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ConFlow - A new space-based Application for complete Conformational Analysis of Molecules

**The work of F. Cordes and H. Meyer
has been supported by the German Federal Ministry of education and research
(grant no. 031U109A/031U209A, Berlin Center for Genome Based
Bioinformatics).**



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Abstract

Biochemical interactions are determined by the 3D-structure of the involved components – thus the identification of conformations is a key for many applications in rational drug design. *ConFlow* is a new multilevel approach to conformational analysis with main focus on completeness in investigation of conformational space.

In contrast to known conformational analysis, the starting point for design is a space-based description of conformational areas. A tight integration of sampling and analysis leads to an identification of conformational areas simultaneously during sampling. An incremental decomposition of high-dimensional conformational space is used to guide the analysis. A new concept for the description of conformations and their path connected components based on convex hulls and *Hypercubes* is developed. The first results of the *ConFlow* application constitute a ‘proof of concept’ and are further more highly encouraging. In comparison to conventional industrial applications, *ConFlow* achieves higher accuracy and a specified degree of completeness with comparable effort.

Keywords: Conformational space, Metastability, Hypercubes,

Mathematics subject classification. 65U05, 62H30.

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Introduction

Molecules can adopt an infinite number of different geometrical conformations based upon their flexibility defined by their degrees of freedom. But not every geometry is as well “favourable” as the other. Based upon the energy landscape of a molecule depending on its conformational space, molecules adopt only a small subset of all the possible geometries. These geometries are often referred to as “conformations”, like for example in n-Butane the *trans*- or the two *gauche*- conformations (Fig. 1). In this sense, the *trans/gauche*-conformations are seen as single representative geometries.



Fig. 1: Conformations of n-Butane (trans, +gauche, -gauche).

The function of a molecule is determined by its form or structure. For biochemical processes the three dimensional structure and moreover the set of possible structures a molecule can adopt is the origin for interaction with other molecules. Hence, in molecular modelling, the knowledge of the conformations a molecule adopts is important when performing structural comparisons of molecules or molecular docking. Especially the latter can be seen as a key-lock-system where the key (the ligand) differs by changes in the conformations. In this sense, the conformation of the ligand in a molecular docking simulation decides whether the ligand docks to the target or not.

Any possible geometry a molecule can adopt represents one point in the conformational space. The exploration of this space is performed by conformational analysis.

Assuming a continuous conformational space, there is an infinite number of geometries for a molecule. Although all these geometries can possibly be adopted by the molecule, their occurrence is finally connected to the potential energy resulting from forces depending on the 3-dimensional structures. Any geometry of the conformational space is corresponding to a point on the energy landscape of the molecule or the whole molecular system. Geometries correspond to energy minima as well as to high energy states of a molecule.

The identification of the conformations of a molecule is done by conformational analysis. The main problem in conformational analysis is the so called *curse of dimension*. The expense for the analysis of the conformational space of a molecule grows exponentially with the number of degrees of freedom. Assuming a geometry generator that varies the torsion angles of a molecule by 30 degrees and define the number of degrees of freedom (dof) by the number of the torsion angles, a molecule with 4 dof can adopt at maximum (ignoring steric clashes) 20,736 possible geometries. A molecule with 8 dof has around 430 million possible geometries and a molecule with 20 dof could adopt circa $3.8 \cdot 10^{21}$ geometries. An efficient exploration of a molecule's conformational space is necessary indeed. In the last years, three main methodological approaches for conformational analysis became established in molecular modelling:

1. stochastic methods

These methods are based upon a random generation of molecular coordinates or velocities. Monte Carlo or Molecular Dynamics often aim at generation of conformations and the correct thermodynamical weighting [4, 10,13], whereas Genetic Algorithms offer a heuristic

scheme for an efficient exploration of the combinatorial diversity of conformational space [5].

2. rule based construction methods

The different molecular geometries are built up by constructing the molecular structure depending on rules defined from chemical knowledge, e.g. energy criteria or electrostatic considerations. The main problem of this approach is the warranty of a good coverage of the conformational space for molecules with many degrees of freedom, since the rules are generally derived for small molecules or fragments [1,3,6,7].

3. systematic approaches

Based upon a systematic exploration of the molecule's conformational space, for example by systematic rotation of the torsion angles of a molecule this *brute force* approach suffers from the curse of dimension. To circumvent this problem, chemically motivated fragmentation of the molecules is introduced [9], heuristic penalty terms forces the sampling into the new conformers [8] of generalized degrees of freedom reduce the dimension of conformational space [11,12].

