Comparative Performance Assessment of the Conformational Model Generators Omega and Catalyst: A Large-Scale Survey on the Retrieval of Protein-Bound Ligand Conformations

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In continuation of our studies to evaluate the ability of various conformer generators to produce bioactive conformations, we present the extension of our work on the analysis of Catalyst's conformational subsampling algorithm in a comparative evaluation with OpenEye's currently updated tool Omega 2.0. Our study is based on an enhanced test set of 778 drug molecules and pharmacologically relevant compounds extracted from the Protein Data Bank (PDB). We elaborated protocols for two common conformer generation use cases and applied them to both programs: (i) high-throughput settings for processing large databases and (ii) high-quality settings for binding site exploration or lead structure refinement. While Catalyst is faster in the first case, Omega 2.0 better reproduces the bound ligand conformations from the PDB in less time for the latter case.

INTRODUCTION

3D virtual screening performed on precomputed conformation databases has proved to be a very efficient way for virtual screening[1] and therefore is frequently preferred over technologies that treat the ligand to be flexible during the matching process. For this approach, accurate and efficient generation of representative multiconformational models is an essential precondition. Multiconformer generation programs recently have been assessed in terms of how accurately generated conformers correspond to low-energy conformations. Several studies though disagree with the assumption that a bioactive conformation closely resembles the global energy minimum conformation,[2−8] and the energy threshold of protein-bound conformers is often considerably above the global energy minimum. The structure resulting from X-ray analysis of a homogeneous crystal might be less useful for comparative performance tests, since during induced fit of the ligand to the receptor both molecules experience a certain degree of conformational strain resulting in the bioactive conformation that may differ from that observed in crystals of the pure unbound ligand. For virtual screening using 3D methods, the reproduction of bound conformations and the sampling of possible bioactive poses are therefore more interesting. One of the largest pools of experimentally determined bioactive conformations is the PDB.[9] We are aware that especially low molecular weight compounds stored in this database may have quality shortcomings. However, our analysis of a small sample of ligands that are represented in the PDB and CSD (Cambridge Structural Database) suggests that there is only minor difference in the predictability of both conformations for Catalyst.[4] Average root mean square deviation (RMSD) measured between the best fitting generated conformer and the CSD conformer was found to be only 2.4% lower than for the respective PDB conformation. This observation encourages us to use the PDB as a starting point for further studies although there is a necessity to take into account that some single cases of low quality and unreasonable conformations due to historic reasons or problems in the refinement process might occur. These cases must be corrected manually or omitted if correction is impossible due to lack of available information.

With our recent examination of Catalyst's conformational model generator CatConf (also known as ConFirm),[4] we presented the first extensive large-scale survey on the in silico retrieval of bioactive conformations: A representative sample of 510 pharmacologically relevant ligands was extracted from the PDB for quality assessment of the generated conformational models. Both Catalyst subsampling algorithms, FAST and BEST, were investigated: While FAST works well for reliable sampling of large databases, BEST outperforms its faster counterpart especially when sampling very flexible or macrocyclic compounds with large conformational space. FAST and BEST were examined with maximally 50, 100, and 250 generated conformers. The RMSD between the best fitting generated conformer and the experimentally determined bioactive conformation retrieved from the PDB was used as the quality benchmark for the generator. We proved that Catalyst's conformational model generation is capable of reproducing the bioactive conformation with high quality ensembles that are most of the time well suited for virtual screening techniques most of the time and provided a reliable user-guide for best CatConf performance. In more than 80%

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of all investigated cases, fittings of the best generated conformer and the bioactive conformation below RMSD 1.50 were achieved and in 93% of all cases below RMSD 2.0. RMSD greater than 3.0, which is not suitable for in silico drug research, was found only in 0.6% of all investigated geometries. The computational time considerably rises between maximally 50 conformers per ensemble generated with FAST and a maximum of 250 conformers calculated with BEST by a factor of 230, while the RMSD drops from 1.06 to 0.93 (−12.5%). Therefore, we recommend a limit of 50 conformers and FAST mode for database sampling resulting in the best cost/performance ratio. As expected, the examination showed that both the number of rotatable bonds and molecular weight, which represent the torsional degrees of freedom and in part the size of molecules, have significant impact on the resulting RMSD. The average RMSD rises from 0.4 to 1.6 between the lightest and the heaviest fraction of molecules investigated. Moreover, also the average energy value estimated by Catalyst's CHARMm force field[10] mainly depends on these molecular parameters: While the average calculated energy with both FAST and BEST lies between 4.6 and 6.5 kcal/mol, the heaviest representatives of the sample (MW > 500) have a three times higher average energy threshold (9 kcal/mol) than the lightest fraction. This fact corroborates that the default energy threshold limit of 20 kcal/mol performs best for CatConf. During our examination we found significantly higher RMSD (34−45%) and energy values (4−42%) for sulfonamides. Though Catalyst 4.8 is able to retrieve conformations that are fitting to the bioactive conformation within the 20 kcal/mol energy interval, CatConf penalizes the sulfonamide conformation with too high energy by assuming a wrong torsion angle R−S−N−R1/R2. The generated lowest energy conformation often adopts a 'trans'-like conformation, where two substituents at nitrogen and sulfur share a torsional angle τ of 180°. This is in contrast to crystallographic data from the PDB and CSD,[11] which ensures a very high bias toward about 60°.

In this ongoing investigation, we now present a comparative analysis of two state-of-the-art conformational model generators with different algorithmic approaches: Omega,[12] which employs a rule-based algorithm in combination with variants of the Merck force field 94, and Catalyst,[13−15] which uses a poling algorithm to maximize conformational diversity. While we focused our work on the examination of several user-adaptable Omega parameters, we also adapted the Catalyst settings for comparability reasons.

An important point to mention is that catConf was developed to create conformer databases for 3D pharmacophore searches only, while Omega is a stand-alone product used for several applications. It is a part of the default protocol for OpenEye's docking program Fred as well as for their shape fitting tool ROCS and the electrostatics scoring program EON. A more recent application even uses Omega conformations for exhaustive enumeration of possible poses for electron density fitting in OpenEyes's tool "afitt". The requirements for pharmacophore searching and the other approaches are slightly different: Shape fitting seems to require more conformational poses due to the higher constraint level necessary for comparison, while pharmacophore features typically have tolerance spheres of up to 1.5 Å, allowing the retrieval of matches with fewer conformations.

Until now, there have been very few evaluative studies on Omega. The most important one was published by Boström et al., who carefully examined 36 Omega conformer models of high-quality PDB ligands in 2003.[16] The examination was based on a very small set with no ligands exceeding 11 rotatable bonds and two-thirds having less than six. Since our prior study proved that Catalyst's BEST algorithm shows a better performance compared to FAST for molecules with more than 12 rotatable bonds in particular, we believe that this small and only medium-flexible test set is not sufficient for a representative examination of several generator options that are supplied by Omega. Furthermore, both Omega and Catalyst have been enhanced in the meantime and are available in new versions.

Our primary aim was therefore to work out a precise evaluative comparison of both conformer model generators in their latest versions and to provide an up-to-date comprehensive user-guide for best Omega performance.

METHODS

This continuing assessment on the retrieval of the bioactive conformation with conformational model generators is based on the careful analysis of 778 drug molecules and pharmacologically relevant structures that have been selected manually from the PDB to meet several requirements for a representative survey (see 'Test Set Assortment'). We used shell scripts and Perl for workflow automation. Nine hundred nineteen compounds were submitted to Omega and Catalyst for model generation with several different parameters; 778 of these passed all process steps without issues. One hundred forty-one ensembles dropped out, mostly within the Citest RMSD calculation process that would require manual interaction within the Catalyst GUI. More detail on this issue is given below under 'Computation of the RMSD'.

Hardware Specifications and Runtime Environment. All operations considering calculation time were processed on a single Intel Pentium IV 2800 MHz PC equipped with 1 GB RAM running Linux Fedora Core 3. A fifteen nodes Linux cluster equipped with Intel Pentium IV and AMD Athlon XP processors was used for distributed computing.

Software Specifications. The following program versions were used in this study: Babel3 2.0[12] for file conversion, Catalyst 4.11, and Omega 2.0. LigandScout 1.0[17−19] was used for the retrieval, preprocessing and inspection of the sample. Pipeline Pilot 5.0.1.100[20] was used for substructure search of sulfonamides as well as CSD and PDB duplicate elucidation.

CONFORMATIONAL MODEL GENERATORS

Omega. Omega was designed for high-throughput generation of conformational models to reproduce the bioactive conformation. It consists of two parts: one for model building and one for torsion driving. The generator uses fragment templates along sigma bonds to assemble initial models. Conformations of these fragments are either retrieved from previously generated libraries or gained on the fly. Molecule assembly is accomplished by simple vector alignment. As the seed conformation of a molecule has been determined, Omega performs ring and invertible nitrogen

enumeration to construct additional models. Omega attempts to generate every possible combination of ring conformations for a given geometry.

