half-width of 0.3 Å was used in subsequent calculations on this system.

To compare the conformational penalties obtained with the different force fields, we calculated the average conformational penalty for agonists in all crystal structure chains for AMBER, MMFFs, and OPLS2005 using the 0.3 Å well half-width. From Figure 8 it can be seen that there is a large variation in conformational penalties predicted by the different force fields.

OPLS2005 gives rather large penalties, all above 12.6 kJ/mol, which was previously proposed as the maximum value for high-affinity binders.⁴ AMBER gives more varied penalties, from 2 to 24 kJ/mol. This may indicate that for at least this specific class of small molecules, these force fields are not suited for calculating the conformational energy. MMFFs give the lowest values in the 0–7 kJ/mol range and thus is in line with the 12.6 kJ/mol cutoff suggested earlier.⁴ More worrying than the actual penalties is that there seems to be no general agreement of the relative penalties between the force fields. For instance, *S*-2-me-tet-AMPA, which binds with a K_i of 5 nM, is predicted to have the highest conformational penalty when using AMBER. With a penalty of 24 kJ/mol, this compound would be predicted not to bind to the receptor; despite that it is the compound with the lowest K_i in the

set. When comparing to the Boström et al. test set, this force field dependency is much more pronounced with the charged compounds, underscoring the difficulty using these methods to make accurate energy calculations when charges are in close proximity.

Using Conformational Penalties for Post-Docking Filtering. To validate the applicability of the method, a docking study of the GluA2 receptor was performed. The GluA2 ligands for which crystal structures of the ligand–protein complex are available all have a chiral center. The majority of the compounds bind only in the *S* form, with the exception of TDPA, where both *R* and *S* forms bind.¹¹ The *R* and *S* forms of the ligands were redocked into the parent receptors with all waters removed and multiple conformations generated for each compound. The standard glide XP protocol yields docking scores in the same range for all the tested compounds (cf. Table 5). In this case, all the nonbinding *R* forms are false positives, as it is impossible to separate the binders from the nonbinders based on XP score alone. A subsequent step of calculating the conformational penalties, as described above, using the MMFFs force field and a 0.3 Å well half-width was performed. Eliminating all conformations with calculated penalties higher than 15 kJ/mol removes all but one of the false positives without removing any true positives

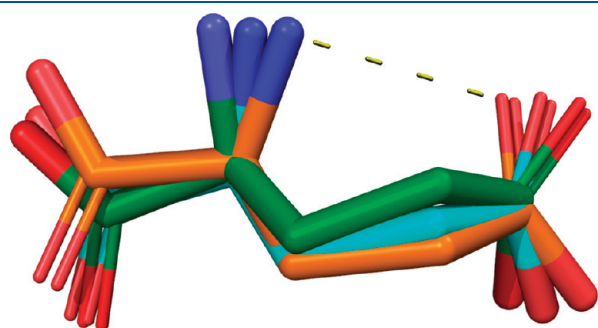


Figure 7. Constrained minimization of the *S*-glutamate binding conformation (green) with 0.4 Å (cyan) and 0.8 Å (orange) well half-widths. The internal hydrogen bond after minimization with a well half-width of 0.8 Å is indicated with a dotted yellow line.

Table 5. Best XP Docking Scores for the *R* and *S* Forms of Eight GluA2 Agonists Without and With Conformational Penalty Filter Applied

	best XP score		best XP score and $\Delta E_{\text{conf}} < 15$ kJ/mol	
	<i>R</i> form	<i>S</i> form	<i>R</i> form	<i>S</i> form
TDPA	−11.2	−12.7	−11.2	−11.5
2-me-tet-AMPA	−11.4	−10.5	N/A	−10.5
ACPA	−12.2	−11.9	N/A	−11.9
AMPA	−11.5	−12.3	N/A	−12.3
ATPA	−10.7	−11.5	N/A	−11.5
Br-HIBO	−11	−10.5	N/A	−10.5
des-me-AMPA	−1.3	−11.5	N/A	−8.9
Glu	−11.6	−11.6	−11.5	−11.5

