A Path Planning Approach for Computing Large-Amplitude Motions of Flexible Molecules

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ABSTRACT

Motivation: Motion is inherent to molecular interactions. Taking into account the molecular flexibility is necessary to develop accurate computational techniques for interaction prediction. Energy-based methods currently used in molecular modeling (i.e. molecular dynamics, Monte Carlo algorithms) are, in practice, only able to compute local motions. However, large-amplitude motions often occur in biological processes. We investigate the application of geometric path planning algorithms to compute such large motions on flexible molecular models. Our purpose is to exploit the efficacy of a geometric search as a filtering stage before refining with energies.

Results: Two kinds of large-amplitude motions are treated in this paper: protein loop conformational changes (involving protein backbone flexibility) and ligand trajectories to the active site of a protein (involving ligand and protein side-chain flexibility). The first simulations performed using our two-stage approach (geometry + energy) show that, compared to classical molecular modeling methods, quite similar results can be obtained with a significant performance gain, ranging from 100 to 1000. Furthermore, the geometric treatment can provide by itself highly valuable information to biologists.

Availability: The algorithms presented in this paper have been implemented in the general-purpose motion planning software Move3D (Siméon et al., 2001). We are currently working on an optimized stand alone library that will be available to the scientific community.

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1 INTRODUCTION

The interest of the computational analysis of molecular motions is well known. Molecular flexibility remains the main challenge for accurate docking approaches (Janin et al., 2003) or for studying molecular pathways (e.g. protein folding, structural rearrangements). Classical biomolecular modeling methods (e.g., Leach, 1996) are too computationally expensive for generating large motions while accounting for molecular flexibility. Molecular dynamics simulations are in practice applicable to compute motions in the time range of nanoseconds. Techniques based on Monte Carlo algorithms permit to compute larger motions, but they are also restrained. In practice, the two limiting factors of these methods are the CPU time cost of energy computation and the high tendency to fall in local minima of the energy landscape.

Motion planning algorithms (Latombe, 1991) originally developed in robotics, have already been proposed as new tools for computational biology (see Section 2). While these techniques have been applied to explore the molecular force field, our aim is to exploit the efficacy of a geometric treatment of molecular constraints before dealing with energies. The goal is to better handle the complexity introduced by flexible molecular models. We investigate a two-stage approach for computing large-amplitude motions. In a first stage, motion planning algorithms are applied to explore the portions of the conformational space that satisfy a set of geometric constraints corresponding to strong energetic restrictions (see Section 3.1). The interest of this geometric filtering is that high-dimensional conformational spaces can be globally explored in an efficient way. The geometric path is then used in a second stage as a filtered input for a more accurate analysis using classical energy-based modeling techniques. This paper focuses on the geometric stage of the approach.

Note that previous works in computational biology already rely on a geometric treatment of molecular constraints. The geometric complementarity of molecular surfaces is a widely used criterion to predict protein-ligand or protein-protein interactions (e.g., Kuntz et al., 1982; Rarey et al., 1996; Jackson et al., 1998), especially in rigid docking approaches. Also, techniques for the conformational sampling of polypeptide segments (e.g., Moult and James, 1986; DePristo et al., 2003)
often apply geometry-based approaches to the loop closing problem and to atom overlap detection.

The proposed approach is applied to two kinds of computational biology problems. Section 4 deals with the analysis of protein loop mobility. Section 5 concerns the study of accessibility problems in protein-ligand interactions, considering both the flexibility of the ligand and that of the protein side-chains. Our studies show the efficacy of the approach on such problems involving large-amplitude motions and molecular flexibility which are both poorly treated by energy-based methods. Finally it highlights the interest of the approach for guiding the rational design of proteins.

2 ABOUT MOTION PLANNING

Motion planning is a classical problem in robotics (Latombe, 1991). In the last years, motion planning techniques have undergone considerable progress. Recent techniques have been successfully applied to challenging problems in diverse application domains and have provided promising results in computational biology.