Next, Omega begins torsion search with an assessment of freely rotatable bonds. RMSD heavy atom distance deviation provides the measure for final ensemble selection, and SMARTS matching is used for the assignment of favored dihedral angles for each rotatable bond (RB). Geometric symmetry checks and pattern recognition avoid multiple searches for equivalent dihedral angles. After torsions are altered by 120 and 180 degrees and assorted into fragments of sets of up to five contiguous rotatable bonds, exhaustive depth-first torsion search generates conformers which are listed with energy thresholds. Complete molecules are constructed by selection of the lowest energy set of fragments and then the next lowest set, until a termination limit is reached (as e.g. defined by the total number of generated conformers or the maximum energy threshold). The final conformational model is assembled by conformers that comprise a certain RMSD to each other, with respect to the energy threshold limit and the ensemble size.

In this study, we investigated several user-adaptable torsion driving and force field settings supplied in Omega as explained below. If not mentioned otherwise, default settings were used for conformational model generation. Both nitrogen and ring enumeration were kept true (default). Unless mentioned otherwise, the fragment library supplied by Omega was used for this examination.

Omega Parameters. ewindow: The *ewindow* parameter defines the strain energy range within which conformers are considered as candidates for the final ensemble. Consistent with Catalyst, conformers that exceed this energy limit are rejected. The default *ewindow* setting is 25.0 kcal/mol.

maxconfs: The *maxconfs* setting defines the maximum number of conformers (NOC) that will be elected for the final conformational ensemble. After energy threshold and RMSD check, conformers are stored as part of the final model up to the *maxconfs* limit (default = 400).

maxpoolsize: The *maxpoolsize* option sets the maximum number of conformers to be stored temporarily before RMSD duplicate check. The default value is 10 000, which in general drastically exceeds the necessary number of intermediate solutions unless e.g. the RMSD cutoff is set very low.

rms: Besides *ewindow*, the *rms* flag defines the second criterion on the number of conformers considered for the final ensemble. *rms* sets the minimum root mean square deviation of coordinates below which two conformers are considered to be identical. While a lower *rms* limit causes Omega to generate larger ensembles, a higher *rms* promotes conformational diversity. The default *rms* value is 0.8.

buildff: The *buildff* flag defines the force field used for the generation of fragments that are subsequently assembled to build the initial model of the input molecule. There are two predefined force fields (mmff: Merck molecular force field[21] and mmff94s (i.e. the 94s variant of the Merck molecular force field)) with two additional modifications each available.[21,22] The *noestat* variant includes all force field terms except coulomb interactions; the *trunc* option excludes both coulomb and the attractive part of van der Waals interactions. The default force field used for ensemble generation is *mmff94s_noestat*.

searchff: The *searchff* parameter is used to specify the force field used to calculate strain energies of conformers generated during torsion driving. Consistent with *buildff*, both force fields with two flavors each are available (default = *mmff94s_noestat*).

For concise distinction between both force field parameters in the following figures and text, we use a prefixed 'b' for *buildff* and 's' for *searchff*, respectively: e.g. *bmmff94s_noestat* stands for *buildff mmff94s_noestat*.

maxtime: The *maxtime* limit defines the maximum amount of time to be spent for conformer generation per molecule (default = 30 s).

eRange, maxConfRange, and rmsRange. These parameters have been introduced to Omega 2.0 limiting the maximum energy window, the maximum number of conformers per ensemble, and the RMS duplicate cutoff with respect to the number of rotors of the molecule. Since in this first assessment of Omega we were primarily interested to find settings for the best overall performance of Omega within a given dimension of flexibility, we passed a detailed analysis on these parameters for now. We are looking forward to an in-depth analysis of this issue.

fraglib: The *fraglib* parameter defines a OEBinary V2 molecule file containing the coordinates of prebuilt acyclic fragments as well as multiple conformations of cyclic systems. As a default, we used the supplied fragment library, but we also built a fragment collection for our test set to estimate the benefit in computation time.

Two optimized settings for *high-quality (HQS)* and *high-throughput (HTS)* conformational model generation with multiple user-adapted parameters were elaborated: *HTS* stands for *maxconfs* 50 and *bmmff94s_trunc*; *HQS* is consistent with *HTS*, except *maxconfs* 500 and *rms* 0.4.

Catalyst. The Catalyst software suite includes the CatConf module for conformational model generation using the CHARMm force field. The Catalyst CHARMm force field parameter set, which was used in our study, is based on the CHARMm parameters with several refinements. The program is designed for comprehensive conformational space sampling. The integrated poling algorithm described by Smellie et al.[14,15] repels too similar conformers and therefore provides models of highly diverse conformations. There are two prominent options available for quality/computational time ratio management in Catalyst: the maximum number of generated conformers setting and the FAST/BEST algorithm selection. FAST's calculation speed is largely improved by the internal fragment library of ring conformations. For an analysis of these generator options the reader is referred to our prior study on Catalyst.[4]

For all calculations, the energy threshold value was kept at 20 kcal/mol, which has been proven to ensure optimum performance.[4] Conformational models were generated with 50 FAST, 100 FAST, 250 FAST, 500 FAST, 50 BEST, 100 BEST, and 250 BEST. 500 BEST exceeds internal memory for several very flexible molecules and thereby causes Catalyst to automatically restrict the exploration of conformational space. However, the emerging flood of data of 500 conformers per molecule would not be convenient for in silico screening of millions of compounds; computational costs in processing time and data storage are not in reasonable relationship with the improvement of model quality.

Hereafter, Catalyst settings are abbreviated with "CF" or "CB" for the respective algorithm and the limit of generated conformations per ensemble. For example, CB250 stands for Catalyst BEST algorithm with a maximum of 250 generated conformers.

WORK FLOW SCHEME

Proceeding with our former study, we subdivided our work flow into four major steps: (a) extraction of a ligand set from the PDB, (b) conformational search with different methods and settings, (c) computation of fit values between the protein-bound ligand conformation and all generated conformations by usage of the Catalyst software module Citest, and (d) data analysis of the best fitting conformation of each ligand.

Test Set Assortment. We refined and enhanced our prior 510 compounds test set and built up a 919 PDB compound sample by manuals election to fulfill several needs. Focus was shifted from pharmacologically relevant structures toward drug molecules. The molecules retrieved are of complexes with reasonable resolution (the vast majority has resolution below 2.5 Å) and quality. LigandScout, a pharmacophore management and visualization software, was used to assign bond characteristics to all PDB entries and to extract the small organic molecules from their respective protein environment. Since inaccuracies in PDB geometry data may lead to false interpretation of atom hybridization states and bond types, both properties were verified manually. The experimentally determined bioactive conformations were stored in MDL MOL files without charges; tautomers were derived from the optimum protein−ligand interaction pattern. Special care was taken on both molecular weight as well as rotatable bond distributions. The respective histograms of all 778 assessed compounds, which passed the whole automated work flow without any issues, show representative property distributions (Figure 1).

Compared to our prior study, the revision and extension of our test set led to moderately decreased overall flexibility of the investigated compounds. This is a direct result of the quantitative depreciation of e.g. very flexible yet pharmacologically less relevant substructures and the adjustment of our focus toward drug molecules. However, one-third of the compounds shows a minimum of nine rotatable bonds and therefore guarantees a reliable examination of molecules with large conformational space.

Input Preparation. To avoid any influences on conformational model generation by presenting 3D seed structures, isomeric SMILES notation was used as program input. We tested SMILES code generated by Catalyst and Babel3 as program input for each conformational model generator. For compatibility reasons, adaptations in the MOL-to-SMILES conversion process were inescapable to ensure the best performance for each program. Omega was found to perform most reliably with the isomeric SMILES code converted via Babel3. Nine hundred eight of all 919 compounds could be computed without difficulties. Though we validated Babel3 SMILES notation, Catalyst showed a number of issues when parsing the code. Therefore, we chose to use the Catalyst export function in the graphical user interface for the retrieval of isomeric SMILES. Nine hundred ten molecules were processed by CatConf without issues. Catalyst SMILES could not be parsed by Omega in multiple cases.