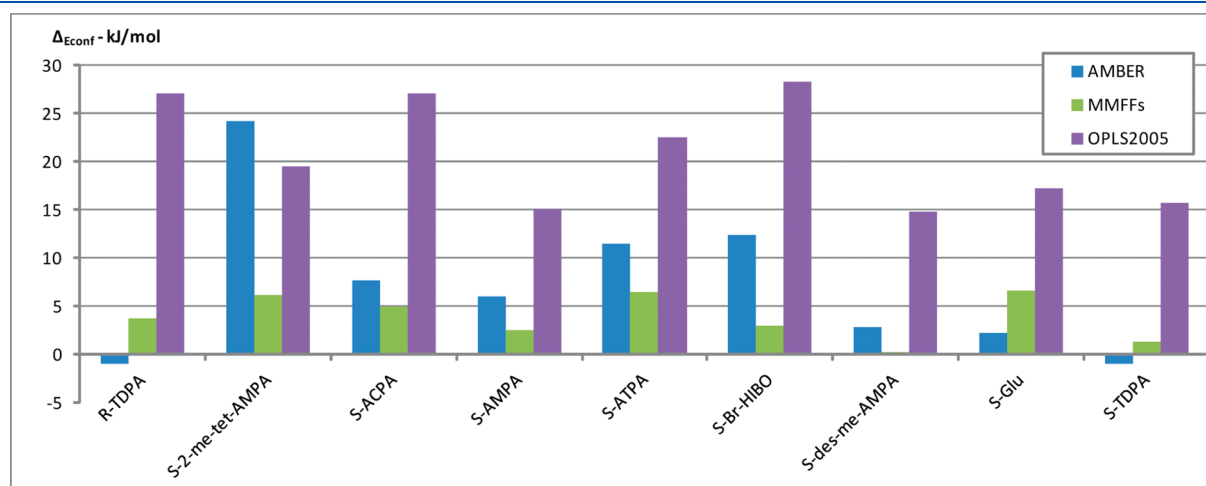


Figure 8. Conformational penalties (ΔE_{conf}) averaged over all chains from the GluA2 agonist crystal structures and calculated with the modified force fields, using a 0.3 Å well half-width for constrained minimization with AMBER, OPLS2005, and MMFFs. For clarity, the conformational energy for compounds *R*-TDPA and *S*-TDPA has been set to −1 kJ/mol for the AMBER force field, which lacked parameters for these compounds.

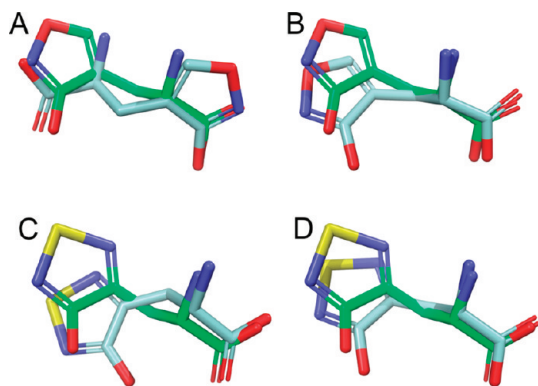


Figure 9. Comparison between crystal structure binding mode (green carbons) and docking conformations (cyan carbons) in the GluA2 receptor. (A) top scoring *S*-des-me-AMPA docking conformation. (B) Top scoring *S*-des-me-AMPA docking conformation with conformational penalty below 15 kJ/mol. (C) Top scoring *S*-TDPA docking conformation (D) Top scoring *S*-TDPA docking conformation with conformational penalty below 15 kJ/mol.

(cf. Table 5). Even though multiple conformations were reported for most of the nonbinding *R* forms, only *R*-glutamate had a conformation with a conformational penalty below 15 kJ/mol. It is not surprising that both forms of glutamate have permissible conformational penalties, as this small, flexible molecule can be imagined to fit in the binding cavity in numerous ways when the water molecules are removed. Applying the conformational penalty filter removed the highest scoring conformations for *S*-TDPA and *S*-des-me-AMPA leading to a less optimal score, but identification of a confirmation that is closer to the binding mode in the crystal structure (cf. Figure 9).