2.1 Sampling-Based Motion Planning Algorithms

Sampling-based motion planners have demonstrated to be efficient tools to explore high-dimensional spaces. Most of these planners are variants of the Probabilistic RoadMap (PRM) approach (Kavraki et al., 1996). The basic principle of PRM is to compute a connectivity graph (the roadmap) encoding representative feasible paths in the search space (e.g. the molecular conformational space). Nodes correspond to randomly sampled points that satisfy a certain feasibility requirement (e.g. collision-freeness) and connections represent feasible subpaths computed between neighboring samples. Once the roadmap has been constructed, it can be used subsequently to efficiently process multiple motion planning queries between two given positions of the moving system or to determine ensemble properties of mobility.

Variants of the PRM framework have been designed for solving single-query problems without preprocessing the complete roadmap. For example, the Rapidly-exploring Random Trees (RRT) algorithm (LaValle and Kuffner, 2001) expands random trees rooted at the query positions and advancing toward each other through the use of a greedy heuristic. Such variants are adapted when solving highly constrained problems for which the solution space has the shape of a long thin tube. While random sampling within the tube would require a high density of points, the random tree variant may benefit from the shape of the tube to naturally steer the expansion.

2.2 Applications to Computational Biology

Recently, PRM-based algorithms have been successfully applied for studying molecular motions involved in biological processes such as protein-ligand interactions (Singh et al., 2001; Apaydin et al., 2004), protein folding (Amato et al., 2003; Apaydin et al., 2002) and also RNA folding (Tang et al., 2004). The main difference of the molecular adaptation of the PRM framework is that the binary collision detection, used in robotic applications, is replaced by a molecular force field. Sampled conformations are accepted on the basis of their potential energy and by weighting the roadmap edges according to their energetic cost. The major strength of these sampling-based techniques is their capability to explore high-dimensional spaces and to circumvent the local minima problem encountered by other classic simulation techniques (like Monte-Carlo algorithms or molecular dynamics) which waste a lot of time trying to escape from the local minima of the molecular energy landscape. Although the techniques are general enough to use any method to compute the potential energies, the proposed planners consider simple potentials for efficiency purpose (e.g. van der Waals and electrostatic terms, using thresholds to avoid performing all pairwise computations on the potential terms).

Singh et al. (2001) and Apaydin et al. (2004) show promising results on the study of binding sites for flexible ligands, assuming, as most of the docking methods, a rigid model of the protein to maintain a reasonably low dimension of the conformational space. Protein flexibility, which however plays an important role in protein-ligand interactions, is partially considered for protein folding applications using simplified articulated models (articulated backbone with bounding spheres approximating side-chains (Amato et al., 2003), or a vector-based approximation of secondary structure elements (Apaydin et al., 2002)).

Apart our previous work on long protein loop conformational studies (Cortés et al., 2004), RRT-like methods have never been applied to computational biology problems.

3 THE GEOMETRIC APPROACH

This section describes our geometric path planning approach. It first discusses how geometric constraints allow us to model the strongest molecular constraints considered by energy-based methods. It then describes our articulated model of proteins and the main algorithms we developed for the efficient computation of geometrically feasible paths.

3.1 Geometric View of Molecular Constraints

3.1.1 Molecular degrees of freedom. Molecular mechanics force fields consider separately bonded and non-bonded atomic interactions. Bonded interactions concern the variation of the relative position of bonded atoms which is usually given in internal coordinates: bond lengths (stretching), bond angles (bending) and dihedral angles (torsion). Slight variations of bond lengths and bond angles from their ideal values produce a large increase of energy. Thus, in first approximation, these parameters are generally assumed to be constant. Under this assumption, a molecular chain is an articulated mechanism with revolute joints that correspond to bond torsions.

In many studies, the global molecular architecture is known and only segments of the molecular chain (e.g. protein loops)
are assumed to be flexible. The first and last atoms of the flexible segments must remain bonded with their neighboring atoms. Hence, kinematic loop closure constraints considerably reduce the subset of feasible conformations of the articulated molecular model. Such constraints also appear in cyclic molecules and in the presence of disulphide bonds.

3.1.2 Main repulsive constraints. Concerning non-bonded interactions, the strongest contributor (in short distance interactions) corresponds to the van der Waals repulsive term. A large amount of energy is required to get two non-bonded atoms significantly closer than the van der Waals equilibrium distance. Acceptable conformations must then satisfy a geometric constraint: the distance between pairs of non-bonded atoms must be greater than a given contact distance.