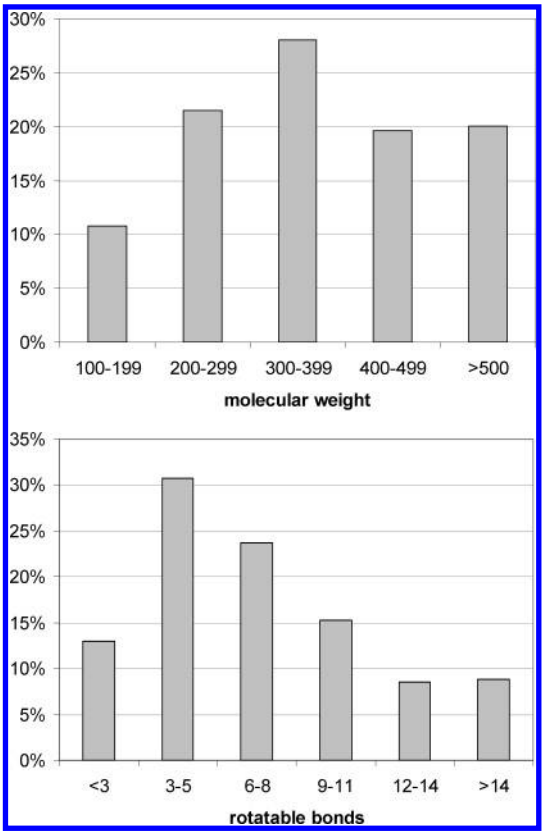

Figure 1. Molecular weight and rotatable bonds distributions of the 778 assessed compounds. Both diagrams prove our sample's diversity in terms of structural dimensions and flexibility.

Computation of the RMSD. RMSD between the bioactive conformation and the best fitting conformer of the calculated ensemble has been demonstrated to provide a meaningful quality benchmark for conformational models. In this procedure, the structures are aligned using superposition of the matching heavy atoms between each pair of models. The function to be minimized is defined as the sum of squares of the distances between all pairs of heavy atoms to be superimposed. Catalyst's Citest module was used to compare conformations in a fully automated way. Citest allows the user to compare two conformers of both same or different molecules as well as fit-value calculation of molecules on feature-based hypotheses. For about 7% of all tested geometries, Citest requires defined tethers between equivalent heavy atoms for RMSD comparison. Since this feature is only available by manual interaction within the Catalyst GUI and our aim was to develop a fully automated and unbiased workflow protocol, we rejected these molecules. In most of these problematic cases, Citest is not able to fit a certain ligand with every generator setting; in a minority of cases, automated fitting is not achieved for conformational models generated with a certain user setting. To provide reliable conclusions on the generator quality, we investigated the RMSD values considering ensemble size.

Data Analysis. Data were elaborated for 778 compounds with Perl and shell scripts. As described above, about 1% of the basic 919 compounds set failed due to parsing issues, and in about 7% of all cases manual tether definition

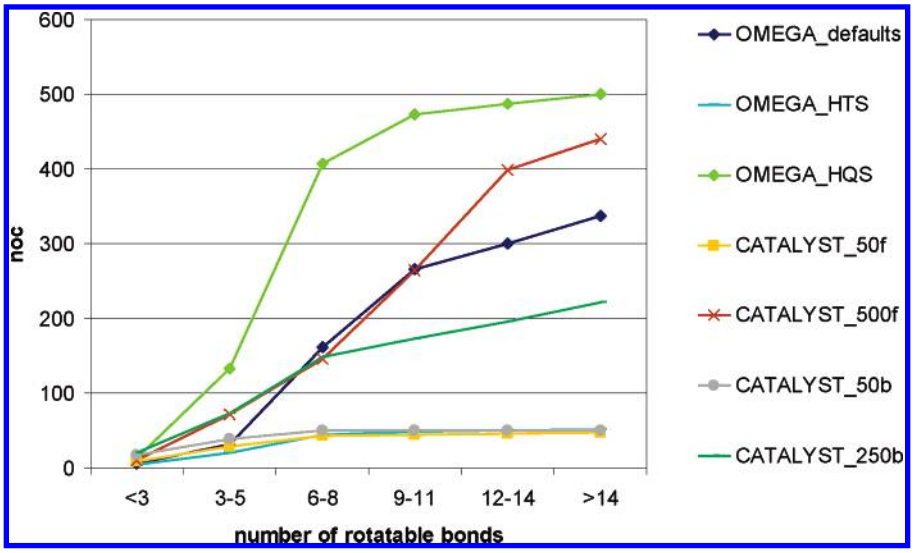

Figure 2. Average NOC depending on the number of rotatable bonds. Omega shows a steeper increase of generated conformers per ensemble with respect to the number of rotors in the HQS mode.

would be needed for fitting with Citest. Therefore, finally 778 compounds that passed the workflow with every generator setting were assessed.

RESULTS AND DISCUSSION

Number of Generated Conformers (NOC). The average NOC has a major impact on the accuracy of conformational ensembles in terms of representing the bioactive conformation and is directly related to the number of rotatable bonds (Figure 2). Very rigid compounds with less than three rotatable bonds are represented in small models of up to 50 conformers by all model generators with all investigated settings; more flexible ligands show extended conformational variability and subsequently an average NOC approaching the respective upper limit.

A higher NOC limit implicates lower overall RMSD between the best fitting generated conformer and the experimentally determined bioactive conformation. However, calculation time and data file size enlarge dramatically with respect to the moderate gain in accuracy, which is the reason that for larger databases only ensembles of maximally 50 conformers may be convenient. Also the substantial increase in computational demands in consecutive virtual screening applications must not be underestimated. Thus, the ability to generate conformational models representing the bioactive conformation with only a few calculated conformations is of special interest.

Omega's default limit of conformations is 400 per ensemble, which results in an average of 145 generated conformations per model (Figure 3). We tested Omega's performance with NOC constrictions of 50, 250, 400 (default setting), and 500 conformers per ensemble. A limit of 50 conformations produces 33 (CF 35, CB 42) solutions per compound on average and a cutoff at 250 conformations 109 (CF 115, CB 123), respectively. A maximum of 500 conformations give rise to an average of 165 (CF 170) conformers. Overall, Omega produces very similar average NOCs as Catalyst FAST, which makes both programs best comparable in terms of representing the bioactive conformation with the least generated conformers.

As mentioned before, Omega assembles the final conformational models out of temporary geometries with respect to the energy threshold window (*ewindow*) and the RMSD (*rms*) deviation between already selected conformers and the currently considered candidate. Figure 3 proves the decisive correlation of these two parameters and the respective size of the ensembles: A low-energy threshold cutoff of 10.0 kcal/mol results in an average of only 113 conformers per model, and a 40.0 kcal/mol limit raises the average value to 157. Though the average RMSD decreases with higher energy threshold limits (as described in the RMSD analysis section below), it seems to be more advisable to maintain the *ewindow* default of 25 kcal/mol and to tune alternative parameters instead. A lower energy threshold than 25 kcal/mol may reject valuable conformations, and a higher energy threshold may lead to high-energetic conformers that are not likely to represent the bioactive one; any lowering or rising of this parameter will decrease model quality and is therefore not recommended. Another powerful generator parameter is *rms*. A lower *rms* limit decreases the minimum RMSD required for two conformers to be considered as unique. Thus, *rms* 0.2 produces 283, *rms* 0.4 produces 248, and *rms* 1.2 produces 55 conformers per ensemble, respectively. While the various seed structure build force fields as well as the *maxpoolsize* flag have no effect on the NOC, the search force field parameter has a minor influence on the average NOCs: The basic *smmff* and *smmff94s* torsion search force fields produce an average of 124 and 125 conformers, respectively, and both modifications of both force field types have a higher average conformations output between 143 and 145. The highest average NOC (296 conformers) was achieved with *HQS*.

Evaluation of the Conformational Models Generated with Single User-Adapted Settings. This section provides insight into the impact of several user-adaptable parameters on the quality of model generation. By the help of this survey, we elucidated optimized settings with respect to different application scenarios, which are described in detail below in the section 'Optimum Omega Performance for High-Throughput and High-Quality Screening'.

