LowModeMD—Implicit Low-Mode Velocity Filtering Applied to Conformational Search of Macrocycles and Protein Loops

Paul Labute*

Chemical Computing Group, Inc., 1010 Sherbrooke St. W., Suite 910, Montreal H3A 2R7, Quebec, Canada

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We present a method for conformational search of complex molecular systems such as macrocycles and protein loops. The method is based on perturbing an existing conformation along a molecular dynamics trajectory using initial atomic velocities with kinetic energy concentrated on the low-frequency vibrational modes, followed by energy minimization. A novel Chebyshev polynomial filter is used to heavily dampen the high-frequency components of a randomly generated Maxwell−Boltzmann velocity vector. The method is very efficient, even for large systems; it is straightforward to implement and requires only standard force-field energy and gradient evaluations. The results of several computational experiments suggest that the method is capable of efficiently sampling low-strain energy conformations of complex systems with nontrivial nonbonded interaction networks.

INTRODUCTION

The physical and biological properties of a molecule (or collection of molecules) often depend on its conformation. The conformation of a molecule determines its shape and electrostatic properties; consequently, thorough conformational analysis is critically important in many areas of research, such as drug discovery, protein engineering, and the design of catalysts. This problem is complex, because (a) the number of degrees of freedom is large, even for relatively small organic molecules; (b) nontrivial nonbonded interactions such as salt bridges, hydrogen bonds and hydrophobic contacts are often important; (c) steric hindrance and rigidity properties of rings can have important consequences; and (d) environmental effects (such as the solid state, aqueous state or protein bound state) can stabilize conformations that would otherwise exhibit high strain energy.

Molecular dynamics (MD), Monte Carlo (MC) simulations, or hybrids thereof[1] are general methods to generate a physically meaningful ensemble of conformations. These methods rely on a suitably well-parametrized force field that describes the forces to which the atoms in the system are subject. A trajectory is generated, typically at constant temperature, and the resulting conformations can be subjected to clustering and further analysis. These methods are general and can work with large, complex, multicomponent systems. However, one serious drawback is that they explore the conformation space very slowly, since most of the simulation time is spend around the equilibrium energy state(s) and low-frequency conformation transitions are observed relatively rarely. Special techniques can been used, e.g., for polypeptide chains,[2] to greatly improve the sampling properties of Monte Carlo; however, the long simulation times make MD and MC simulations more suitable for the thermodynamic analysis of molecular systems rather than conformational analysis.

Many methods have been developed for the conformational analysis of small organic molecules.[3] Greater efficiency than MD or MC can be achieved by assuming that (a) most conformations are combinations a fairly small number of dihedral angles about rotatable bonds and a small number of conformations of small rings, and (b) small molecules in isolation do not have a tendency to form very complex nonbonded interaction networks. Stochastic or systematic sampling of these combinations, often derived from small molecule crystal data,[4] followed by coordinate refinement or elimination of conformations with steric clash, has been the basis of many methods, such as MIMUMBA[5] and OMEGA,[6] especially for very rapid generation of conformations of small organic molecules. These methods are not suitable for macrocycles, molecules with more complex intramolecular nonbonded interactions, polypeptides, or sterically hindered systems, because the low-energy landscape is not sufficiently well-approximated by rotatable bond combinations. In these systems, "folding" effects and concerted motions become important. In addition, if additional constraints are imposed upon the system (for example, distance bounds from NMR spectroscopy) the *a priori* collections of dihedral angles may no longer be valid, thereby reducing the effectiveness or efficiency of the methods.

Methods based on distance geometry[7−9] are attractive for complex systems, because of their generality and ability to handle large systems. Briefly, the input to these methods is a collection of upper and lower bounds of specific interatomic distances. These distance constraints are used to (stochastically) generate several three-dimensional (3D) coordinate collections consistent with the constraints. Typically, the resulting coordinates are refined with force field energy minimization to produce a collection of conformations. The distance geometry methods are capable of handling large systems with complex networks of constraints. The methods are very good for well-determined systems (large numbers of constraints) and less suitable for underdetermined systems, because they are fundamentally uninformed about nonbonded

* E-mail: paul@chemcomp.com.

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interaction networks, complex steric hindrance, or "folding" effects: they become uniform samplers of a very large conformation space with little chance to sample a low-energy conformation.

In 1996, Kolossváry and Guida reported on Low Mode Search (LMOD), which is a conformational search method based on low-frequency mode-following.[10] In this method, on each iteration, the molecular system is perturbed along low-frequency vibrational mode directions in a systematic way (along with an optional stochastic exploration of combinations of mode directions). The perturbed conformation is then subjected to coordinate optimization. The investment in the costly vibrational analysis paid dividends because the concerted motions of complex systems were elucidated; conformation production in LMOD is a trajectory of low-frequency mode transitions. For large systems, the vibrational analysis is very costly; for such systems, the LLMOD variation of LMOD was developed,[11] which is based on using sparse matrix techniques to determine the low-frequency modes.[12,13] Even with the sparse matrix techniques, the vibrational analysis was so costly (at the time, requiring several hours of CPU time) that it was conducted only once, at the outset, and the same modes were used throughout the search. Even with the increase in speed of computers in the past decade, such a calculation would be prohibitive on each iteration of a conformational search. More importantly, although a single vibrational analysis of a protein can provide a wealth of information about its gross motions,[14−16] it is not necessarily the case for individual loops and nearby interacting residues where conformational details may be important, e.g., for molecular recognition.

The fundamental principle of Low Mode Search is very appealing: use low-frequency mode transitions to "hop" from one low-energy state to another. An assumption is made that such transitions will lead to a full exploration of the low-energy conformation ensemble; however, this assumption is physically motivated and appears reasonable. Moreover, the details of complex nonbonded interactions are taken into account, making the method suited for macrocycles, protein loops, and sterically or topologically hindered systems (including arbitrary force-field restraints and tethers), where the assumptions of other methods may break down. However, the problem with (L)LMOD is the costly vibrational analysis for large systems.