3.1.3 Main attractive constraints. Two other important types of interactions are hydrogen bonds and hydrophobic interactions. Intrinsically, such interactions maintain the globular shape of macromolecules and are also involved in molecular docking. Both kinds of interactions are possible when specific relative locations of the concerned atoms are satisfied. Thus, they imply geometric (distance/orientation) constraints on the molecular model.

These geometric constraints are the driving forces affecting molecular conformational changes. It is assumed that energetically acceptable conformations are contained in the subset of the geometrically feasible ones.

3.2 Geometric Modeling

As in many other approaches we handle all-atom models of molecules, that are considered as articulated mechanisms with atoms represented by spheres. Groups of rigidly bonded atoms form the bodies and the articulations between bodies correspond to bond torsions. A cartesian coordinate frame is attached to each group. The relative location of consecutive frames is defined by an homogeneous transformation matrix that is a function of the rotation angle between them. We follow a similar method to that of Zhang and Kavraki (2002) to define such frames and matrices between rigid groups. Figure 1 shows the mechanical model for a particular amino acid residue of a protein.

Our modeling can also take advantage of a known secondary structure. In this case, the rigid secondary structure elements (alpha helices and beta sheets) are modeled as rigid groups of backbone atoms with articulated side-chains. Since secondary structure elements are fixed in the model, loop closure constraints are introduced in the in-between segment. Similar closure constraints can also be introduced in the model of a molecule to consider nonbonded interactions, such as hydrogen bonds, that impose the spatial proximity between some atoms of the protein.

3.3 Motion Planning Tools

Several geometric tools are combined within our motion planner. The main algorithm fulfills the conformational space exploration. Two principal functions called into this algorithm concern the sampling of points in the search space (i.e. conformational sampling) and the avoidance of steric clashes (i.e. collision detection). We describe below the tailored algorithms we developed for molecular models.

3.3.1 Collision Detection. The overall performance of motion planning techniques highly relies on efficient algorithms for accepting or rejecting the sampled conformations and paths. This performance requirement is especially important for molecular applications because of the quadratic cost of enumerating all non-bonded atom pairs in models with thousands of atoms.

For this purpose, we developed a tailored algorithm BioCD (Ruiz de Angulo et al., 2005) for efficient self-collision and distance computations in highly articulated molecular models. BioCD uses, as Lotan et al. (2002), hierarchical data structures that approximate the shape of the protein at successive levels of details, allowing to significantly reduce the number of interacting pairs to be tested for collision. However, while the former algorithm was designed to situations in which only a few randomly selected degrees of freedom (DOF) of the kinematic chain are slightly changed at each step, BioCD is more adapted to our sampling-based motion planning situations in which preselected but larger sets of DOF are modified simultaneously and arbitrarily when sampling new conformations during conformational space exploration.

BioCD is inspired by the dual kd-tree traversal algorithms initially developed for N-point correlation problems in statistical learning (Moore et al., 2001). The algorithm maintains two levels of bounding volume hierarchies grouped according to spatial proximity. The first level organizes the rigid parts
of the articulated model according to the selected DOF, and the second level organizes the atoms inside each rigid part of the first level. Such data-structure can be efficiently tested for collision and also updated with a moderate cost. Experimental tests performed with BioCD show its efficacy to process thousands of collision tests per second on articulated protein chains with hundreds of DOF (Ruiz de Angulo et al., 2005).

3.3.2 Conformational Sampling. We also developed an algorithm to sample molecular conformations that satisfy loop closure constraints. The algorithm relies on the general technique called Random Loop Generator (RLG) (Cortés et al., 2002). Its application to protein loops is discussed by Cortés et al. (2004). RLG is based on a decomposition of the closed-chain mechanism. The kinematic chain corresponding to the loop backbone is divided into an active subchain and a passive one. The passive subchain is a backbone portion involving 6 rotational bonds. The parameters (dihedral angles) of the active subchain are progressively sampled using a simple geometric algorithm that notably increases the probability of obtaining a conformation of the passive subchain satisfying loop closure. The conformation of the passive subchain is computed by a general $6R$ inverse kinematics method (Renaud, 2000). RLG performs efficiently with long protein loops, which are a challenge for most other related techniques.