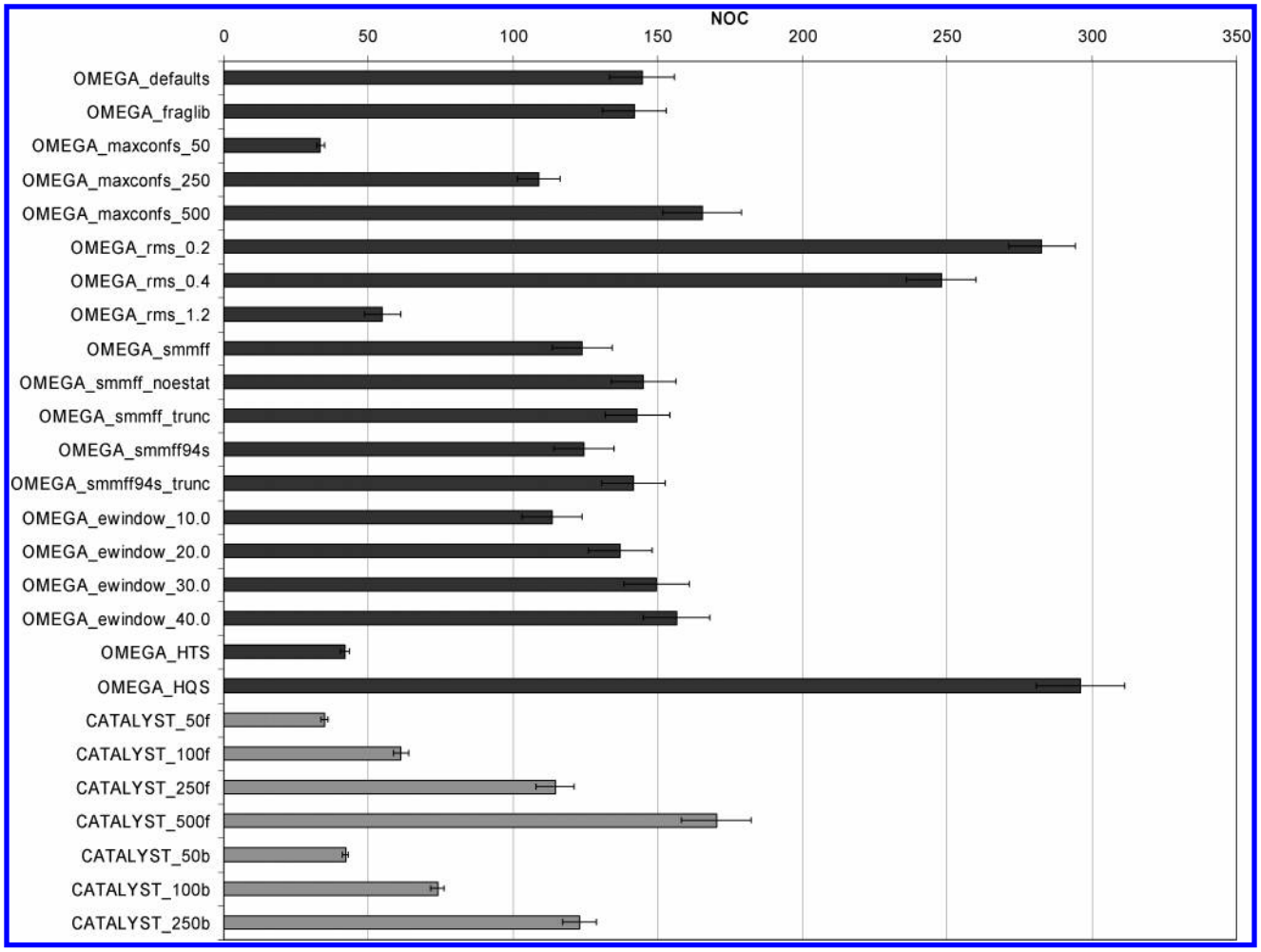

Figure 3. The average number of generated conformers as a function of the settings used for model generation with Omega and Catalyst. Omega's most important control parameters for the number of conformers generated per ensemble are the *maxconfs* and *rms* flags.

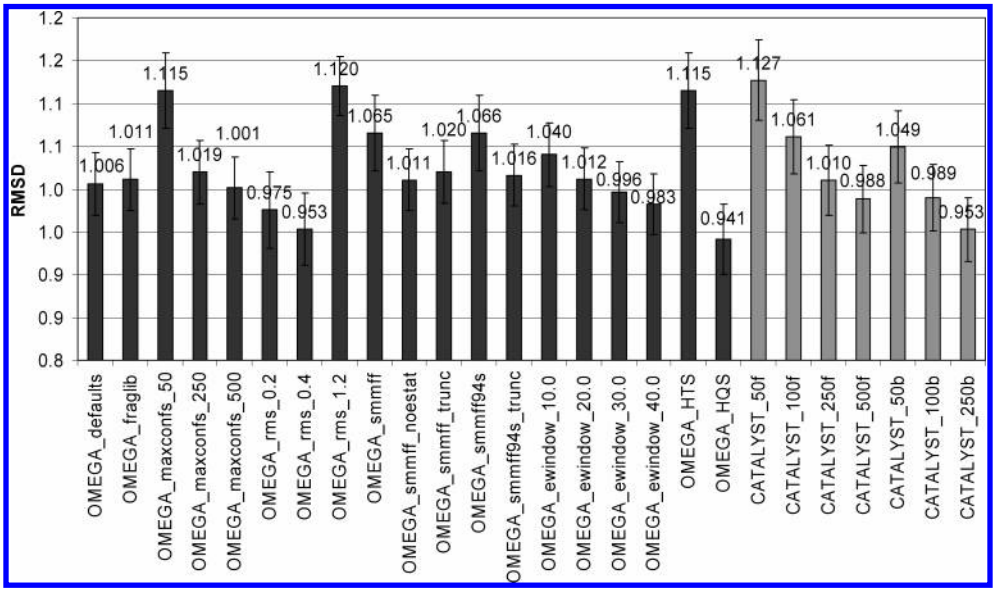

Figure 4. Average RMSD for the best fitting conformation. This histogram shows the impact of several user-adaptable generator settings on the average RMSD of the best fitting conformers.

The average RMSD with Omega's default settings (*maxconfs* 400) is 1.006 (Figure 4). Compared to Catalyst's FAST algorithm, Omega appears to perform marginally better in reproducing the bioactive conformation with small conformational ensembles: Maximally 50 generated conformers result in an average RMSD value of 1.115 (CF50 RMSD 1.127; +1.1%). When generating large ensembles, Catalyst FAST (CF500 RMSD 0.988) slightly outperforms Omega (*maxconfs* 500 RMSD 1.001; +1.3%). While the minor differences discussed above are not statistically significant, Catalyst's more exhaustive and time-consuming BEST algorithm clearly shows increased conformational

Table 1. Spreading of the RMSD of the 778 Compounds Test Set

settings\rms_class	<0.1	<0.5	<1	<1.5	<2	<3	≥2
OMEGA_defaults	0.9%	10.9%	45.6%	30.3%	7.8%	3.2%	4.4%
OMEGA_fraglib	0.9%	10.3%	45.0%	31.9%	7.7%	3.2%	4.2%
OMEGA_maxconfs_50	0.9%	10.0%	38.4%	32.0%	10.0%	6.3%	8.6%
OMEGA_maxconfs_250	0.9%	10.9%	44.5%	30.5%	8.4%	3.7%	4.9%
OMEGA_maxconfs_500	0.9%	10.9%	46.1%	30.3%	7.6%	3.0%	4.1%
OMEGA_rms_0.2	0.9%	23.9%	33.9%	25.3%	8.9%	5.4%	7.1%
OMEGA_rms_0.4	0.9%	22.9%	36.8%	24.9%	8.7%	4.6%	5.8%
OMEGA_rms_1.2	0.8%	6.9%	33.8%	43.6%	10.2%	3.6%	4.8%
OMEGA_ewindow_10.0	0.9%	10.2%	42.2%	32.9%	9.0%	3.6%	4.9%
OMEGA_ewindow_20.0	0.9%	11.2%	44.5%	30.6%	8.5%	3.2%	4.4%
OMEGA_ewindow_30.0	0.9%	10.9%	46.0%	30.7%	7.5%	3.0%	4.0%
OMEGA_ewindow_40.0	0.9%	11.7%	47.3%	28.8%	7.2%	3.2%	4.1%
OMEGA_maxtime_5	0.9%	10.9%	45.6%	30.5%	7.7%	3.2%	4.4%
OMEGA_maxtime_10	0.9%	10.9%	45.6%	30.5%	7.7%	3.2%	4.4%
OMEGA_HTS	0.8%	10.7%	37.5%	32.4%	10.3%	6.2%	8.4%
OMEGA_HQS	0.8%	23.1%	38.0%	23.9%	8.7%	4.5%	5.4%
CATALYST_50f	1.2%	16.8%	29.0%	26.7%	17.1%	7.6%	9.1%
CATALYST_100f	1.2%	18.4%	30.3%	28.5%	15.4%	5.7%	6.2%
CATALYST_250f	1.2%	19.2%	32.3%	29.8%	12.6%	4.5%	5.0%
CATALYST_500f	1.2%	19.3%	33.8%	30.1%	11.2%	4.0%	4.5%
CATALYST_50b	1.2%	16.8%	33.4%	28.3%	12.5%	7.5%	7.8%
CATALYST_100b	1.2%	18.5%	35.6%	27.5%	11.4%	5.7%	5.8%
CATALYST_250b	1.2%	19.5%	38.7%	25.8%	10.2%	4.6%	4.6%

sampling accuracy compared to Omega: average RMSD with CB50 is 1.049 (−5.9%) and CB250 0.953 (−6.5%), respectively. Overall, our data suggest a slightly higher accuracy for small ensembles within a moderate calculation time for Omega, while Catalyst is better regarding the generation of large ensembles, especially in the BEST algorithm mode.