This proof-of-principle experiment demonstrates that conformational penalties can be used to distinguish between binders and nonbinders with similar docking scores. Furthermore, in some cases it is possible to use the conformational penalty filter to identify a conformation that is closer related to the conformation in the crystal structure than the one with the best docking score.

CONCLUSIONS

In this paper, we have shown that for charged compounds a force field based conformational search in implicit water can lead to generation of folded conformations with internal hydrogen bonds and that the energies for these conformations are too low compared to the extended conformations. Conformations generated by a conformational search using a modified force field, where energy contributions from internal hydrogen bonds are turned off, are useful representatives of the global minimum conformations present in solution, as the stability of the folded conformations are no longer exaggerated. Instead, the extended conformations with the lowest energy are reported in agreement with NMR and *ab initio* data from previous studies as well as the molecular dynamics results presented here. As the NMR data also show the folded conformations to occur in solution, it is important to note that the modified protocol does not give the correct distributions—folded structures are often ignored. What is achieved is a good approximation of the minimum energy conformation.

When using the generated global minima to calculate conformational penalties, our results show that using the force field modification is beneficial. It specifically eliminates artificially high

penalties with no detected side effects. Our results also show that care should be taken to choose the force field and the force field settings. From the conformational penalties for the GluA2 ligands, we observe that energy well half-widths of 0.4 Å and higher during constrained minimization of the binding conformation may lead to formation of internal hydrogen bonds. More important is the choice of force field, as the conformational penalties of the GluA2 agonists vary significantly between force fields. We attribute these differences to the different ways in which the force fields handle charge—charge interactions in the context of the implicit solvation model. As a consequence, we do not recommend comparing the calculated penalties quantitatively to gain insight into the relative affinity of different compounds, whether the force field modification is used or not. When used for rejecting putative bioactive conformations with conformational energy above a certain threshold, it is important to test the performance of the available force fields before deciding on a cutoff energy.

As conformational penalties are critically dependent on docked conformations, directly applying a conformational penalty cutoff to a virtual screening result may be dangerous, as virtual screening protocols often only report a single conformation for each compound. Active compounds for which the best scoring docking conformation is different from the actual binding mode may give rise to high penalties and result in subsequent elimination of the compound, as was seen in the case of *S*-des-me-AMPA and *S*-TDPA. Our docking results demonstrate that the introduction of an intermediate step using multiple conformations for each compound followed by a conformational penalty cutoff or reranking can be useful. Compared to simply evaluating a compound based on the top scoring conformation, evaluating the penalty of the top 10 best scoring conformations only introduces a number of extra minimizations, which are computationally inexpensive compared to the conformational search itself. This method will improve the general quality of the reported binding conformations, as conformationally prohibitive binding modes are no longer reported, even if they have the best docking score. This allows for a ranking by docking score of conformations that are physically possible.

ASSOCIATED CONTENT

S Supporting Information. Chemical structures of the 98 random compound sets; O–N distances during 100 ns MD simulation of *S*-aspartate, β -alanine, *S*-glutamate and *S*-ACPA; and chemical structures of the compounds in the external test set. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*E-mail: japo@farma.ku.dk.

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REFERENCES

- (1) Nielsen, P. A.; Liljefors, T. Conformational analysis of kainate in aqueous solution in relation to its binding to AMPA and kainic acid receptors. *J. Comput.-Aided Mol. Des.* **2001**, *15* (8), 753–63.