In this work, we present a novel method for conducting a low-mode type conformational search called LowModeMD. It is intended for (large) complex systems and cases where nonbonded interactions or other constraints are important. Fundamentally, we have developed a method to perturb a molecular system along low-frequency modes entirely implicitly, without calculating second derivatives, eigenvectors, or vibrational frequencies. LowModeMD is not intended to be a high-throughput method, and emphasis is placed on the efficient production of low-strain-energy local minima of a potential energy function. The method is stochastic, in that there is no attempt to systematically follow vibrational mode directions (which rapidly becomes impractical once several tens of low-frequency modes are present in the system, in any case). In the subsequent sections, we shall describe the method and present the results of several experiments intended to explore the properties of LowModeMD; conclusions are drawn in the final section.

THEORY AND METHODS

The LowModeMD Search method is a stochastic conformation generation protocol that proceeds as follows (given an initial 3D conformation):

(1) [Initialize] Energy minimize the system and set the conformation list C to empty.

(2) [Velocities] Generate a random set of atomic velocities from the Maxwell−Boltzmann distribution and analytically remove rigid body rotational and translational velocity, if appropriate.[1] Filter the velocities so that there is little kinetic energy along high-frequency vibrational modes (see below). Scale the resulting velocities to 300 K.

(3) [Dynamics] Perform a constant temperature molecular dynamics simulation for ∼0.5 ps.

(4) [Minimization] Energy minimize the system.

(5) [Save] If the conformation is not in C (taking symmetry into account), then add the conformation to C.

(6) [Terminate] If sufficient conformations have been generated or an iteration limit is exceeded then terminate; otherwise, go to Step 2.

The velocity generation procedure in the Velocities step is the main thrust of this work and is more thoroughly described below. The length of the simulation in the Dynamics step is determined stochastically (with adjustable parameters), with a mean of ∼0.5 ps (which is determined empirically to be a reasonable value). In the Save step, duplicates are determined by the root-mean-square (rms) distance, taking molecular symmetry into account.

Structurally, LowModeMD and the (L)LMOD procedures are quite similar, except for the manner in which the system is perturbed and the details of low-frequency motion identification. In LMOD, the low-frequency modes are calculated explicitly and a search along each single mode is conducted, perturbing the coordinates linearly. After each mode is searched (in both positive and negative directions), an MC procedure is used to construct a random linear combination of several low-frequency modes and perturb the system in a similar manner to the single-mode perturbation. The LLMOD procedure is similar to LMOD, except that, for large systems, identification of the low-frequency modes is performed once, at the start of the entire calculation; i.e., *the same low-frequency mode vectors are used throughout the search procedure*,[11] under the assumption that, for proteins, the modes do not change very much. In contrast, LowModeMD skips the systematic single mode search, favoring, instead, a stochastic velocity generation step, followed by MD perturbation. In addition, LowModeMD makes use of a novel projection method so that low-frequency motions can be identified *on every iteration*, even for large systems. We shall now present the LowModeMD velocity generation procedure in detail.

Let $U(\mathbf{r})$ be a potential energy of a set of n atoms as a function of the $3n$-dimensional atomic coordinates $\mathbf{r}$. Let $\mathbf{M}$ denote the $3n \times 3n$ diagonal matrix of atomic masses. We suppose that $\mathbf{r}$ is sufficiently near a local minimum of U so that $|\nabla U(\mathbf{r})| \approx 0$ and, consequently,

$$U(\mathbf{r} + \mathbf{h}) \approx u + \frac{1}{2}\mathbf{h} \cdot \mathbf{G}\mathbf{h}$$

where $u = U(\mathbf{r})$ and $\mathbf{G}$ is the second derivative matrix of G ($\mathbf{G} = (\mathrm{d}^2U/\mathrm{d}\mathbf{r}^2)(\mathbf{r})$. Under these suppositions, the motions

approximately follow a classical Hooke system and, consequently, the vibrational modes are the eigenvectors of $\mathbf{U} = \mathbf{M}^{-1/2}\mathbf{G}\mathbf{M}^{-1/2}$ and the force constants are its eigenvalues. Since $\mathbf{U}$ is a real symmetric matrix, it can be expressed in the form $\mathbf{U} = \mathbf{Q}^{\mathrm{T}}\mathbf{D}\mathbf{Q}$, where $\mathbf{D}$ is a diagonal matrix of force constants and $\mathbf{Q}$ is an orthonormal matrix of eigenvectors (the rows).

A given velocity vector $\mathbf{v}$ can be *filtered* to remove the kinetic energy along modes associated with high-frequency vibrations, using the explicit diagonalization of $\mathbf{U}$ as follows. Let $\mathbf{P}$ be a diagonal matrix with $\mathbf{P}_{ii} = f(\mathbf{D}_{ii})$ for some filter function f (to be described more precisely below). For example, f could be a low-pass Heaviside function $f(x) = \delta(|x| < x_0)$ for some threshold x_0, which would map force constants with magnitudes less than x_0 to 1 and all other force constants to 0. The velocity filtering operation is then defined to be $f(\mathbf{v}) = \mathbf{Q}^{\mathrm{T}}\mathbf{P}\mathbf{Q}\mathbf{v}$, which removes all kinetic energy along the modes with force constants of magnitude greater than or equal to x_0. Using the notation $\mathbf{P} = f(\mathbf{D})$, we can write the filter operation as

$$f(\mathbf{v}) = \mathbf{Q}^{\mathrm{T}} f(\mathbf{D})\mathbf{Q}\mathbf{v}$$

For systems with few degrees of freedom, the filtering can be effected in $O(n^3)$ operations for the dense matrix diagonalization and $O(n^3)$ to estimate the second derivative matrix $\mathbf{G}$ with finite differences (if analytical second derivatives are not available). $O(n^2)$ memory is required to store the matrix $\mathbf{G}$; e.g., for 500 atoms, $\mathbf{G}$ is a 1500×1500 matrix and 1500 evaluations of the potential energy U are required to estimate its contents with finite differences. For large systems, the explicit diagonalization technique is prohibitive, because of the storage requirements; moreover, implicit iterative methods[12]—based only on multiplications of the form $\mathbf{G}\mathbf{x}$—can be slow to converge, requiring thousands of evaluations of U (at least one per iteration).