In the upper mentioned methods, especially the first and the last approach suffer from the curse of dimension. Nevertheless this problem arises in the rule based methods too but with lower influence because of neglect of unfavourable geometries better than in the other approaches. For a deeper understanding on the pros and contras of each method, a recommended overview is given by Gasteiger & Engel [1].

All approaches aim at finding subsets within the high dimensional conformational space which share common properties. The method described in the following paper is based on the central common property of a subset, that a structural change of the molecule leads to a geometry of the same subset with high probability and that transitions between different subsets occur with low probability. In the following, these subsets are referred to as *metastable* subsets. Furthermore, the term "conformation" is now extended in the way, that a conformation is no longer one single representative. A *conformation* of a molecule is now defined as a metastable set of geometries with the upper mentioned property.

The conformation analysis tool "ConFlow" uses a systematic approach to explore the conformational space of a molecule. The algorithm is based on the hypothesis:

For every molecule exists a complete decomposition of its conformational space in unreachable regions, conformations and the Path Connected Components of these conformations.

Conformational analysis with "ConFlow" – an overview

The exploration of the conformational space of a molecule and the analysis of the potential energy landscape with "ConFlow" is based upon a systematic decomposition of the conformational space by an evolving grid with restriction to thermodynamically accessible areas. As a result, "ConFlow" can be seen as a "thermodynamically inspired" conformation analysis. Thermodynamics are used by "ConFlow" to restrict its exploration of the conformation space to a small subset of high information. "ConFlow" uses a deeper analysis of the molecule to identify torsion angles essential for conformational metastabilities. An energy threshold depending on the molecule is used to restrict the exploration of the conformational space.

A multidimensional adaptive evolving grid is the playground for all processes described below. The grid is an abstraction for the conformational space of the molecule under investigation.

The dimensions spanning the grid are derived from physical and chemical properties of the molecule. Simplifying the dimensions are a selection from the dihedral angles of the molecule satisfying the condition to be a source of metastability. Each vertex of the grid defines a unique combination of these relevant dihedrals. The grid resolution varies due to the grade of restriction in a dimension (e.g. dependent on the number and size of ring structures containing that dihedral).

Considering the high dimensionality of the grid a straight forward approach by spanning first the grid and doing then operations based on that grid is not very useful. In contrast the evolving grid approach based on a tight integration of sampling and analysis utilized all actual available insights about the conformational landscape to steer the sampling process to *vertices* where the information gain is maximal. As a result the very most of all *vertices* can be excluded from direct analysis.

The investigated area is starting from an initial *vertex* successive expanded. The next *vertex* to analyze is always chosen from the clash-free neighborhood of all known *vertices*. This proceeding ensures the restriction to clash-free accessible areas in the grid and avoids jumps into areas which are not clash-free accessible.

Neighborhood: The neighborhood of a *vertex* (blue) contains all *vertices* which can be reached from origin by changing a sole dimension exactly one step (Fig. 2). By considering that the grid spanned by dihedral angles is circular (mathematically a torus) there are no borderlines limiting the grid. Thus every *vertex* has $2d$ neighbors, where d is the number of dimensions of the grid. The neighborhood can be iterative enhanced by building the neighborhood of the first level neighborhood (yellow). If there are clash areas (red line) *vertices* generating clashes (grey) are not further expanded. Thus the neighborhood-driven evolving of the grid is restricted to clash-free connected clash-free areas – that is to say clash-free areas in the conformational space which are isolated by clash-areas are excluded from investigation.

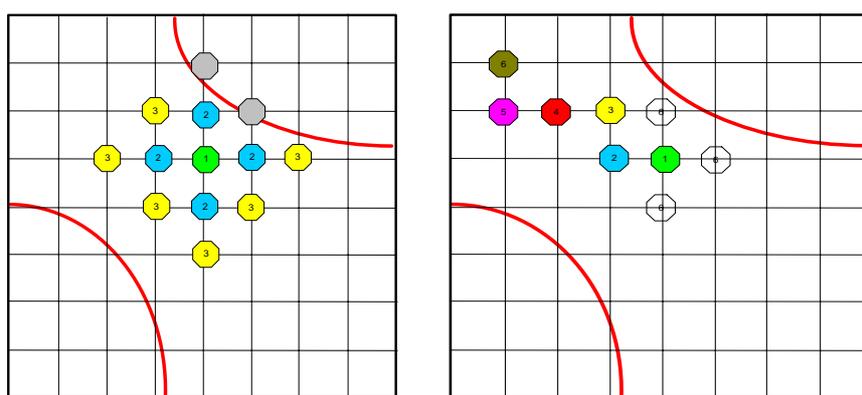


Fig. 2: Neighborhoods of a vertex (left) and depth-first sequence order (right)