Analysis of Omega's *rms* parameter proves that the default value of 0.8 is too restrictive for large ensembles; it leads to repression of valuable geometries. The highest accuracy is achieved with *rms* set to 0.4, which results in an average RMSD of 0.953. A further decreased RMSD similarity condition to *rms* 0.2 reraises the RMSD to 0.975; *rms* 1.2 pushes the average RMSD to 1.120.

*smmff94*s*_noestat* seems to be the best force field flavor for torsion search, which we assume to be caused by the fact that switching off electrostatics prevents strongly folded vacuum conformations (with intramolecular interactions such as hydrogen bonds) from being generated. *buildff* and *maxpoolsize* have no impact on the quality of the generated ensembles.

Corresponding to our Catalyst experiences, constricting the Omega *ewindow* default (25.0 kcal/mol) may have negative effects on the ensemble quality: While a larger energy threshold limit decreases the average RMSD (*ewindow* 30.0 kcal/mol 0.996; *ewindow* 40.0 kcal/mol 0.983 kcal/mol), conformational model quality is reduced by a low threshold of 10 kcal/mol (1.040). An unreasonably low-energy threshold of 2 kcal/mol would increase average RMSD by 25%, compared to the default settings. The highest RMSD ever measured was 4.649 for ptm-1jyg with *smmff*. We found that the default energy threshold limit of 25.0 kcal/mol is necessary for the acceptance of valuable geometries in the final conformational models. Too restrictive energy settings lead to rejection of high quality conformers that represent the bioactive conformation. However, instead of a more tolerant energy level than the default one of 25 kcal/mol it seems to be more advisable to adjust alternative flags for the retrieval of larger ensembles, as discussed above.

The average RMSD values do not provide full information on the comparability of conformational ensembles. Determination of the RMSD distribution of the respective computational settings gives more insight to this. Therefore, RMSD values were assigned to the classification we adapted from our prior study: RMSD below 0.5 means excellent fit. This range of RMSD approximately represents the experimental accuracy of protein X-ray crystallography; therefore, the compared molecules are in general declared identical. RMSD between 0.5 and 1.0 means good fit; RMSD below 1.5 is still acceptable. Fittings within $1.5 \leq \text{RMSD} < 2.0$ are more or less acceptable; some features may already drift apart. Conformations with RMSD above 2.0 cannot be considered as appropriate representations of the biologically active one.

Table 1 shows the gradation of RMSD values. Most fittings are achieved within the $0.5 \leq \text{RMSD} < 1.0$ intervall. Overall, excellent fit ($\text{RMSD} \leq 0.5$) was achieved in 8−25% of all cases with Omega and in 18−21% of all cases with Catalyst. For more than three-quarters of all compounds (Omega: 76−89%; Catalyst 74−85%) the best fitting conformer has RMSD below 1.5, and only in a few cases (Omega 7−14%; Catalyst 10−17%) RMSD is greater than 2.

The Impact of the Molecular Weight and the Number of Rotatable Bonds on the Average RMSD. Size and flexibility of molecules have major impact on the quality of generated conformational ensembles as they in part reflect the depth of conformational space. Both characters are in part represented by the molecular weight and the number of rotatable bonds. The molecular weight and the rotatable bonds distribution show increasing average RMSD with increasing compound size and flexibility.

Within the interval of MW 100−300, there is a minor decrease in ensemble quality (Figure 5). Above 300, the average RMSD increases from about 0.7 for MW 200−299 to about 0.9 with MW 300−399 and with similar gradient to about 1.1 for MW 400−499. Above Lipinski's oral bioavailability limit of MW 500, further increase to about

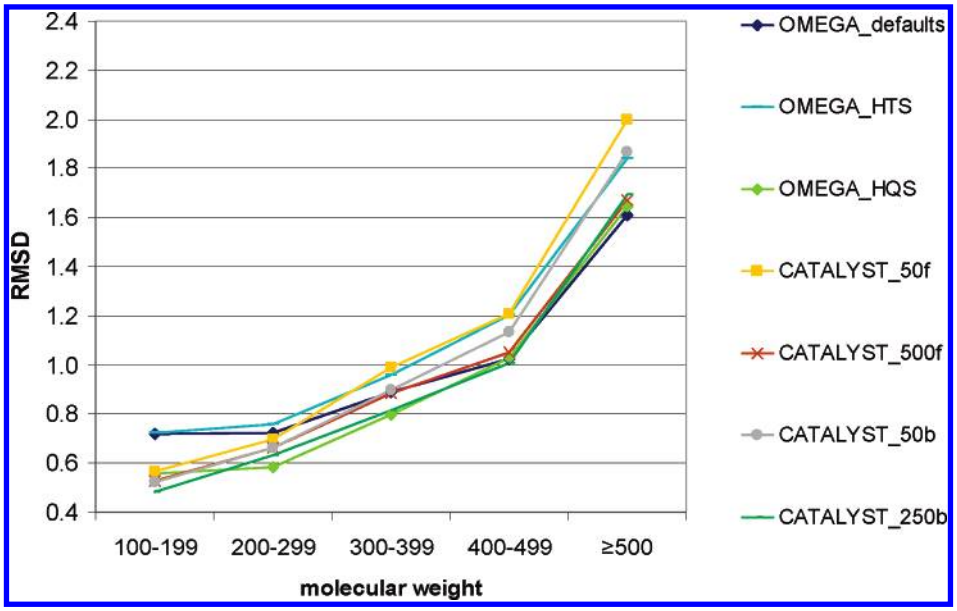

Figure 5. Average RMSD depending on the molecular weight. The size of the compounds, which is in part reflected by the molecular weight, shows significant impact on the average RMSD.

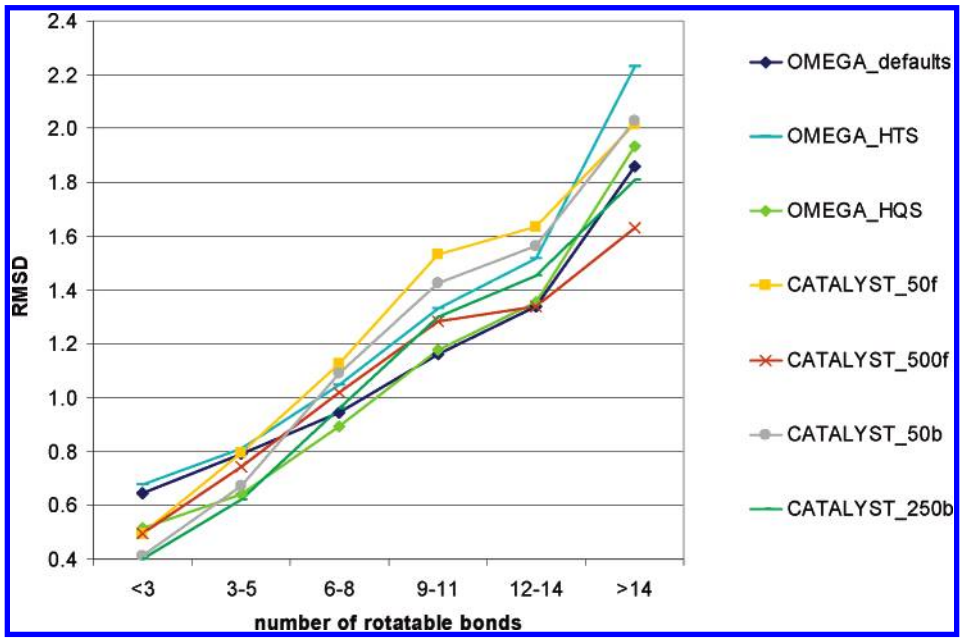

Figure 6. Average RMSD depending on the number of rotatable bonds. RMSD is in direct relation to the number of rotors of the molecules.

RMSD 1.8 is observed. Despite the decreasing 'drug-likeness', this fraction is yet of high interest for conformer ensemble investigations, although less important for drug research.

The number of rotatable bonds and the resulting average RMSD show an almost linear relationship (Figure 6). Average RMSD values start at about 0.5 in the RB < 3 class and rise to a level of about 1.9 for the most flexible investigated molecules.

Conformational Space Subsampling. It is an important criterion for conformational model generators whether a low-energy X-ray structure conformation can be reproduced. However, coverage of low-energy conformational space, i.e., the geometric distribution, is another point of interest particularly relevant when a molecule is used for 3D pharmacophore searches. We therefore attached all possible pharmacophore points to the X-ray structure of the ligand and aligned the generated conformers using the reference structure pharmacophore as a query. The pharmacophore feature definitions include hydrogen bond acceptors, donors, and positive and negative ionizable groups and were generated by LigandScout. After the alignment, which standardizes the relative position of each generated conformer, we projected all atom coordinates into two dimensions to create a printable scatter plot that gives an impression about the distribution of atom positions (Figure 7; further examples are provided as Supporting Information).