Rather than use a method that formally calculates the eigenvectors and eigenvalues, followed by an explicit projection of the velocities, we use a Chebyshev polynomial[17] filter that directly projects out the high-frequency kinetic energy from the velocity vector, $\mathbf{v}$, using a *constant* number of evaluations of U. Consider a degree m polynomial filter function f, so that

$$\begin{aligned} f(\mathbf{v}) &= \mathbf{Q}^{\mathrm{T}} f(\mathbf{D})\mathbf{Q}\mathbf{v} = \mathbf{Q}^{\mathrm{T}}\left[\sum_{i=0}^{m} a_i \mathbf{D}^i\right]\mathbf{Q}\mathbf{v} \\ &= \sum_{i=0}^{m} a_i [\mathbf{Q}^{\mathrm{T}}\mathbf{D}\mathbf{Q}]^i \mathbf{v} \\ &= \sum_{i=0}^{m} a_i \mathbf{U}^i \mathbf{v} \end{aligned}$$

Now, for $i > 0$, we have that $\mathbf{U}^i\mathbf{v} = \mathbf{M}^{-1/2}(\mathbf{G}(\mathbf{M}^{-1/2}\mathbf{U}^{i-1}\mathbf{v}))$, and, because $\mathbf{G}\mathbf{x} \approx (\nabla U(\mathbf{r} + h\mathbf{x}) - \nabla U(\mathbf{r}))/h$, we can estimate $f(\mathbf{v})$ with finite differences using m evaluations of U. All that is needed is that f approximately map low magnitude values to $\sim$1 and other values to $\sim$0 over a finite domain containing all of the force constants; e.g., a polynomial approximation to $\delta(|x| < x_0)$ over the domain $[-3000, 3000]$ would be suitable for force-field parameters in common use. There is a large degree of freedom in choosing f, since all that is required is near-unit mapping for low-magnitude values and near-zero mapping for high-magnitude values: perfect {0,1} mapping is not required because the molecular dynamics will quickly transfer kinetic energy to other (nonstationary) modes as the coordinates deviate from $\mathbf{r}$.

However, some care is needed in evaluating the filter polynomial. A straightforward evaluation is numerically unstable, especially for the high-degree polynomials needed to dampen all force constant values above, e.g., $\sim$1 kcal/g/Å^2. For this reason, we use Chebyshev polynomials, which have excellent numerical evaluation properties[18]—even for very high degrees—and a filter function that does not contain a hard cutoff such as $\delta(|x| < x_0)$. For a given filter function f, the Chebyshev interpolation coefficients are (for $i = \{1, 2, \ldots, m\}$)

$$c_i = \frac{2}{m}\sum_{j=1}^{m} f(x_i)\cos(x_i(j-1))$$

$$x_i = \cos\frac{\pi(2i-1)}{2m}$$

Using these coefficients, f can be interpolated accurately at an arbitrary point x in the range $[-x^*, x^*]$, using a well-known iterative formula. This formula generalizes naturally to provide for the evaluation of the velocity filter function $f(\mathbf{U})\mathbf{v}$ in the following pseudo-code, taking as input a velocity vector $\mathbf{v}$, a domain bound x^*, and m Chebyshev coefficients $\{c_i\}$:

```
d = 0; prev_d = 0;
for i = m, 2 do
  temp = d;
  d = 2 Umul(d/x*) − prev_d + c_i v;
  prev_d = temp;
end;
return Umul(d/x*) − prev_d + c_1/2 v;
```

Umul($\mathbf{x}$) is a function that estimates $\mathbf{M}^{-1/2}(\mathbf{G}(\mathbf{M}^{-1/2}\mathbf{x}))$ with finite differences as described above. In the present work, we take $f(x) = (\sin x)/x$ for the filter function and approximate it with an $m = 1500$ degree Chebyshev interpolation polynomial. This filter function does not require the specification of an arbitrary threshold x_0 and, empirically, has excellent high-frequency damping properties. Using the foregoing, a given velocity vector, $\mathbf{v}$, can be filtered in a numerically stable way using m evaluations of the potential energy function U. It is straightforward to implement and can use standard force-field function implementations, because no analytical second-derivative formulas are required. Naturally, for very small molecules, the dense matrix method can be used with the $(\sin x)/x$ transformation of the force constants to construct the filter function $f(\mathbf{v})$.

Computational Methods. The foregoing was implemented in the Scientific Vector Language of the Molecular Operating Environment[19] (MOE), version 2009.10. LowModeMD constant-temperature molecular dynamics was conducted using the 300 K Berendsen thermostat[20] and the velocity Verlet algorithm.[21] Calculations were conducted with Intel compatible computers that were running Windows XP or Solaris 10.