Nevertheless is the sequence of investigation for the *vertices* not only determined by the neighborhood. Due to the neighborhood criterion the next vertex is selected only from the neighborhood of all known *vertices* – but just a small fraction of all neighborhood-vertices is analyzed. As shown in Fig. 2 the sequence of analyzed *vertices* follows a depth-first strategy.

This strategy is mainly driven by a new concept called *Hypercubes* which is revealed in the following chapter.

Due to the evolving grid approach, the conformational space to be analyzed is a priori restricted to a fraction of the whole space which is spanned up by all degrees of freedom. Remembering the upper example of a molecule with 20 degrees of freedom and at maximum around 10^{21} possible geometries, the restriction of the conformational space made by “ConFlow” is in the magnitude of 10^{10} geometries. This is only a “small” subset of the overall geometries, but in fact even this subset is far too large for a systematic analysis. Within the resulting subset now, the importance level of the geometries to be analyzed is of very high importance. “ConFlow” uses an approach maximizing the amount of information earned by the analysis of any geometry by the concept of expanding hypercubes in conformational space allowing the exclusion of a large amount of geometries from analysis. Additionally, the approach of the expanding hypercubes is used to define the order of the remaining geometries for analysis (discussed below). The conformational analysis tool “ConFlow” uses thermodynamical considerations combined with a completely new concept of exploration of the conformation space of a molecule to reduce the number of geometries to be analyzed to an essential subset with maximum amount of information.

Details of the conformational analysis with “ConFlow”

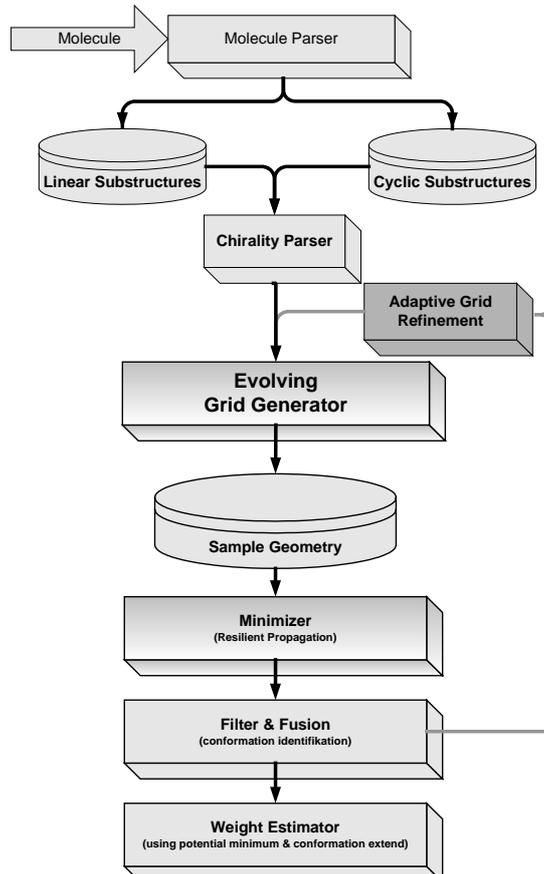
The main components of the “ConFlow” version used in this work are shown in Fig. 3.

In the first step of the conformation analysis, an investigation on the properties of the molecules is performed. Here, a set of essential torsion angles representing potential metastable degrees of freedom is selected. Cyclic structures are deeper analysed and the minimum set of torsion angles required to describe the ring’s geometry is selected.

The chiral atoms of the molecule are identified. Here, numerical and real chiral centres are treated different. Numerical chiral centres result when defining chirality upon the index of an atom in the molecule. A carbon atom for example can be bonded to two hydrogen atoms. Each hydrogen atom has another index. When exchanging both hydrogen atoms, the chemical properties of the molecules would not be changed, but the indexing would. This leads to a pseudoenantiomere in numerical sense. In difference, real chiral centers are defined in chemical sense as asymmetric centers.