- (2) Nielsen, P. A.; Norrby, P.-O.; Liljefors, T.; Rega, N.; Barone, V. Quantum Mechanical Conformational Analysis of β -Alanine Zwitterion in Aqueous Solution. *J. Am. Chem. Soc.* **2000**, *122* (13), 3151–3155.
- (3) Gregoire, F.; Wei, S. H.; Streed, E. W.; Brameld, K. A.; Fort, D.; Hanely, L. J.; Walls, J. D.; Goddard, W. A.; Roberts, J. D. Conformational Equilibria of β -Alanine and Related Compounds as Studied by NMR Spectroscopy. *J. Am. Chem. Soc.* **1998**, *120* (30), 7537–7543.
- (4) Bostrom, J.; Norrby, P. O.; Liljefors, T. Conformational energy penalties of protein-bound ligands. *J. Comput.-Aided Mol. Des.* **1998**, *12* (4), 383–96.
- (5) Schrödinger MacroModel, 9.8; Schrödinger, LLC: New York, 2010.
- (6) Irwin, J. J.; Shoichet, B. K. ZINC—a free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.* **2005**, *45* (1), 177–82.
- (7) Schrödinger LigPrep, 2.4; Schrödinger, LLC: New York, 2010.
- (8) Kevin, J. B. In *Scalable Algorithms for Molecular Dynamics Simulations on Commodity Clusters*; Edmond, C., Huafeng, X., Ron, O. D., Michael, P. E., Brent, A. G., John, L. K., Istvan, K., Mark, A. M., Federico, D. S., John, K. S., Yibing, S., David, E. S., Eds.; Association for Computing Machinery: New York, 2006; pp 43–43.
- (9) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47* (7), 1739–49.
- (10) Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J. Med. Chem.* **2006**, *49* (21), 6177–96.
- (11) Beich-Frandsen, M.; Pickering, D. S.; Mirza, O.; Johansen, T. N.; Greenwood, J.; Vestergaard, B.; Schousboe, A.; Gajhede, M.; Liljefors, T.; Kastrup, J. S. Structures of the ligand-binding core of iGluR2 in complex with the agonists (R)- and (S)-2-amino-3-(4-hydroxy-1,2,5-thiadiazol-3-yl)propionic acid explain their unusual equipotency. *J. Med. Chem.* **2008**, *51* (5), 1459–63.
- (12) Hogner, A.; Kastrup, J. S.; Jin, R.; Liljefors, T.; Mayer, M. L.; Egebjerg, J.; Larsen, I. K.; Gouaux, E. Structural basis for AMPA receptor activation and ligand selectivity: crystal structures of five agonist complexes with the GluR2 ligand-binding core. *J. Mol. Biol.* **2002**, *322* (1), 93–109.
- (13) Armstrong, N.; Gouaux, E. Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. *Neuron* **2000**, *28* (1), 165–81.
- (14) Lunn, M. L.; Hogner, A.; Stensbol, T. B.; Gouaux, E.; Egebjerg, J.; Kastrup, J. S. Three-dimensional structure of the ligand-binding core of GluR2 in complex with the agonist (S)-ATPA: implications for receptor subunit selectivity. *J. Med. Chem.* **2003**, *46* (5), 872–5.
- (15) Kasper, C.; Lunn, M. L.; Liljefors, T.; Gouaux, E.; Egebjerg, J.; Kastrup, J. S. GluR2 ligand-binding core complexes: importance of the isoxazolol moiety and 5-substituent for the binding mode of AMPA-type agonists. *FEBS Lett.* **2002**, *531* (2), 173–8.
- (16) Todeschi, N.; Gharbi-Benarous, J.; Girault, J. P. Structure of kainic acid totally elucidated by NMR and molecular modelling. *Bioorg. Med. Chem.* **1997**, *5* (10), 1943–57.
- (17) Todeschi, N.; Gharbi-Benarous, J.; Acher, F.; Larue, V.; Pin, J. P.; Bockaert, J.; Azerad, R.; Girault, J. P. Conformational analysis of glutamic acid analogues as probes of glutamate receptors using molecular modelling and NMR methods. Comparison with specific agonists. *Bioorg. Med. Chem.* **1997**, *5* (2), 335–52.
- (18) Kainosho, M.; Ajisaka, K. Letter: Conformational analysis of amino acids and peptides using specific isotope substitution. II. Conformation of serine, tyrosine, phenylalanine, aspartic acid, asparagine, and aspartic acid beta-methyl ester in various ionization states. *J. Am. Chem. Soc.* **1975**, *97* (19), 5630–1.
- (19) Morton, A.; Matthews, B. W. Specificity of ligand binding in a buried nonpolar cavity of T4 lysozyme: linkage of dynamics and structural plasticity. *Biochemistry* **1995**, *34* (27), 8576–88.