The Alkane Ring, Bonnet Macrocycle, and Molecular Dynamics experiments (see below) were conducted using the MMFF94s force field[22] with gas-phase electrostatics and

Table 1. LowModeMD versus Stochastic Search on Alkane Rings[a]

	LowModeMD						Stochastic Search					
name	N	R_{min}	R_{max}	E_{min}	V	I	N	R_{min}	R_{max}	E_{min}	V	I
K8	16	1.90	1.96	12.15	538274	647	17	1.90	1.96	12.15	127457	438
K10	50	2.24	2.30	17.58	595354	1447	51	2.24	2.30	17.58	535357	1350
K12	29	2.56	2.60	11.40	430731	447	17	2.56	2.60	11.40	354659	681
K14	47	2.84	2.93	6.04	1475438	1338	17	2.84	2.93	6.04	611747	935
K16	49	3.08	3.25	3.21	1985166	1663	15	3.08	3.25	3.21	1116358	1487
K18	874	3.29	3.65	3.32	12930786	10000	408	3.31	3.64	3.61	8589836	10000
K20	1189	3.53	4.07	3.37	13924150	10000	390	3.53	4.00	3.37	9610936	10000

[a] Table legend: N, unique conformations; R_{min}, smallest radius of gyration (Å); R_{max}, largest radius of gyration (Å); E_{min}, lowest-energy conformation (kcal/mol); V, number of force-field evaluations; and I, iteration count.

no nonbonded interaction cutoff. Coordinate refinement was conducted with the Truncated Newton method,[23] using force-field parameters implemented in MOE. The energy minimization gradient threshold was 0.001 kcal/mol/Å. The MOE "Stochastic Search" method is a modified version of Random Incremental Pulse Search,[24] which randomly assigns dihedral angles to all bonds, including rings, followed by energy minimization. LowModeMD and Stochastic Search were configured to terminate after 200 contiguous failed attempts to generate a novel conformation up to a maximum of 10 000 iterations. Conformations rejected on strain energy grounds count as failure to generate a novel conformation. Conformations were deemed duplicates if the root-mean-square distance (rmsd) between them, optimized over rigid body rotation and translation, taking molecular symmetry into account, was less than a threshold. The rmsd threshold was 0.5 Å for the Alkane Ring experiments and 0.75 Å for the Bonnet Macrocycle[25] and Molecular Dynamics experiments. The diversity of a conformational ensemble was assessed by calculating the total number of unique conformations (N), the minimum and maximum of the radius of gyration (R_{min}, R_{max}), the minimum energy observed (E_{min}), and the number of unique binned three-point pharmacophore features (N_p), as described by Bonnet et al.[26]

The Rho-Associated Protein Kinase experiment was conducted with the AMBER 94[27] parameters using Reaction Field electrostatics[1] with a solvent dielectric of $\epsilon = 80$. AM1-BCC[28] charges for the fasudil ligand and water molecules in the active sites were removed. The crystal structure from PDB:2F2U was prepared with Protonate3D[29] to add protons, ionize groups, flip terminal amide and histidine groups, and orient waters. During the conformational analysis, no strain energy cutoff was imposed and conformations were deemed duplicates if their unfixed heavy atom rmsd was <0.75 Å.

RESULTS AND DISCUSSION

LowModeMD is designed to produce local minima of a potential energy surface; it relies on the force field's parameters to implicitly encode reasonable geometries and strain energies. For complex molecules, reliance on a force field is unavoidable. It is not our objective to test the correctness of any particular force field; consequently, our computational experiments shall focus on testing LowModeMD's conformation space exploration abilities and efficiency. We present results for conformational searches of alkane rings and macrocycles, as well as an application of LowModeMD to protein loop conformations.

Alkane Rings. We compared LowModeMD to MOE Stochastic Search, whose algorithmic protocol is identical to LowModeMD, except for the manner in which coordinates are perturbed prior to energy minimization. We assembled a small collection of even-sized alkane rings (with sizes 8−20) denoted K8−K20. The same starting conformations and termination criteria were used for both methods (see the Theory and Methods section). Conformations within 5.0 kcal/mol of the observed minimum were retained (the low-energy conformations).

Table 1 presents the results of the foregoing calculations and reveals that (a) both methods found comparable minimum energies; (b) Stochastic Search required fewer force-field evaluations than LowModeMD; and (c) as the ring size increased, LowModeMD found more low-energy conformations than Stochastic Search. If we consider the efficiency, which is measured by the number of force-field evaluations per unique conformation (V/N), we find that LowModeMD is more efficient for the larger rings (K14−K20), while Stochastic Search is more efficient for the smaller rings (K8−K12). In fact, beginning at K12, LowModeMD found significantly more low-energy conformations than Stochastic Search.

These results strongly suggest that LowModeMD is more biased toward lower-energy conformations than Stochastic Search. While there may be appreciable variation in the number of unique conformations from run to run—both methods are stochastic in nature—the results confirm the assertion that, as the total conformation space grows, Stochastic Search has less and less chance of generating a low-energy conformation. Alkane rings do not contain strong intramolecular electrostatic interactions; for more-complex molecules, such as peptides, folding interactions are stronger and Stochastic Search (as well as other geometry sampling methods) will have even more difficulty in generating low-energy conformations (see below).

Bonnet Macrocycles. Bonnet et al.[26] recently compared 12 conformational search methods, including Stochastic Search and a variant of LMOD, on a collection of macrocycles. Bonnet et al. conclude that the SOS2 variant of Stochastic Proximity Embedding (SPE) was the most effective at generating diverse low-energy structures (with strain energies under 20 kcal/mol) and superior to both the Stochastic Search method and to the LMOD variant. SPE is a stochastic distance geometry method and it is a geometric sampler of conformation space without considering energetics (similar to the Stochastic Search method). We used the Bonnet et al. macrocycle collection to test the assertion that,

Table 2. Comparison of LowModeMD and Stochastic Proximity Embedding[a]