The identification of chiral centers is very important, because the conformational analysis is performed by rotating the torsion angles of the molecule followed by a reconstruction of the other parts of the molecule upon the Z-matrix. The Z-matrix is a set of interval degrees of freedom necessary to reconstruct the 3D-structure of

Fig. 3: Components of ConFlow



a molecule. Here, it is possible that the reconstruction switches two atoms connected to a chiral atom leading to a different enantiomere. If this switch is not detected and that molecule is used as starting point for further sampling, the conformation space of a different enantiomere is analyzed. Normally, this is not wanted in conformation analysis. But if one is interested in analysing the whole enantiomere conformational space, “ConFlow” can allow inversion of chiral centers.

After the identification of the essential torsion angles and chiral centers, the generation of sample geometries in the “neighborhood” of the starting geometry is performed by rotating the selected torsions by a predefined value, e.g. 30 degrees. The resulting backbone structure of the generated sample molecule is then hold fixed and the rest of the molecule – for example new positions of the hydrogen atoms in methyl or hydroxyl groups – is reconstructed. If the reconstructed molecule is free of steric clashes and energetically below the used threshold, it is used in the geometry generation of the next loop as starting geometry.

After the reconstruction an energy minimization is performed on the resulting molecule using the R-Prop minimization algorithm [16]. The RPROP (Resilient Propagation) algorithm was chosen for the constrained and the unconstrained minimization because of robustness and implicit smoothing of the energy landscape.

First, a constrained minimization process minimizes all degrees of freedom which are not used to span the grid, while fixing all the grid-based dihedrals. The selection of the grid-spanning dihedrals aims to choose exactly those which indicate flexibility. The constrained minimization provides the potential energy of the vertex. Clashes were detected. The following unconstrained minimization shows the way to a minimum that is chosen as representative for the metastable conformation.

A hypercube is spanned between the starting point of the minimization and the found minimum.

The hypercube is a rough approximation of the convex hull surrounding a conformation. This approximation was chosen due to the condition that the calculation of the convex hull in an n-dimensional space is an NP hard problem. This approach ensures that the convex hull is always covered by the cube.
The concept of expanding hypercubes leads to a gradual expanding decomposition of the conformation space reducing the areas to analyze.

The concept of the expanding hypercubes allows a fast decomposition of the conformation space. The expansion of the hypercubes is based upon the starting points of the energy minimization trails. The energy minimization of any sample geometry finally leads to three possible results:

1. the minimization ends in a minimum found before
2. the minimization ends in a new minimum of known conformation
3. the minimization ends in a new minimum of a new resulting conformation

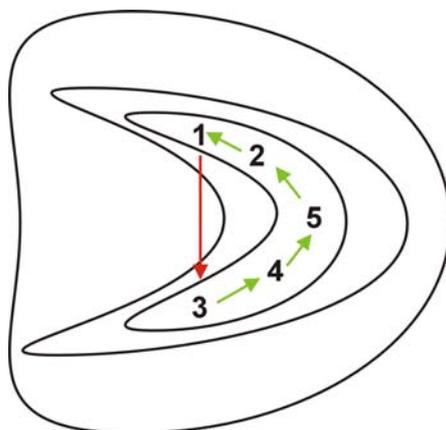


Fig. 4: Scheme of the work of the „Fusion“ component of “ConFlow”

In the first case, the minimum is ignored because it was found previously, but the minimization trail is saved for further use. In the second case, the minimum is added to the conformation and the hypercube spanned around this conformation is expanded to the starting and ending point of the energy minimization.

The identification and handling of these cases is done by the “Fusion” component of “ConFlow”. The “Fusion” component does the assignment of minima to conformations and the merging of conformations. It uses a Lipschitz-based constraint maximum search in one direction in higher dimensional space¹. The heuristic is based upon a Lipschitz-constant defining the maximal slope of a function. The goal is to use a quasi binary interval dissection method to detect exceed of a defined function value.

Using the upper mentioned Lipschitz approach, a barrier free path to another minimum can be found in a fast way, like for example in Fig. 4, where the minimum “1” was found first and then minimum “2”. Here, a barrier free path between both minima was found. If such a path exists, both minima belong per definition to the same conformation.

In the example of the third found minimum (“3”), such a path would not be found, because the search is performed only in a linear direction. Here, minimum “3” would build a new conformation at first. Later, minima “4” and “5” are found, and now, a barrier free path can be found by “Fusion” from minimum “3” to minimum “1”, so minimum “3” belongs to the same conformation like minimum “1”. The bottom line is that minima 1-5 are merged to the same conformation.