Projection was done using principal component analysis (PCA) taking the first two components as *x*- and *y*-axis and thus minimizing overlap; the rotation within the *xy*-plane is not relevant and therefore random. The more nonclustered points appear in the scatter plot, the more diverse the distribution of atom positions in 3D space. This parameter is not necessarily related to the quality of the model, but it

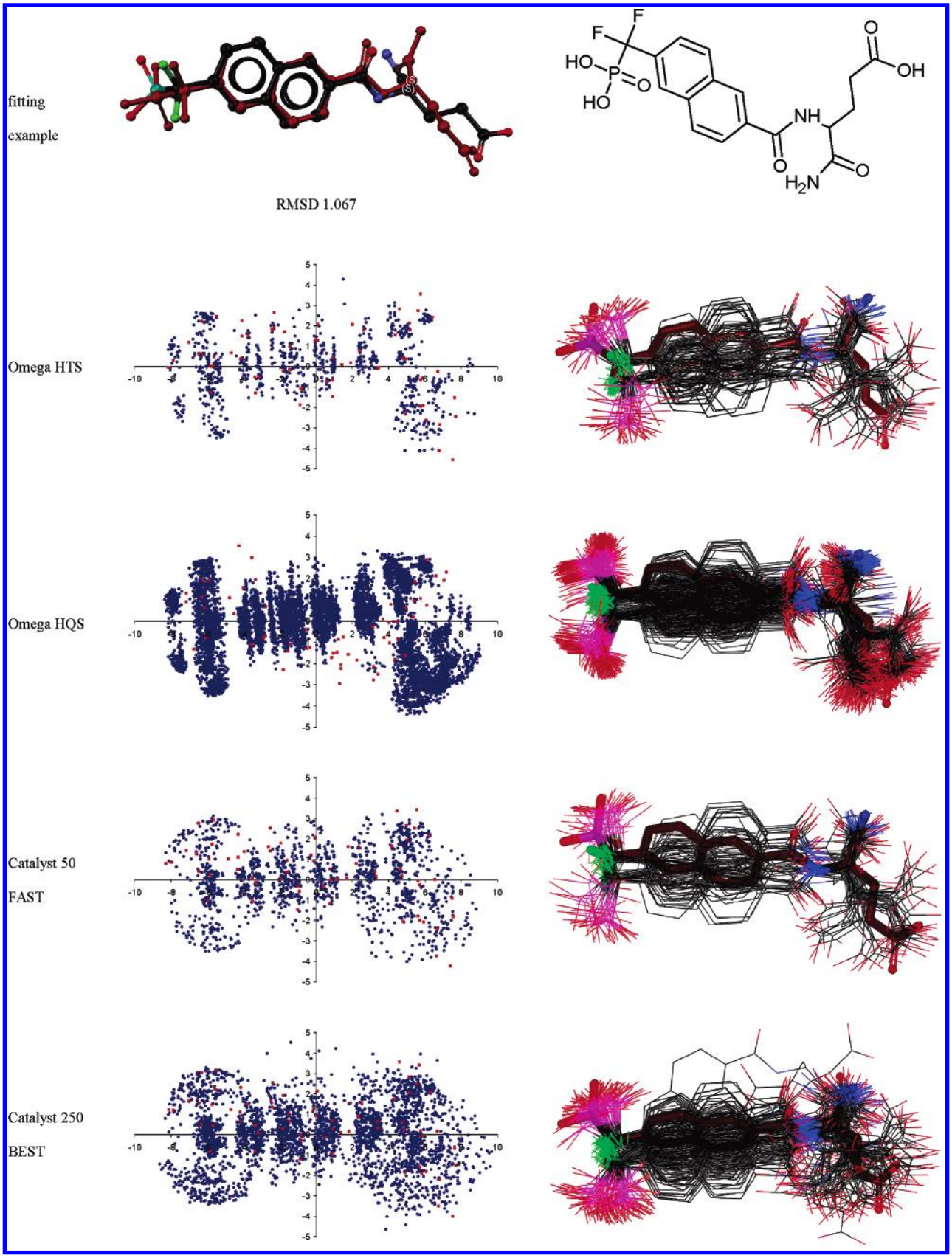

Figure 7. Conformational space subsampling of PDB ligand tpi-1bzc with Omega and both Catalyst FAST and BEST.

gives an impression how the different algorithms place atoms and how different conformers are with respect to the X-ray structure. In this sense Omega seems closely related to Catalyst FAST, while Catalyst BEST shows increased conformational space sampling depth.

Data File Size and Computational Resources. The following statistics present a benchmark on computational efforts and storage. Processing time of both model generators was assessed on a single Intel Pentium IV 2800 MHz workstation.

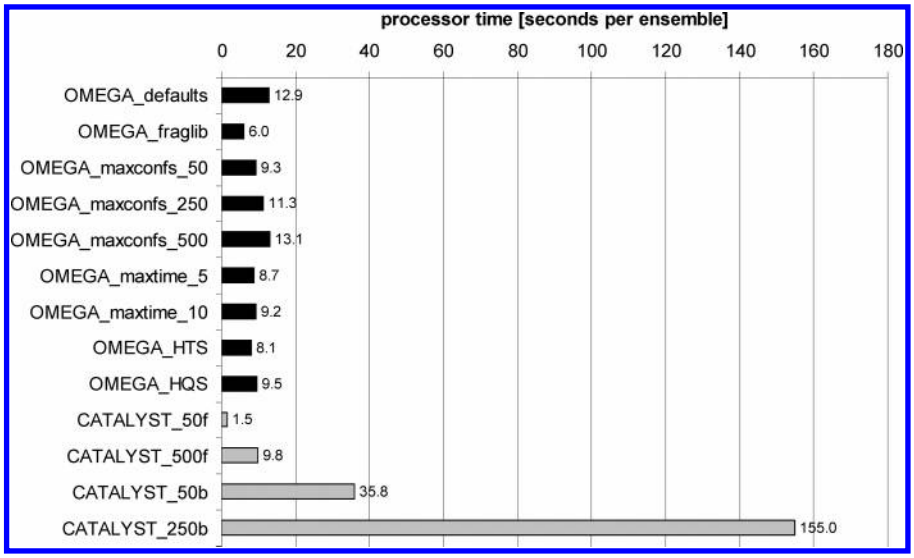

Figure 8. Processing times per ensemble. Catalyst FAST shows the best efficiency in conformational space subsampling.

Omega supports various output file formats as well as data compression on the fly. For best compatibility with Citest, we chose the Tripos MOL2 file format for data export: The initial set produces 150MB of data using *maxconfs* 50, 530 MB with *maxconfs* 250, and 800 MB using *maxconfs* 500. Catalyst's conformational models in CPD file format occupy less than one-third of disk space, and Omega oeb.gz file format only about one-sixth, respectively. With factory defaults and the supplied default fragment library, Omega's conformational model generation of the 778 compounds takes 12.9 s per ensemble (Figure 8); fragment library precalculation of the test set with Omega's *makefraglib* halves conformer generation time (6.0 s per ensemble). The number of conformers generated has a major impact on the calculation time: While *maxconfs* 50 requires 9.3 s per ensemble of processor time, *maxconfs* 500 raises the average generation time to 13.1 s. Omega's speed seems to be affected especially by macrocyclic systems: Five of the top 10 time-consuming ligands have macrocycles; the longest calculation time measured for Omega was found for the *HQS* model of rap-1pbk (277 s). Also some very flexible molecules take significant calculation time (e.g. alq-1m4h requires 34 s). The newly introduced *maxtime* flag that controls the maximum conformational search time (default is 30 s per ensemble) shows minor impact on the generation time: The quite low limit of 5 s per ensemble still requires 8.7 s of processing time on average. In contrast to that, Catalyst's FAST algorithm requires only 1.5 s for conformational ensembles of maximally 50 conformers, which is 18.5% of the respective speed-optimized Omega setting *HTS* (see below). Moreover, the generation of large conformational ensembles of maximally 500 conformers with Catalyst FAST consumes a similar amount of time as Omega's *HTS* setting. However, Catalyst's BEST algorithm requires significantly more computer power to gain high-quality conformers: A maximum of 50 conformers per ensemble takes 35.8 s, and maximally 250 conformers take 155.0 s, respectively.