	LowModeMD						SPE (SOS2)					
name	N	R_{min}	R_{max}	E_{min}[b]	N_p	I	N	R_{min}	R_{max}	E_{min}	N_p	I
C6	53	3.09	3.63	28.75	560	732	23	3.04	3.54	28.74	416	10000
C8	275	3.40	4.30	26.21	1294	1917	132	3.39	4.23	26.20	1085	10000
C10	4484	3.78	5.57	35.08	3158	10000	1335	3.81	5.56	36.12	2781	10000
C12	5106	4.08	5.72	35.13	4141	10000	1618	4.11	5.80	39.09	3779	10000
C14	5594	4.34	7.11	40.69	6287	10000	1753	4.35	7.09	40.07	5859	10000
C16	3429	4.63	6.63	41.73	6323	10000	300	4.63	6.02	36.94	4770	10000
C18	2314	4.84	7.98	**44.34**	8019	10000	395	4.79	7.72	49.57	7276	10000
C20	474	5.00	6.39	**42.14**	6812	10000	328	5.08	7.30	51.33	7541	10000
D6	232	4.79	5.44	522.33	105	10000	20	5.02	5.35	522.64	82	10000
D8	26	6.16	6.79	686.70	189	10000	19	6.23	6.80	687.32	194	10000
D10	121	5.86	8.27	871.94	601	10000	59	6.49	8.24	**863.37**	616	10000
D12	132	6.35	8.88	1045.94	829	10000	14	6.73	9.55	1045.60	702	10000
D14	24	6.73	8.87	**1207.18**	700	10000	1	7.68	7.68	1213.23	219	10000
P1	217	5.74	7.38	**7.94**	21902	4904	77	5.81	7.60	26.17	20799	10000
P2	199	5.61	7.77	**145.15**	24698	3415	52	5.77	7.55	157.00	19174	10000
P3	6168	4.03	5.53	118.80	10210	10000	1276	3.99	5.46	119.83	9010	10000
P4	1294	5.05	7.10	**−158.05**	23144	10000	214	5.14	7.40	−146.44	22157	10000
P5	980	4.74	6.63	**383.10**	15006	10000	93	4.94	6.20	399.45	12695	10000
P6	325	5.71	6.87	**13.73**	23801	4149	22	5.78	6.80	32.35	18197	10000

[a] Table legend: N, unique conformations; R_{min}, smallest radius of gyration (Å); R_{max}, largest radius of gyration (Å); E_{min}, lowest energy conformation (kcal/mol); N_p, number of unique pharmacophore triplets; and I, iteration count. [b] Bold-faced entries indicate a lower E_{min} value in excess of 5 kcal/mol.

for complex molecules, LowModeMD is more efficient than unbiased geometric sampling. Our experiments also served to compare LowModeMD directly to SPE and by inference to Stochastic Search and LMOD.

Table 2 presents the results of LowModeMD and the SOS2 variant of SPE and reveals that LowModeMD (a) found equivalent or lower global minimum conformations in almost all cases; (b) found greater numbers of unique conformations with comparable or better diversity;[30] and (c) was on average 10 times more efficient measured with *I*/*N* (since *V*/*N* was not available for Bonnet data). LowModeMD found substantially lower energy global minima for P1, P2, P4, P5 and P6 (boldfaced in the table) rendering the conformation count and diversity comparison somewhat meaningless in these cases; however, it is quite clear that LowModeMD is searching the low strain conformations more thoroughly than SPE (and by implication the Stochastic Search method and the LMOD variant). In all cases, LowModeMD appears to be finding many conformations that SPE missed.

Methods such as LowModeMD and (L)LMOD are quite different from other conformational search methods, in that they extract information about the potentially complex nonbonded interactions from the molecular system and do not rely on *a priori* rules or uniform sampling procedures to cover the seed space for coordinate optimization. Instead, low-mode techniques rely on the assumption that low-energy conformations are connected by low-frequency mode transition pathways involving concerted motions. In this sense, we concur with Kolossváry's observation [LMOD] that that low-mode techniques "generate their own search space".

Complete enumeration of the low-energy conformations of a large molecule is not practical. Systematic searches cannot be undertaken; consequently, stochastic sampling is required. Stochastic methods can be viewed on a spectrum. One end has the purely geometric samplers (such as distance geometry) and the other end has methods that are force- or energy-directed (such as molecular dynamics and Monte Carlo); LowModeMD and (L)LMOD lie somewhere in the middle. Geometric samplers rely on the fact that, with enough (unbiased) sample attempts, many low-energy conformations will result from coordinate refinement. The computational results on the Bonnet et al. macrocycles suggest that these molecules are beyond the capabilities of purely geometric samplers: the odds of generating a low-energy conformation are too low. Our experiments strongly suggest that some element of force or energy bias is required to efficiently generate low-energy conformations of complex molecules.

Comparison to Molecular Dynamics. In some sense, LowModeMD is similar to "steer and quench" molecular dynamics; i.e., alternating molecular dynamics and energy minimization. Were it not for the periodic reassignment of the velocities along low-frequency vibrations, LowModeMD would be practically identical to performing a molecular dynamics calculation followed by energy minimization of the trajectory. Therefore, it is natural to compare the exploration capabilities of LowModeMD and molecular dynamics. To this end, we subjected P1−P6 of the Bonnet macrocycle collection to a 10-ns constant-temperature (300 K) molecular dynamics simulation (using MOE) sampled every picosecond and refined the 10 000 resulting conformations under identical conditions to the previously described LowModeMD calculation. The starting structures were identical to those given to LowModeMD. Thus, we allocated substantially identical computational resources and identical starting structures to both methods run under identical simulation and analysis conditions.