¹ In fact, the problem to find a barrier-free path from one minimum to the other in high dimensional space is an NP hard problem. The usage of a linear approach is a simple but efficient approximation of it.

This way, the membership of every found minimum (and its minimization trail as well) to a given conformation can be tested, allowing a fast expansion of the hypercubes and exclusion of conformational space from analysis².

The performance of the “Fusion” component is strongly connected to the coverage of the conformational space and the used energy threshold. The first mark especially points out when considering a larger molecule, where rotations of the torsion angles accumulate. Here, often geometries are sampled that minimize into new minima or conformations not found yet. Often, those minima cannot be connected to the conformations found before although they possibly belong to them. This happens due to a rare coverage of the conformational space at the beginning of the analysis, but can be avoided by longer simulation times or smaller changes of the torsion angles in each geometry generation. Here, a compromise between simulation time and conformation space coverage has to be made in both cases.

The concept of the hypercube expansion is schematically shown in Fig. 5. In the first picture the minimization trail from the starting points to their local minima define the expansion of the first hypercube. In the second run, the generated sample geometry minimizes to the same conformation as well but to another minimum, leading to a further expansion of the hypercube of this conformation. In the second picture, the minimization trail starting from a different sample geometry leads to another minimum that could not be connected to the conformation found before. As a result, a new hypercube for the found conformation is created. Picture three shows the further expansion of the new hypercube due to new found minimization trails (not shown).

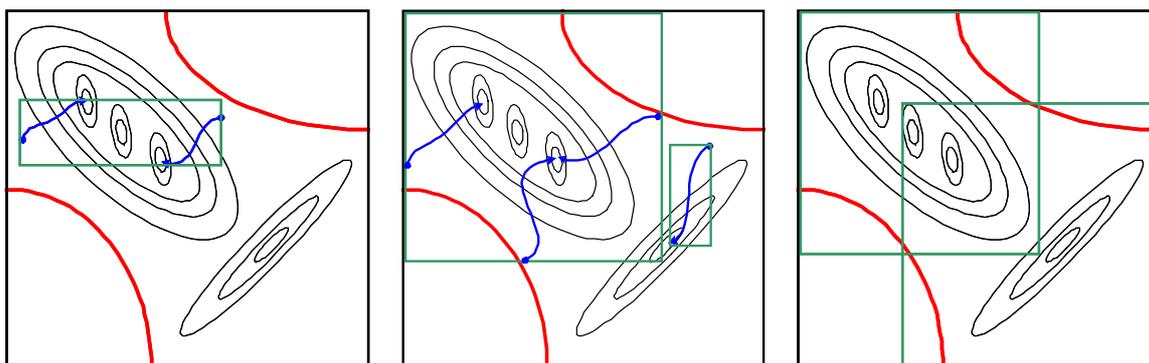


Fig. 5: Scheme describing the expanding hypercube concept

Fig. 5 illustrates, that the resulting hypercubes may finally overlap. This effect results from the algorithms which guides the investigation always to starting point outside the cubes – adding the new point to an existing conformation or creating a new one. If one would use the convex hull instead of hypercubes, no intrusion would exist.

² The exclusion of the area of conformational space in this step is not final. In the enrichment phase of the “ConFlow” algorithm (here not discussed), all geometries at the rim of each hypercube are revisited and analyzed according to their distance to the next hypercube.

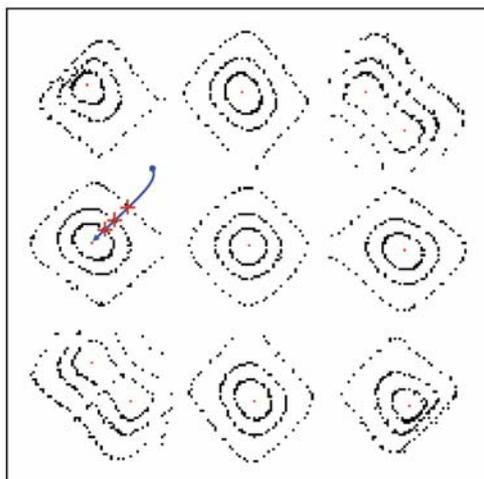


Fig. 6: Pentane with an illustrating minimization trail and trailpoints at 2, 6, and 14kJ/mol generated by ConFlow.