Though these demands of CPU cycles may not represent any constraints for our test set, there is a need for efficient resource management when processing large molecule libraries with millions of compounds. For better expression of the conformational model generator efficiency we define the

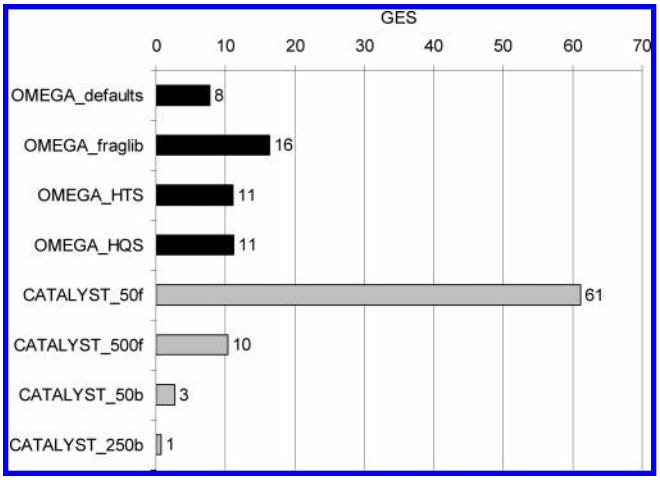

Figure 9. Generator efficiency score. Catalyst FAST clearly outperforms Omega and its BEST counterpart in high-throughput screening. For high-quality screening, Omega proves to be a very good choice.

GES (Generator Efficiency Score), a very simple yet to our experience a meaningful measure that considers both quality and computing time of the conformational ensembles:

$$\text{Efficiency} = \frac{\text{RMSD}^{-1}}{\text{calculation\_time[seconds]}} \times 100$$

Catalyst FAST clearly outperforms Omega and its BEST counterpart in high-throughput screening and achieves the best GES, which is 61 (Figure 9). For high-quality screening, Omega proves to be a very good choice (GES 11), showing comparable efficiency as CF500 (GES 10). Indeed, Omega *fraglib* (i.e. Omega using a fragment library of the respective compounds to be generated) shows an improved GES of 16; however, consideration of the fragment library processing time preceding the actual conformational model generation consumes this efficiency advantage. With a GES of 1, Catalyst 250 BEST is only recommended for most exhaustive conformational searches.

Figure 10 illustrates the rotatable bonds/time ratio and the NOC/time ratio. Omega *HQS* shows nearly no increase in

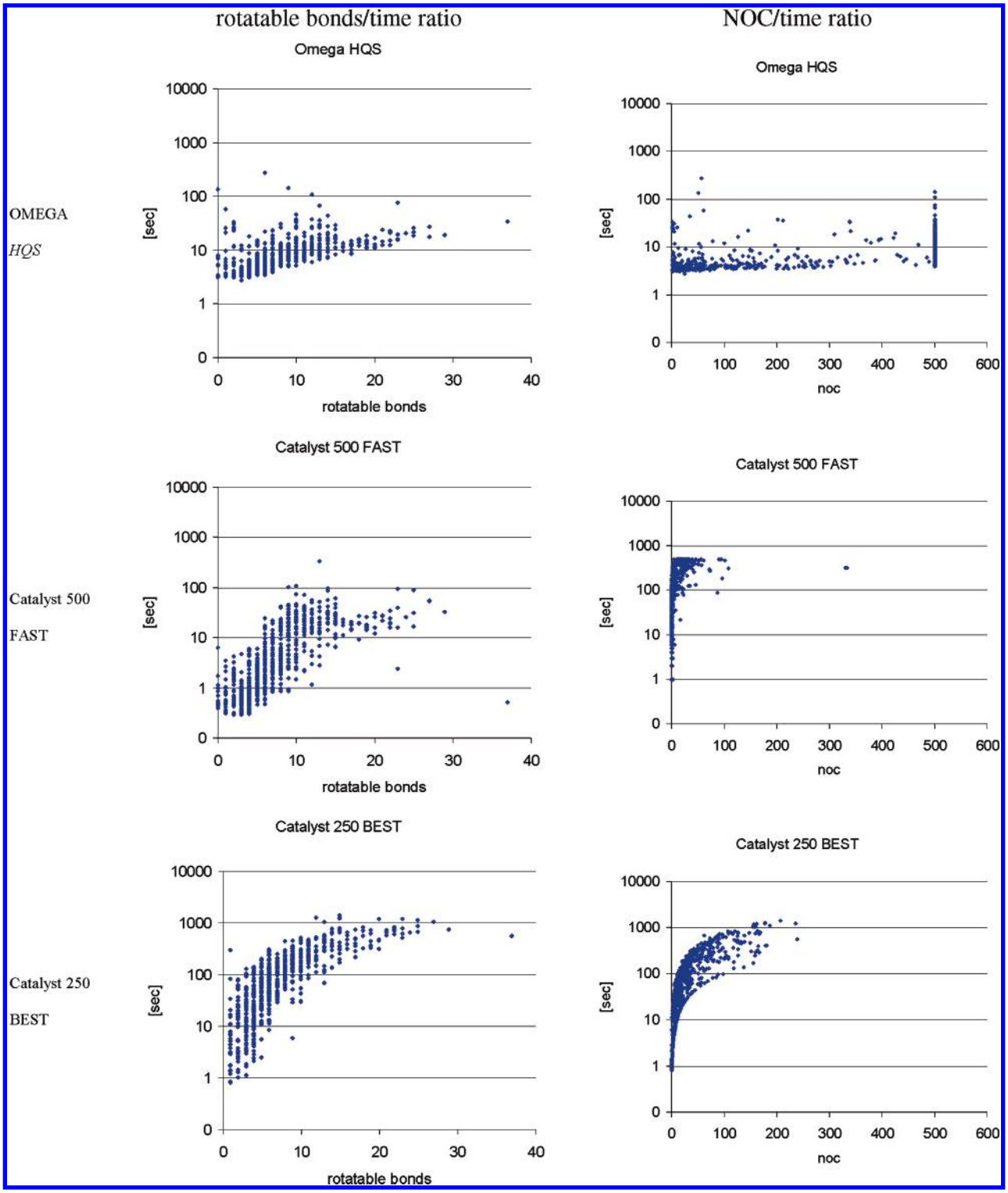

Figure 10. Rotatable bonds/time relationship and NOC/time relationship. Catalyst BEST mode in particular shows significant correlation between the size of conformational space and computation time.

computing time for very flexible compounds. In contrast to that, Catalyst BEST shows a significant correlation between the size of conformational space and calculation time.

Optimum Omega Performance for High-Throughput and High-Quality Screening. Considering the results we got with single user-adapted settings with Omega, we additionally processed three runs using combined adapted generator options to optimize Omega's conformational model generation quality for high-throughput (*HTS*) and high-quality screening (*HQS*): *HTS* stands for *bmmff94s_trunc* and *maxconfs* 50; *HQS* stands for *bmmff94s_trunc*, *maxconfs* 500, and *rms* 0.4. Therefore, the best average RMSD ever detected in our test runs was found in the *HQS* mode: RMSD 0.941, which is 6.5% lower than with Omega's manufacturer defaults, 6.1% lower than with CF500, and 1.3% lower than with CB250. When comparing with CB250 it has to be considered that the average ensemble is for sure smaller and thereby less cost intensive in subsequent screening applications than Omega *HQS*. However, Omega's *HQS* mode shows an 11 times higher GES than CB250.

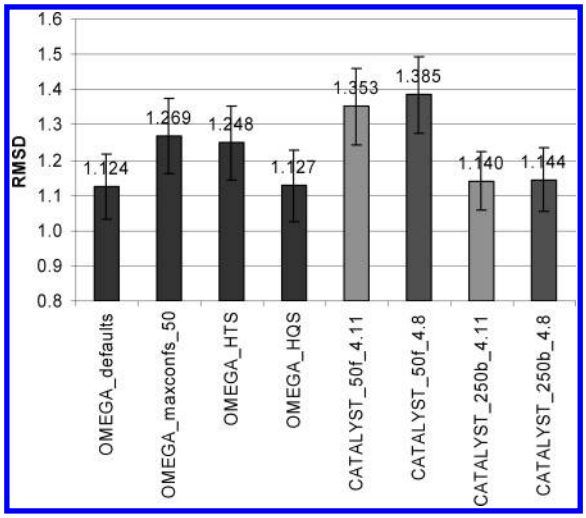

Figure 11. Investigation of sulfonamides containing compounds of the test set. The diagram documents the slight improvement of Catalyst's CatConf module since our uncovering of this force field weakness.

Even more interesting for us was how difficult it was to gain the best accuracy with maximally 50 conformers per ensemble, which is in our opinion currently the best choice for sampling large databases of several million compounds with respect to acceptable computational costs—especially with regard to subsequent in silico screening applications. We maintained the approved *HQS* settings and repeated the same run with *maxconfs* 50. However, the average RMSD of 1.135 was higher than the respective run with *maxconfs 50* (and all other flags kept default). It is clear that for this low NOC *rms* 0.4 is not suitable. A higher *rms* setting is needed to ensure adequate conformational diversity to represent conformational space. Best performing *rms* setting for *HTS* is *rms* 0.8, which achieves an average RMSD of 1.115. Although this RMSD is identical to the quality achieved with *maxconfs* 50, we prefer this setting since it was shown to have a higher efficiency.