Table 3 presents the results of the MD simulation, alongside the results for LowModeMD from Table 2. Table 3 clearly shows that LowModeMD (a) found minimum energy conformations of significantly lower energy; (b) found greater numbers of unique conformations, except for P2; (c) generated more-diverse ensembles; and (d) was, on average,

Table 3. Comparison of LowModeMD and Molecular Dynamics[a]

	LowModeMD						Molecular Dynamics					
name	N	R_{min}	R_{max}	E_{min}[b]	N_p	I	N	R_{min}	R_{max}	E_{min}	N_p	I
P1	217	5.74	7.38	**7.94**	21902	4904	74	5.85	6.15	17.34	11216	10000
P2	199	5.61	7.77	**145.15**	24698	3415	354	5.74	7.11	162.90	20013	10000
P3	6168	4.03	5.53	**118.80**	10210	10000	35	4.28	4.86	127.31	3624	10000
P4	1294	5.05	7.10	**−158.05**	23144	10000	87	5.24	6.34	−146.47	13137	10000
P5	980	4.74	6.63	**383.10**	15006	10000	24	5.61	6.11	391.60	8526	10000
P6	325	5.71	6.87	**13.73**	23801	4149	260	5.97	6.56	30.70	17589	10000

[a] Table legend: N, unique conformations; R_{min}, smallest radius of gyration (Å); R_{max}, largest radius of gyration (Å); E_{min}, lowest energy conformation (kcal/mol); N_p, number of unique pharmacophore triplets; and I, iteration count. [b] Bold-faced entries indicate a lower E_{min} value in excess of 5 kcal/mol.

more than 40 times more efficient. A higher-temperature MD simulation, or a longer simulation, would lead to greater conformational diversity; however, the same can be said for LowModeMD. This experiment clearly demonstrates the effectiveness of LowModeMD's velocity filtering method at inducing conformational change.

For macrocycles such as P1−P6, one would not expect a full exploration of the conformation space whether by LowModeMD or molecular dynamics and such a full exploration was not expected given the lengths of our calculations. However, we can make some qualitative observations. The main problem with MD as a conformational search method is that it must "wait" for kinetic energy to occasionally concentrate on the low-frequency vibrational modes to effect a gross conformational change. As structures get larger, the waiting time gets longer. For most of the simulation, kinetic energy is spread over all vibrational modes, leaving relatively little kinetic energy on the low-frequency modes. MC simulations can also have difficulty making gross conformational changes, despite special techniques[2] and hybrid schemes.[31] LowModeMD, on the other hand, directly induces the concentrations of kinetic energy on the transition modes from the details of the forces acting upon each particular conformation. Our MD experiment with P1−P6 confirms the assertion that LowModeMD is more efficient at conformation exploration than MD.

Of the numerous methods developed to enhance the sampling of MD, we feel that the Replica Exchange method[32] is most noteworthy here (keeping in mind that our comments are speculative, because we have not made any direct comparison). In Replica Exchange, multiple simultaneous simulations are conducted of the same atomic system, but at different temperatures (e.g., 200−600K). Periodically, the (scaled) velocity vectors of different replicas are stochastically exchanged so that, for example, the velocities of the replica at 300 K are replaced by the (scaled) velocities of the replica at a different (nearby) temperature. The idea is that the equilibrated high-temperature velocities will more likely correspond to larger conformational motions. Replica Exchange is, in some respect, similar to LowModeMD, in that the velocity vector is the focus of the attempt to induce conformational change. However, Replica Exchange is much more costly, from a computational standpoint: multiple simultaneous simulations are required. More importantly, a velocity vector at a higher temperature will not necessarily correspond to low-frequency motions, when the velocities are scaled to a lower temperature. The concentration of kinetic energy on the low-frequency modes is a rare event that is induced directly by LowModeMD, whereas in Replica Exchange, the waiting time for this event is still that of a traditional MD simulation, reduced somewhat because of the velocity exchange.

Comparison to (L)LMOD. LowModeMD is similar in spirit to (L)LMOD, but there are important differences, both theoretical and practical. Both methods generate a perturbation vector from low-frequency vibrational modes. For large systems, both methods use stochastic combination techniques, since an explicit all-possible-mode-combinations approach rapidly becomes impractical, because of combinatorial explosion. The differences between the methods lie in the nature of the coordinate perturbation. (L)LMOD builds up the perturbation vector from an expensive explicit calculation of low-frequency mode vectors whereas LowModeMD removes the undesirable high-frequency modes from a velocity vector with a fast filter, which can be done at every iteration, independent of system size. Aside the practical speed benefit, LowModeMD's coordinate perturbations are guided by each conformation's particular forces, so that, in theory, better perturbations will result. LLMOD performs a single vibrational analysis that is used throughout the calculation and relies on the fact that the low-frequency modes of the input conformation will apply to all generated conformations - which clearly cannot be true if the input conformation is very different from subsequent generated conformations. Another notable difference is the coordinate perturbation method: (L)LMOD uses a linear perturbation that can create gross conformational distortions, whereas LowModeMD uses NVT molecular dynamics, which follows the equations of motion accurately through a conformational change.

BQ123 was used as a test case for the LMOD method. BQ123 is a cyclic pentapeptide (cyclo-D-Trp-D-Asp-Pro-D-Val-Leu) that is potent against the ET_A receptor for endothelin (which is a peptide vasoconstrictor). In addition to the cyclic pentapeptide backbone conformations there are ∼3000 side chain rotamers to consider. NMR studies[33] indicate a type II β-turn with Leu and D-Trp at positions $i+1$ and $i+2$ and an inverse γ-turn at Pro with a preference for contact of the Leu and D-Trp side chains in hydrophilic environments.[34] LMOD was applied to BQ123 with the AMBER force field with the GB/SA implicit solvent model, and the result was 654 conformations with a strain energy of <6 kcal/mol after 5000 iterations. When restraints were applied to enforce the aforementioned hydrogen bonds, the LMOD procedure produced 261 conformations, which was reduced to 209