The usage of the hypercube concept leads to a new description of a conformation. Normally, a conformation is defined upon a point cloud in the conformational space. Instead of this, “ConFlow” uses a description of the associated area in conformational space to define a conformation. Especially in high dimensional spaces (due to larger and/or very flexible molecules) this description based upon spaces is more efficient than a point cloud description. Translated to the cartesian space this leads to a description of a conformation and its flexibility with respect to metastable areas. The inclusion of the minimization paths leading from a sample geometry to a minimum – one could call it the *catchment area* of a conformation – is a new modification of the concept. Fig. 6 illustrates the concept of catchment areas for the nine metastable conformations of *n*-pentane. Every analyzed point in a minimization trail is assigned to a conformation. Even geometries distorted by external forces – for example a ligand in the binding pocket – can thus be assigned at least to the catchment area of a conformation.

A comparative study

Conformational analysis with “ConFlow” was validated on a dataset of 32 ligands published by Boström [17] in a “A Critical Evaluation of Several Popular Conformational Searching Tools”, namely the programs Corina [2], Catalyst from Accelrys, which is based on the Poling-Algorithm [8], Confort [19], Flo99 [20], MacroModel [4] and Omega [21,14]. For all ligands experimental data of the binding modes are available in the Protein Databank [18] (see Tab. 1 and Fig. 7).

1	1A28	9	1C83	17	1DYZ	25	1EJN
2	1TNG	10	1ECV	18	2IZG	26	1CAQ
3	1TNH	11	1FCZ	19	1CBX	27	1MTV
4	1QFT	12	1GR2	20	5STD	28	1MTW
5	1FTM	13	1IAN	21	6STD	29	1PPC
6	1PHG	14	1BZS	22	7STD	30	1FOU
7	3BTO	15	1FRB	23	1CBS	31	1FKG
8	1D36	16	1BJU	24	1DAM	32	1PPH

Tab. 1: Numbering and pdb-entries of the molecules from the publication of Boström [17].