- (20) Kurinov, I. V.; Harrison, R. W. Prediction of new serine proteinase inhibitors. *Nat. Struct. Biol.* **1994**, *1* (10), 735–43.
- (21) Sacchettini, J. C.; Gordon, J. I.; Banaszak, L. J. Crystal structure of rat intestinal fatty-acid-binding protein. Refinement and analysis of the Escherichia coli-derived protein with bound palmitate. *J. Mol. Biol.* **1989**, *208* (2), 327–39.
- (22) LaLonde, J. M.; Bernlohr, D. A.; Banaszak, L. J. X-ray crystallographic structures of adipocyte lipid-binding protein complexed with palmitate and hexadecanesulfonic acid. Properties of cavity binding sites. *Biochemistry* **1994**, *33* (16), 4885–95.
- (23) Young, A. C.; Scapin, G.; Kromminga, A.; Patel, S. B.; Veerkamp, J. H.; Sacchettini, J. C. Structural studies on human muscle fatty acid binding protein at 1.4 Å resolution: binding interactions with three C18 fatty acids. *Structure* **1994**, *2* (6), 523–34.
- (24) Poulos, T. L.; Howard, A. J. Crystal structures of metyrapone- and phenylimidazole-inhibited complexes of cytochrome P-450cam. *Biochemistry* **1987**, *26* (25), 8165–74.
- (25) Xu, Z.; Bernlohr, D. A.; Banaszak, L. J. The adipocyte lipid-binding protein at 1.6-Å resolution. Crystal structures of the apoprotein and with bound saturated and unsaturated fatty acids. *J. Biol. Chem.* **1993**, *268* (11), 7874–84.
- (26) Kleywegt, G. J.; Bergfors, T.; Senn, H.; Le Motte, P.; Gsell, B.; Shudo, K.; Jones, T. A. Crystal structures of cellular retinoic acid binding proteins I and II in complex with all-trans-retinoic acid and a synthetic retinoid. *Structure* **1994**, *2* (12), 1241–58.
- (27) Verschuere, K. H.; Seljee, F.; Rozeboom, H. J.; Kalk, K. H.; Dijkstra, B. W. Crystallographic analysis of the catalytic mechanism of haloalkane dehalogenase. *Nature* **1993**, *363* (6431), 693–8.
- (28) Newcomer, M. E. Structure of the epididymal retinoic acid binding protein at 2.1 Å resolution. *Structure* **1993**, *1* (1), 7–18.
- (29) Leslie, A. G. Refined crystal structure of type III chloramphenicol acetyltransferase at 1.75 Å resolution. *J. Mol. Biol.* **1990**, *213* (1), 167–86.
- (30) Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. Quaternary ligand binding to aromatic residues in the active-site gorge of acetylcholinesterase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90* (19), 9031–5.
- (31) Cowan, S. W.; Newcomer, M. E.; Jones, T. A. Crystallographic studies on a family of cellular lipophilic transport proteins. Refinement of P2 myelin protein and the structure determination and refinement of cellular retinol-binding protein in complex with all-trans-retinol. *J. Mol. Biol.* **1993**, *230* (4), 1225–46.
- (32) Hamilton, J. A.; Steinrauf, L. K.; Braden, B. C.; Liepnieks, J.; Benson, M. D.; Holmgren, G.; Sandgren, O.; Steen, L. The x-ray crystal structure refinements of normal human transthyretin and the amyloidogenic Val-30→Met variant to 1.7-Å resolution. *J. Biol. Chem.* **1993**, *268* (4), 2416–24.
- (33) Weber, P. C.; Ohlendorf, D. H.; Wendoloski, J. J.; Salemme, F. R. Structural origins of high-affinity biotin binding to streptavidin. *Science* **1989**, *243* (4887), 85–8.
- (34) Oh, B. H.; Ames, G. F.; Kim, S. H. Structural basis for multiple ligand specificity of the periplasmic lysine-, arginine-, ornithine-binding protein. *J. Biol. Chem.* **1994**, *269* (42), 26323–30.
- (35) Eads, J.; Sacchettini, J. C.; Kromminga, A.; Gordon, J. I. Escherichia coli-derived rat intestinal fatty acid binding protein with bound myristate at 1.5 Å resolution and I-FABPArg106→Gln with bound oleate at 1.74 Å resolution. *J. Biol. Chem.* **1993**, *268* (35), 26375–85.