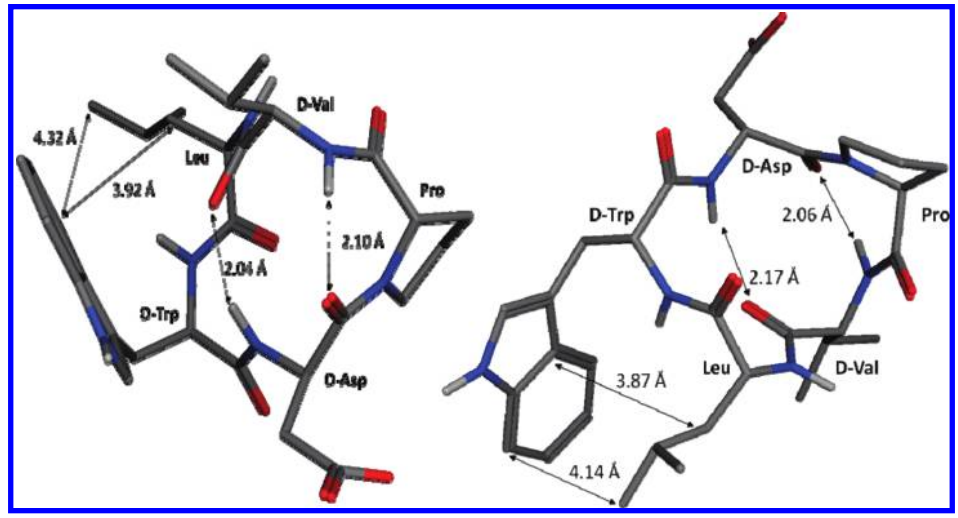

Figure 1. AMBER+GB/VI lowest energy conformation produced by LowModeMD of the cyclic polypeptide BQ123 (left). Eleventh lowest-energy conformation produced with a strain energy of 1.4 kcal/mol. The indicated distances measure the conformation distances, corresponding to NMR spectroscopy analysis.

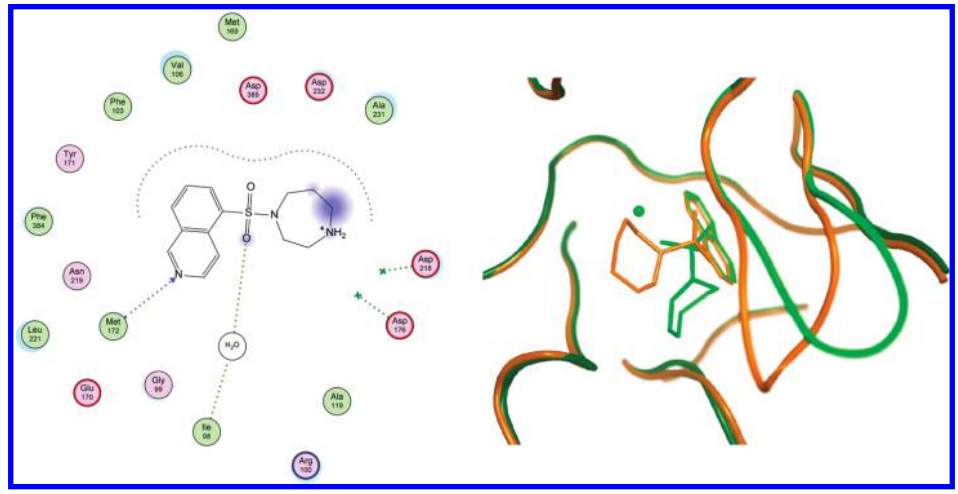

Figure 2. Ligand:receptor diagram for complex A of the homodimer Rho-associated protein kinase complexed with fasudil (PDB:2F2U) (left); green disks represent hydrophobic residues; purple disks are hydrophyilic residues; and red inset rings denote acidic (basic) functionalit. The ligand solvent exposure is depicted with blue clouds. Right: Overlay of Rho-kinase:fasudil complexes A (green) and B (orange); the isolated green sphere is a mediating water in complex A not present in complex B in which the ring amine of fasudil makes hydrogen bonds with Asp175 and Asp218 (blue).

conformations when the restraints were removed and the coordinates reoptimized.[10] We subjected BQ123 to LowModeMD analysis using the AMBER 94 force field without restraints and the GB/VI[35] implicit solvent model; the search was terminated after 5000 iterations. In the resulting collection of 663 conformations, 64 conformations exhibited both of the required turn H···O interatomic distances of <3.5 Å; 48 of which exhibited both interatomic distance values of <2.2 Å. The lowest energy conformation is shown in Figure 1, left. This conformation exhibits contacts consistent with those required for the two turns, as well as a contact between the D-Trp and Leu side chains. The 11th lowest-energy conformation (1.4 kcal/mol strain energy) is shown on the right-hand side in Figure 1; it also exhibits distances consistent with the turns but the side chain contacts between D-Trp and Leu are less oblique than with the lowest-energy conformation. The LowModeMD search produced a similar conformation count to that of LMOD but LowModeMD did not require restraints to find low-energy conformations consistent with the NMR results.

On BQ123, LowModeMD seems to outperform (L)LMOD. It also appears that LowModeMD performed better than an LMOD variant, because Bonnet et al. found that SPE (SOS2) was better than a variant of LMOD in their experiments, and we found that LowModeMD was better than SPE (SOS2). These results, along with the theoretical considerations, suggest that LowModeMD is more effective than (L)LMOD.

Application to Protein Loops. To demonstrate an application of LowModeMD to large systems, we obtained the 2.4-Å-resolution X-ray structure of Rho-Associated Protein Kinase (ROCK2) complexed with fasudil from the Protein Data Bank submission 2F2U.[36] 2F2U is a homodimer with different conformations for the ligand, fasudil, and the nearby 10-residue loop Ile98-Gly-Arg-Gly-Ala-Phe-Gly-Glu-Val-Gln107 in each of complexes A and B. The left-hand side of Figure 2 depicts fasudil and its interactions[37] with nearby residues in both complexes. In complex A, water HOH514 bridges a sulfonamide oxygen of fasudil with the backbone oxygen of Ile98, whereas the water is absent in complex B,