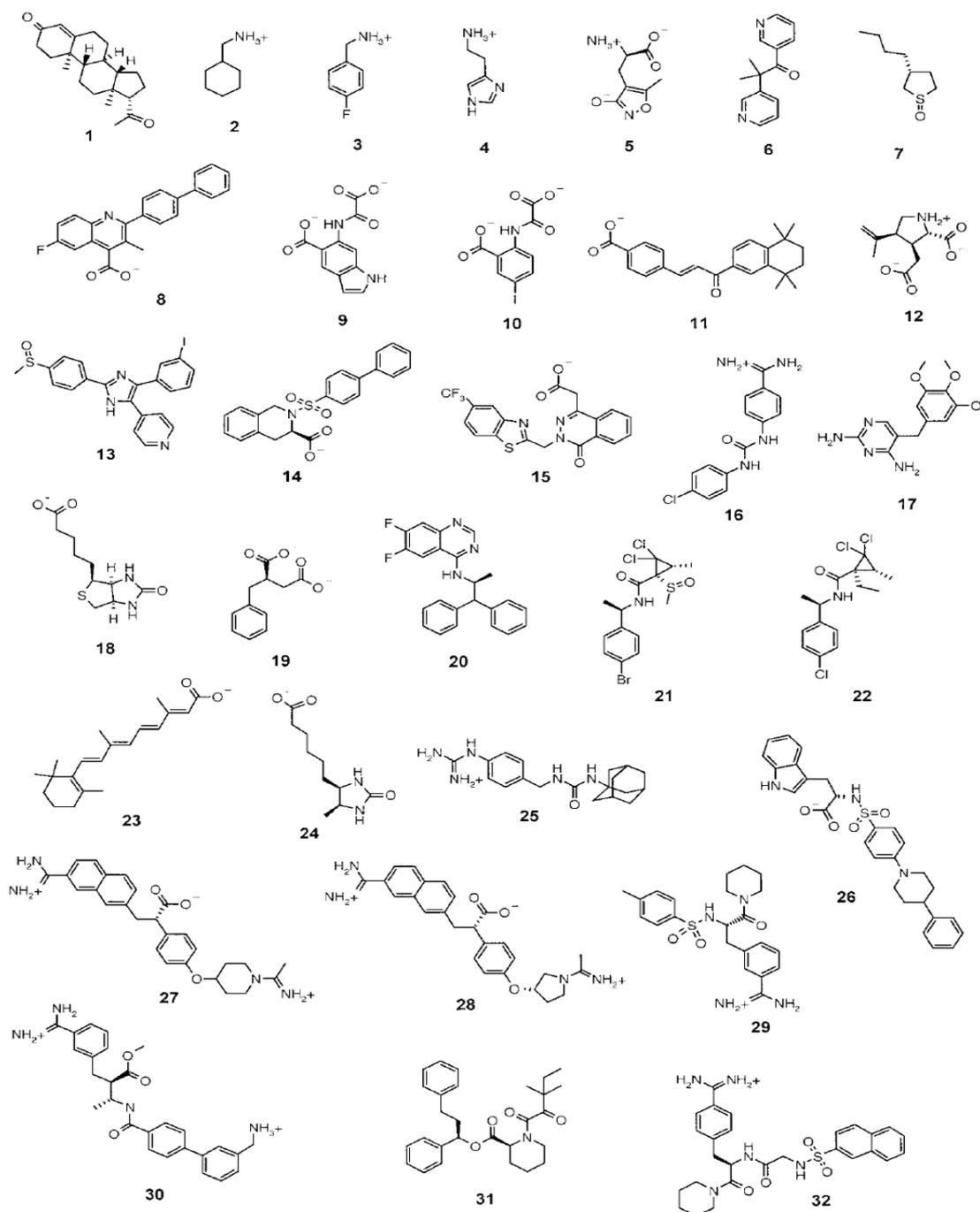


Fig. 7: 32 drug-like molecules from Boström [17]

Starting geometries for the analysis have been the geometries generated by an independent conformational analysis tool [22], which are “far away” from the binding mode.

The generation of “neighbor”-geometries from a starting geometry was done by torsion angle rotation of 30 degrees. The energy minimization was performed with an energy convergence criterion of 0.003 kJ and a maximum number of minimization steps of 50,000. For all energy-dependent calculations the Merck Molecular Force-Field (MMFF) was used [15]. The conformational analysis was performed in vacuum with a maximum runtime of 20 hours.

Additionally to the found local energy minima and their relation to a conformation, so-called *trail points* were saved reflecting the energy minimization trails to their according minimum.

But not all minimization steps were saved, only trail points with energy lower than 200 kJ above the final minimum. Furthermore the points were saved with a doubling point interval starting 4 kJ above the found minimum. The further save points were then 8, 16, 32, 64, 128 and finally 200 kJ (Remark: The points were only saved if they existed. No artificial points were created to the interval). Finally a result trajectory was built including all minima, their according trail points and their relationship to a conformation.

Fig. 8 shows for all programs of the contest the minimal distance between the generated conformational ensemble and the binding mode averaged over all molecules.

Fig. 8: For all programs the minimal RMS distance [\AA] between conformational ensemble and the binding mode averaged over all 32 molecules is shown.