Our RLG-based sampler integrates collision detection in the progressive sampling process. Each time that a dihedral angle is generated, overlaps between atoms in the corresponding rigid group and the rest of the protein (including the previously generated loop portion) are checked. In case of collision, the dihedral angle is re-sampled a given number of times. The computed backbone conformations satisfy loop-closure and avoidance of steric clashes.

The loop conformational sampler also considers distance constraints between different elements of the closed chain. Such constraints allow us to account for the presence of backbone hydrogen bonds, which particularly affect the motion of some loops (e.g. hairpin loops). Loop conformations are sampled such that the position of N and O atom pairs involved in hydrogen bonds remains within a given distance range.

3.3.3 Conformational Exploration. The main algorithm in a motion planner determines the strategy to explore the search space. Molecular motions are in general extremely constrained mainly due to steric clashes. In other application domains (e.g. mechanical disassembly), incremental search planners have demonstrated to be better suited than roadmap-based algorithms to solve very constrained motion planning problems. A recent improved version of the RRT approach presented by Yershova et al. (2005) shows impressive performance in such cases. The basic principle is to iteratively develop a random tree rooted at the initial conformation. In each iteration, the tree is expanded toward a randomly sampled conformation while motion constraints remain satisfied along the path (see Figure 2). We extended this kind of exploration strategy to applications involving protein loops following the approach for closed-chain motion planning described by Cortés and Siméon (2004).

The next sections discuss two applications of our planning techniques on problems involving large molecular motions. They also demonstrate the capability of the approach to efficiently handle articulated models with many DOF.

4 PROTEIN LOOP MOBILITY

Loops are irregular portions of proteins. Such “irregularity” implies a difficulty for structure prediction and available techniques often fail when applied to long loops (Tramontano et al., 2001). Indeed, surface loops are, in many cases, able to undergo significant conformational changes (often corresponding to their functionality), and they can adopt a variety of energetically favorable conformations. The main interest for studying loop conformational changes is due to their importance in protein interactions. For instance, they can adapt the surface topology of antibodies for antigen recognition (James et al., 2003), or play key roles in catalytic mechanisms (Osborne et al., 2001). Despite the interest in protein loops, very limited tools are available to analyze their mobility. Energy-based approaches are only applicable (in practice) to compute slight conformational changes. For larger motions, computationally simpler approaches have to be conceived.

Next we discuss the results obtained with our geometric filtering approach for the study of a particular enzyme, a xylanase. The goal of this study is to validate the approach by comparing our results to those obtained by a classic molecular modeling method and to experimental results.

4.1 Mobility of a Specific Loop of Xylanase

We study endo-$\beta$-1,4-xylanase (EC 3.2.1.8) from Thermobacillus xylanilyticus (XTX) aiming to optimize the conversion of cereal co-products in bio-ethanol fuel. The architecture of
XTX is similar to a right hand, where the thumb is a long hairpin loop. In this protein, the corresponding amino acid sequence spans from 107 to 125. Although maintained by a network of hydrogen bonds, this loop is suspected to be very flexible, like for other xylanases (e.g., de Lemos et al., 1998). An open loop conformation could allow an easier access of the substrate (xylan) to the catalytic pocket. Once the xylan inside the main crevice, a closed conformation of this loop could complete the full docking. Figure 3 shows the structure of XTX and a feasible loop motion computed by our approach.

4.2 Results

4.2.1 Conformational Sampling. We used two methods to sample acceptable conformations for this loop. Both methods include the presence of backbone hydrogen-bond networks (HBN) that maintain the hairpin structure of the loop. Several possible HBN (from known structures of similar anti-parallel beta-sheet loops) were tested.