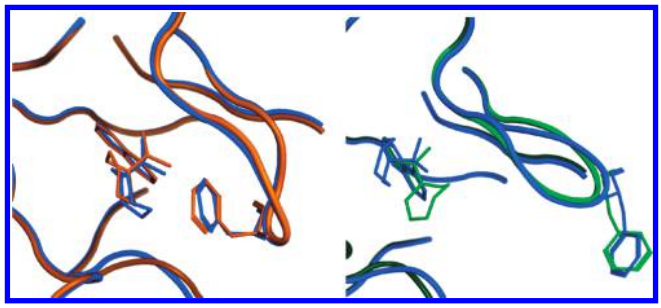

Figure 3. LowModeMD on the Ile98-Glnl107 loop of 2F2U with a Phe103 side chain shown. Results starting with complex A; complex B is in orange and the closest calculated structure is shown in blue. Right: results starting with complex B; complex A is shown in green and the closest calculated structure is shown in blue.

allowing fasudil to adopt a conformation that forms hydrogen bonds between the secondary amine of fasudil and Asp 175 and Asp218. The right-hand side of Figure 2 depicts the superimposed complexes of 2F2U with complex A in green and complex B in orange. In a recent study, Gohda et al.[38] attempted, but failed, to interconvert the conformations using 2 ns of molecular dynamics; they concluded that a much longer simulation would be required to observe a loop conformation change. We applied 1000 iterations of LowModeMD to this task, attempting to convert conformation A into B and vice versa.

Starting with complex A, all receptor atoms in residues within 4.5 Å of fasudil and the mobile loop were free to move while all other atoms where held fixed; the fused aromatic ring block of fasudil was also held fixed. The generation of 913 structures was observed, using a duplicate threshold of 0.75 Å rmsd. The calculated structure closest to complex B had a backbone rmsd of 0.45 Å with a strain energy of 51.0 kcal/mol (see Figure 3, left). The calculated structure closest to complex A (0.5 Å rmsd) had a strain energy of 50.9 kcal/mol. The lowest energy structure was 5.6 Å from complex A and 3.6 Å from complex B. Low AMBER energies were not correlated with crystal coordinates; perhaps a more sophisticated treatment of electrostatics may produce different results. Nevertheless, a broad coverage of the loop conformations was produced by LowModeMD which managed to interconvert complex A to complex B, including the Phe103 sidechain:ligand contact. For comparison, we conducted a 1-ns dynamics simulation, using the same starting structure and conditions, and we observed no appreciable loop movement (results not shown).

We repeated the calculation starting with complex B, and 928 structures were generated. The calculated structure closest to complex A had a backbone rmsd of 0.89 Å with a strain energy of 35.0 kcal/mol (see the right-hand side of Figure 3). The lowest energy structure was 3.8 Å rmsd from both complex A and complex B. The conformation of a seven-member ring of fasudil was not well-reproduced, most likely because of the absence of the mediating water, which stabilizes the fasudil rotamer. As with the first calculation, LowModeMD generated a diverse collection of loop conformations including the conformation of complex A. These results suggest that LowModeMD is an effective method generating protein loop conformations *in situ* with the caveat that force-field energies alone may not be sufficient to identify experimental structures.

Computational Cost. For LowModeMD, the number of force-field evaluations is generally the dominant timing factor. For highly symmetric flexible molecules, such as the larger cycloglycines, conformation duplicate detection can be the dominant factor. For each generated conformation, LowModeMD requires ~1500 evaluations to calculate the initial velocity vector and ~1000 force-field evaluations to perform a thorough energy minimization; this constitutes a total of ~2500 evaluations. Geometry samplers (such as Distance Geometry and Stochastic Proximity Embedding), followed by energy minimization, require ~1500 evaluations, depending on the quality of the starting geometry. A 1 ps MD sample requires 500 evaluations and ~1000 evaluations for the energy minimization; this constitutes a total of ~1500 evaluations. Thus, LowModeMD is most expensive, followed by geometry samplers, followed by the MD method. However, our experiments have shown that LowModeMD is the most efficient of the methods, the higher per-iteration cost notwithstanding; the vibrational analysis (implicit in the velocity filter) seems to be well worth the effort.

Advantages. LowModeMD offers several distinct advantages over other methods for conformational search of complex systems. First, LowModeMD generates conformations using the physical forces involved in conformational changes, rather than purely geometric considerations. For complex systems, conformation-dependent electrostatic and van der Waals interactions are the important factors, rather than local geometry. Second, LowModeMD is more efficient than alternative force-directed methods, such as MD and (L)LMOD. MD does not search conformation space very well, because very long simulation times are required, whereas LowModeMD directly induces conformational change. (L)LMOD is inefficient for large systems, because of a very costly explicit vibrational analysis, whereas LowModeMD uses a fast implicit velocity filter that uses a fixed number of iterations, independent of system size. Third, LowModeMD can be applied to any force field (as well as holonomic restraints and tethers), provided a gradient is available (i.e., standard codes used for energy minimization or molecular dynamics). The velocity filter requires very little additional code (in contrast to large-scale sparse eigenvector solvers) and very little additional storage. LowModeMD is capable of generating low-frequency mode perturbations without forming a large second derivative matrix, without matrix diagonalization or eigenvector calculations and without the specification of a low-frequency threshold value, approximately at the cost of an energy minimization calculation.

CONCLUSION

We have presented a method for conformational search of complex molecular systems such macrocycles and protein loops. The method is based on perturbing an existing conformation along a molecular dynamics trajectory using initial atomic velocities with kinetic energy concentrated on the low-frequency vibrational modes, followed by energy minimization. A novel Chebyshev polynomial filter is used to heavily dampen the high-frequency components of a randomly generated Maxwell−Boltzmann velocity vector. The method is very efficient, even for large systems, is straightforward to implement and requires only standard

force-field energy and gradient evaluations. The results of several computational experiments suggest that the method is capable of efficiently sampling low-strain-energy conformations of complex systems with nontrivial nonbonded interaction networks, and it seems to be more efficient than MD and distance geometry.

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Supporting Information Available: MOL files containing the lowest energy structures generated for the Bonnet macrocycles P1−P6. This information is available free of charge via the Internet at http://pubs.acs.org.

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