Considering all these experiences with both conformational model generators, Omega proves favorable for high-quality conformational space subsampling, while Catalyst outperforms Omega in high-throughput screening: Compared to Omega *HTS*, Catalyst's pragmatic 50 FAST mode requires less than one-fifth of calculation time and reaches GES 61, which is impressive. In the future, faster CPUs and more storage will probably create a trend toward higher quality presampling of molecular databases making Omega a promising choice.

Revision of the Sulfonamide Issue: An Investigation on the 78 Sulfonamides Found in the Test Set. Our previous study on Catalyst's conformational model generator CatConf uncovered a weakness of Catalyst when sampling sulfonamides: It was shown that Catalyst 4.8 penalizes the sulfonamide conformation with too high energy by assuming a wrong torsion angle R−S−N−R1/R2. The generated lowest energy conformation often adopts a 'trans'-like conformation, where two substituents at nitrogen and sulfur share a torsional angle τ of 180°. With Catalyst 4.11, Accelrys announced enhancements on its conformational model generator to eliminate this problem. Figure 11 illustrates the current performance of Catalyst and Omega on sulfonamide structures in detail: Compared to Catalyst 4.8, Catalyst 4.11 shows comparable average RMSD with FAST (−2.3%) and BEST (−0.35%) although the measured values of both program versions differ by 0.207 (FAST) and 0.162 (BEST) on average. Our data suggest a slight improvement of the FAST algorithm, but there is no statistical significance for this. Omega *HQS* achieves an average RMSD of 1.127, which is more accurate than any Catalyst setting examined. However, it has to be considered that *HQS* has a limit of 500 conformers, and Catalyst BEST was tested with half this NOC limit. Omega *HTS* is situated well between Catalyst FAST and BEST (RMSD 1.248).

Comparison of Test Set Ligands That Are Stored in Both PDB and CSD. We screened our PDB ligand test set

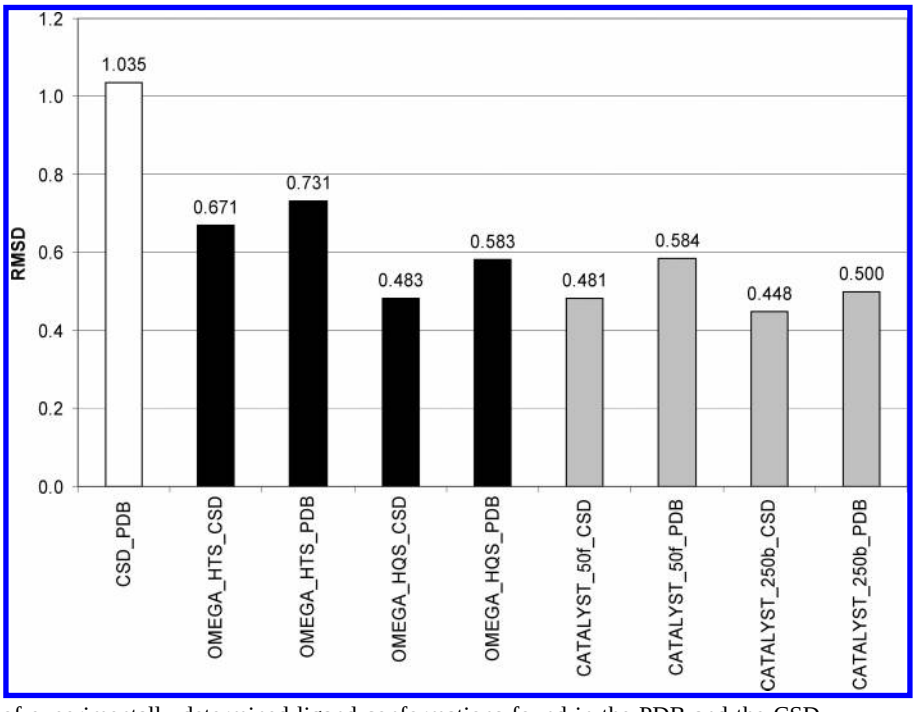

Figure 12. Analysis of experimentally determined ligand conformations found in the PDB and the CSD.

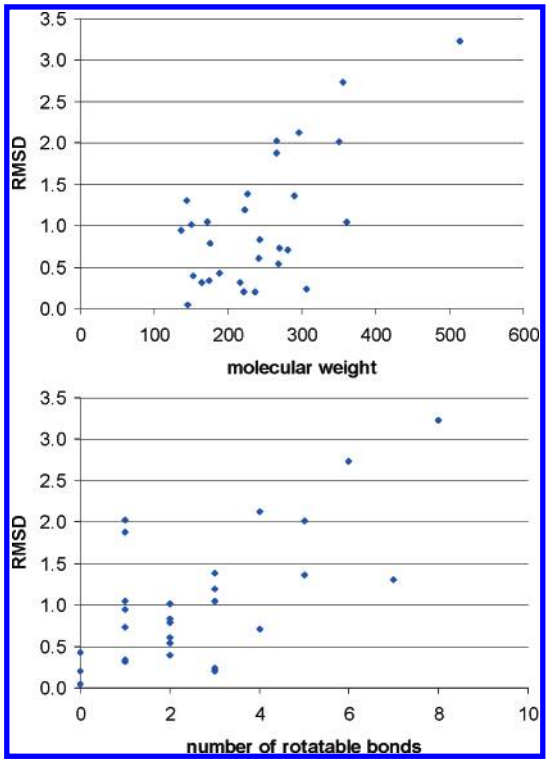

Figure 13. The RMSD between the conformations stored in the CSD and the PDB increases with higher molecular weight and flexibility.

for entries that are also stored in the CSD to be able to estimate the differences in terms of reproducing the experimentally determined conformations (Figure 12). A Pipeline Pilot Script was used to retrieve the 29 duplicates. The average RMSD deviation between the conformers of the PDB and the CSD is 1.035. All three algorithms, Omega, Catalyst FAST, and Catalyst BEST, are able to reproduce the experimentally determined conformations with moderately increased accuracy: The average CSD RMSD values are 8% to 17% lower than the respective PDB conformation.

Figure 13 shows the RMSD between the conformation stored in the CSD and the PDB as a function of the molecular weight and the number of rotatable bonds. As assumed, there is a trend toward higher RMSDs for larger and more flexible compounds.

CONCLUSIONS

We present a continuing large-scale assessment on Omega's conformational subsampling algorithm with respect to Catalyst's generator performance. A test set of 778 drug molecules and pharmacologically relevant structures was gained from the PDB via manual selection, and the respective generated conformational models were investigated in terms of the ability to reproduce the bioactive conformation.

Our examination shows that the quality of conformational models is always a tradeoff between the sampling depth of conformational space and the computational costs—with respect to the algorithm method used. With increasing size and flexibility of the investigated compounds, larger ensembles are needed to represent the bioactive conformation in equivalent quality. Omega's average NOCs generated were examined with several different user-adaptable settings. The *maxconfs* parameter has a major impact on the resulting average NOC but also the *ewindow* as well as the *rms* option are of significant importance. Omega's default energy threshold setting of 25 kcal/mol was proved to be essential for best conformer generation performance since lower limits reject valuable conformations.

Both Omega and Catalyst show reliable performance in terms of retrieving the protein-bound ligand conformations. For Omega, we elaborated optimized settings for maximum performance in high-throughput screening (*HTS*) and high-quality screening (*HQS*), which reflect Catalyst's 50 FAST and 250 BEST modes, respectively. Omega *HTS* and *HQS* show a more accurate representation of the protein-bound ligand conformation than Catalyst FAST, whereas Catalyst BEST proves to be the best choice for the most exhaustive conformational search. Considering generator efficiency, Catalyst FAST surpasses both Omega and Catalyst BEST mode. Therefore, Catalyst 50 FAST, a pragmatic choice for high-throughput screening, shows a 5.5 times higher GES (Generator Efficiency Score) than Omega *HTS*. Omega shows a favorable performance in the high quality domain: It achieves both best accuracy (average RMSD 0.941) and high efficiency.

The revised investigation on sulfonamide moieties contained in our test set suggests that the weakness of Catalyst 4.8 in terms of reproducing the bioactive conformation has been improved for FAST in version 4.11. In addition, a comparison of 29 ligands contained in both PDB and CSD suggests that there are only minor differences in the predictability of the experimentally determined conformations for Omega and Catalyst.

Overall, both Omega and Catalyst provide valuable solutions for conformational model generation. While Omega is the better choice for the generation of high-quality models, Catalyst still shows better performance in high-throughput generation.

Supporting Information Available: PDB code and SMILES notation of all 778 assessed compounds and two investigated examples of the conformational search depth in addition to Figure 7. This material is available free of charge via the Internet at http://pubs.acs.org.

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