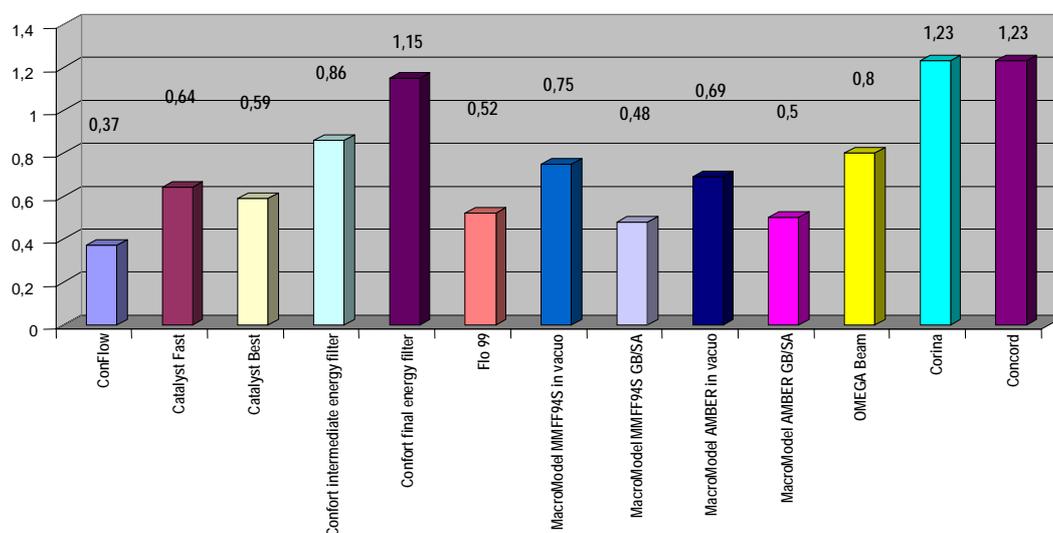
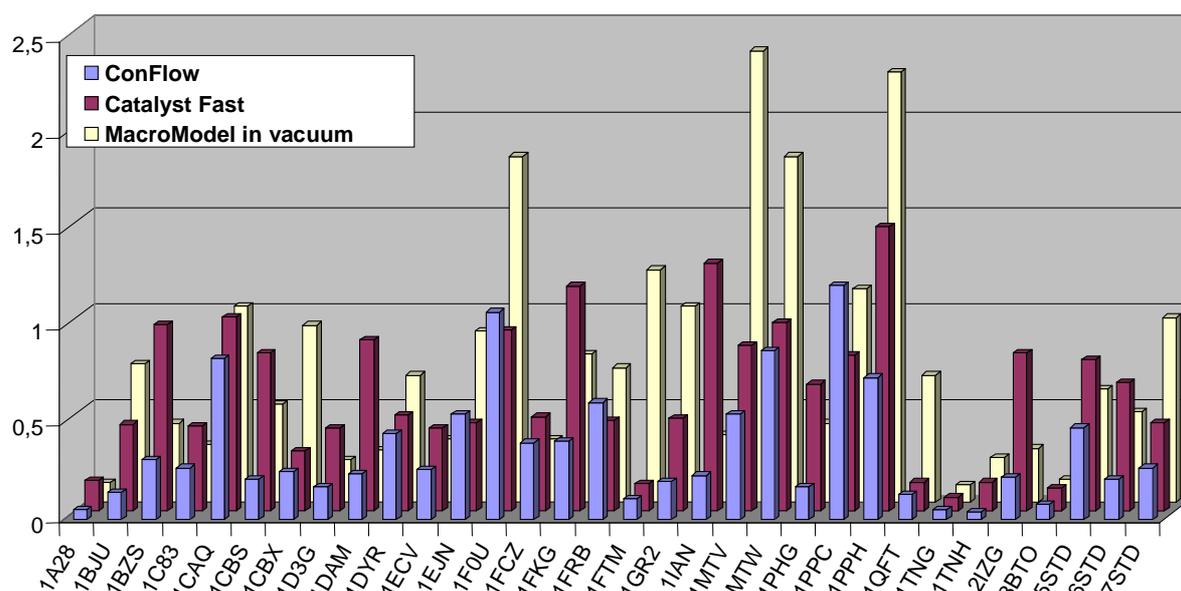


Fig. 9: Minimal RMS-distances [\AA] between the generated ensemble and the binding mode of each molecule. Comparison between ConFlow, Catalyst Fast and MacroModel in vacuum.



ConFlow shows the best averaged results with an RMS of 0,37Å (Fig. 8). Going into detail (Fig. 9) it can be shown, that all programs have some problems with increasing number of flexible degrees of freedom, namely for 1F0U, 1MTV, 1MTW and 1PPH. Besides 1F0U, ConFlow again delivers the best results.

Another interesting observation concerns the worst results of the analysis, which reaches approximately 2,5Å for the MacroModel and 1MTV, 1,5Å for Catalyst and 1PPH, but for ConFlow the worst case for 1PPC is only slightly above 1,0 Å.

Conclusion

The paper has described a new algorithm for conformational analysis in which the sampling routine is tightly controlled by analysis of metastability. Instead of generating point clouds a space-based concept is introduced to identify and describe conformational areas

Hypercubes act as a fast computable upper boundary for the convex hulls of metastable conformations.

Ligands with 5 degrees of freedom can be analyzed completely in only a few minutes. Molecules with 50 degrees of freedom will become easily computable if decomposed in for example 20 overlapping fragments of size 5 dof. Caching of these fragments offers additional performance improvement.

Recent publication [23,24] have investigated only a subset of the above mentioned programs on larger molecular datasets. The main conclusion of these studies correspond to Boström [14,17]. Fast methods as Omega are able to generate impressive results over a set of given molecules, but always generate worst cases, which differ up to 2,0Å and more from a given binding mode. Using conformational ensembles for virtual screening applications, these false-negatives will never be found within the screening.

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