The first method is a modified simulated annealing procedure which is a well described approach in molecular modeling when the number of DOF is too high to perform a systematic conformational search. The initial dynamics simulations were performed at 500 K with CFF91 force field from Accelrys, maintaining constraint distances (around 3.2 Å) between non-hydrogen atoms supposed to be involved in hydrogen bonds. In the second stage, sets of conformations regularly picked along the high temperature trajectories were progressively and slowly cooled and then minimized (more than 10,000 iterations). At the final minimization step, the distance constraints were removed. Only low energy conformations were selected and then clustered in several (10) low energy regions. A complete set of calculations (dynamics + minimization) involving a given HBN needs more than 10 hours on a SGI computer (with MIPS R14000 processor).

The second approach applies a version of the loop conformational sampling algorithm described in Section 3.3.2. The articulated loop model involves 68 DOF (42 for the backbone and 26 for the side-chains). Conformations generated by this geometric algorithm satisfy loop closure and steric clash avoidance. The method is very fast: 10 significantly different conformations are generated in some seconds or, at worst, some minutes, depending on the difficulty to satisfy a given HBN (on a PC with Intel Centrino 1.8 MHz processor).

Obviously, the energy of such geometrically feasible conformations is in general higher than the energy of those obtained by simulated annealing. However, a simple minimization of a geometrically feasible conformation generally yields a low energy one. Since the geometric conformations already satisfy some strong constraints, their energy minimization is also very fast, requiring about 1 minute per conformation. As shown in Figure 4, this minimization only produces a slight deformation of the loop conformation (RMSD < 1 Å).

4.2.2 Incremental Search. We next performed a more accurate study of xylanase loop mobility, considering the conformational change continuity, using the path planning approach explained in Section 3.3.3. We applied our technique to compare the mobility of the thumb loop in native XTX and in a mutant with two deletions: Tyr-111 and Thr-121. Experimentally, this mutant presents no activity.

Tests were made with different HBN. In all the cases, the mutated loop presents a much more restricted mobility in the crevice context compared to the native one. Figure 6 illustrates the possible loop motions computed for the hydrogen-bond networks represented in Figure 5. The search trees (RRT) computed by the planning algorithm contain 5000 nodes. Note that the construction of the trees requires less than one hour on a standard PC. Our tests confirm the impossibility for this mutated loop to go deeply inside the crevice in order to fix the backbone RMSD is 0.67.

![Figure 4. Geometrically feasible random conformation of xylanase loop (blue/dark), and low energy conformation obtained from it by simple minimization (pink/clear). Both conformations are very close, the backbone RMSD is 0.67.](image)

![Figure 5. Hydrogen-bond networks (HBN) for the native/mutated loop of XTX helping to maintain the hairpin-like loop structure.](image)

1 Known from personal communication. Structures of other xylanases of the same family are available (e.g., de Lemos et al., 2004).
Cortés et al

Fig. 6. Graphic representation of the loop mobility computed for native xylanase (left) and the mutated one (right). The small frames (in black) display positions reachable by the Cα atom of the middle residue in the loop. The native loop can move toward the crevice while the mutated loop only undergoes slight conformational changes.

Fig. 7. Trajectory of a ligand ((R)-ph(Br)Et) accessing to the active site of Burkholderia cepacia lipase.

ligand for the catalytic action. This could explain the absence of activity experimentally known for this mutant.

5 PROTEIN-LIGAND ACCESSIBILITY

The active site of many enzymes is located at the bottom of a narrow and deep cavity. In such case, it is reasonable to consider that the docking of the ligand to the binding pocket is influenced by the difficulty of accessing to the active site, this latter affecting the enzyme-ligand affinity. With energy-based methods, computing motions as large as that of a ligand entering from the protein surface to a deep active site (or vice versa) is very expensive in terms of computing time. However, when only geometric constraints are considered, computing the ligand path to go out from the catalytic pocket can be seen as a mechanical disassembly problem on articulated enzyme/ligand models. Our incremental planner described in Section 3.3.3 is well adapted to this kind of problem.

5.1 Study of Lipase Enantioselectivity

The active site of the Burkholderia cepacia lipase (BCL) is placed at the bottom of a narrow and 17Å deep pocket. This lipase is used for the kinetic resolution of racemic 2-substituted carboxylic acid in transesterification reaction. Recently, a classical approach consisting in the modeling of the R and S tetrahedral intermediates of the reaction failed to explain the enzyme enantioselectivity (Guieysse et al., 2003). Thus, a molecular modeling procedure based on pseudo-molecular dynamics simulation under constraints was employed to model the trajectory of each enantiomer from the active site to the protein surface. Figure 7 shows the trace of the trajectory of one of the enantiomers. Remarkably, the energy of the enzyme/enantiomer interaction along the trajectory was found to be always lower for the preferred enantiomer and in agreement with the experimental results of kinetic resolution. Consequently, molecular modeling of each enantiomer trajectory may be very informative to understand the enzyme enantioselectivity and very useful to predict it. However, the modeling protocol designed by Guieysse et al. (2003) has several drawbacks. First, it does not enable an automatic modeling because manual corrections of several side-chain orientations are required to remove the steric conflicts between the active site and the ligand along the trajectory. It is also very much time consuming. Several days are required to generate one trajectory.

5.2 Results

Our incremental search planner was used to compute geometrically feasible paths of articulated (R,S)-enantiomers, also considering the flexibility of 17 side-chains in the catalytic pocket of BCL. The model contains a total of 68 DOF (11 for the ligand and 57 for the side-chains). Paths were computed for several couples of (R,S)-2-halogenophenyl acetic acid ethyl ester (referred to as ph(X)Et) in BCL for which both experimental (in vivo) results and in silico predictions are available. Computing time is, at worst, several minutes (see Figure 9). The first conclusion of the study is that all the ligand paths computed by our geometric approach are very similar to those obtained, after several days of computation,
by pseudo-molecular dynamics. After a simple (fast) energy minimization of intermediate conformations along the geometrically feasible paths, the energetic profiles are also very similar to the curves computed by pseudo-molecular dynamics (see Figure 8).

Figure 9 shows the average computing time calculated from 50 tests and the experimental enantioselectivity for three couples of enantiomers. In general, computing time of sampling-based motion planning algorithms increases with the difficulty of the problem. Thus, assuming that the topology of the catalytic pocket is better adapted to the access of the preferred enantiomer, its path should be computed faster than the path of the slow reacting one. Remarkably, our results show a good correlation between the ratio of the computing time necessary to find the path of (R,S)-enantiomers and the experimental enantioselectivity value.

In addition, analysis of the set of computed paths enables a very fast localization of amino acid residues constraining the access of the enantiomers and involved in BCL discrimination of racemic compounds. Histograms in Figure 10 display the enzyme atoms constraining the motion of the (R,S)-ph(Br,Cl,F)Et enantiomers in four different portions of the path. Note that the (S)-ph(Br)Et enantiomer meets a higher number of atoms restraining its access. This is totally consistent with computing time results. Therefore using this fast technique makes it very easy to pinpoint residues possibly involved in enantioselectivity, this providing highly valuable information for site directed mutagenesis.

6 DISCUSSION

The essential idea behind our approach is that important constraints affecting molecular motions have a geometrical interpretation. Sampling-based motion planning algorithms applied on flexible molecular models are efficient tools to compute the subsets of their conformational space that satisfy such constraints. Our aim is to use such geometric algorithms as efficient conformational filters before a fine energy-based analysis. The geometric filtering is particularly important for large-amplitude motions in high-dimensional spaces, for which the applicability of energy-based approaches is limited. Furthermore, as shown by the results above, a biological interpretation can be directly made from geometrically feasible molecular motions.

Two kinds of applications have been presented. The first one concerns the analysis of protein flexibility, aiming to predict possible conformational changes of polypeptide segments. The other application concerns accessibility problems in protein-ligand interactions. Although the study conducted for this second application only involves protein side-chain flexibility, the backbone flexibility (treated in the first application) could also be considered.

Our geometric algorithms have been tested with several molecules and first results are very promising. However, a deeper study with a larger set of models remains to be done. We are currently looking for examples of protein-ligand interactions involving narrow and deep cavities with available experimental results for a more rigorous validation.

Our current work focused on the geometric level of the approach and classical energy-based molecular modeling methods are used for the second stage. We aim to develop new techniques for this stage to better exploit the geometric path information provided in the first filtering stage.

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