
Harmonic Analysis of Large Systems. I. Methodology

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ABSTRACT

Methods have been developed for the determination of vibrational frequencies and normal modes of large systems in the full conformational space (including all degrees of freedom) and in a reduced conformational space (reducing the number of degrees of freedom). The computational method, which includes Hessian generation and storage, full and iterative diagonalization techniques, and the refinement of the results, is presented. A method is given for the quasiharmonic analysis and the reduced basis quasiharmonic analysis. The underlying principle is that from the atomic fluctuations, an effective harmonic force field can be determined relative to the dynamic average structure. Normal mode analysis tools can be used to characterize quasiharmonic modes of vibration. These correspond to conventional normal modes except that anharmonic effects are included. Numerous techniques for the analyses of vibrational frequencies and normal modes are described. Criteria for the analysis of the similarity of low-frequency normal modes is presented. The approach to determining the natural frequencies and normal modes of vibration described here is general and applicable to any large system. © 1995 by John Wiley & Sons, Inc.[‡]

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Introduction

The dynamics of biological macromolecules are of considerable interest since the internal motions of proteins are known¹ to play an important role in protein function. The application of normal mode analysis to proteins²⁻⁴ has provided considerable insight into the nature of collective motions in these systems. Due to both its relatively modest computer requirements and absence of statistical error, harmonic analysis has emerged as an important complement to molecular dynamics (MD) simulation, but a variety of problems in both the basic methodology and its application to biological systems have yet to be resolved.

Normal mode analysis is a direct way to analyze vibrational motions.⁵ A harmonic approximation that is used assumes that the potential energy can be approximated as a sum of the quadratic terms in the displacements. The coefficients of these terms form a force constant matrix which, together with the atomic masses, can be used to set up a matrix equation for the vibrational modes of a molecule. Next, the eigenvalue problem is solved as follows: For a nonlinear molecule composed of N atoms, $3N - 6$ eigenvalues provide the normal or fundamental frequencies of the vibration and associated vectors, which are called the normal modes of vibration, and give the directions and relative amplitudes of the atomic displacements in each mode. For studies of the motions of proteins, for which N is large, special methods need to be employed.

The coordinate system used in normal mode calculation has a major impact on the complexity of the representation of the energy function as well as on the computation times. Cartesian coordinates must be transformed to internal coordinates for the calculation of individual energy terms and their partial derivatives, but there are compensating advantages. If internal coordinates, such as those based on dihedral angles, are employed in the normal mode calculation, the kinetic and potential energy matrices are smaller than those which result when using Cartesian coordinates to describe the same system. In the Cartesian coordinates in which the kinetic energy matrix is diagonal, only the ordinary eigenvalue problem has to be solved. When using the internal coordinates in which the kinetic energy matrix is not diagonal, the generalized eigenvalue problem must be solved instead.

Normal frequencies and normal modes of vibration for systems of interest can be calculated by harmonic analysis. It is shown in the accompanying articles^{6,7} and elsewhere⁸ that for an isolated protein, the harmonic behavior accounts for roughly half of the simulated protein motion (mean square fluctuation) at room temperature and thus gives significant insight into the internal motions of biological systems. Compared to MD simulation,^{1,9,10} normal mode analysis is much less compute intensive. The quantum mechanical vibrational contributions to the thermodynamic functions are easy to obtain in the harmonic limit. It has been suggested¹¹ that harmonic analysis is of little utility in the study of proteins which, as a result of solvent damping, do not "ring like a bell"; but although solvent damping affects the time scales of the motion,¹² it will not, by itself, affect the amplitude or the energy associated with the motion.¹³

Low-frequency modes of proteins are particularly interesting because they are related to functional properties.¹⁴ It has been shown¹⁵ that for native proteins, the very low-frequency normal modes make major contributions to the conformational fluctuations at thermal equilibrium. In the absence of transitions between different substates (local minima),¹⁶ these low-frequency modes dominate the atomic mean square displacements.

Harmonic analysis has been applied to a number of problems. The dynamics of an α -helix was studied¹⁷ in the harmonic approximation by a vibrational analysis of the atomic motions about their equilibrium positions. The first protein normal mode calculation was performed by Tasumi et al.¹⁸ in 1982 on glucagon (29 residues). This calculation was carried out using a special α -helical force field with no nonbonded terms, approximating sidechains as point masses on a nonminimized structure. Subsequently, several normal mode calculations of a protein were carried out using single-bond torsion angle^{2,3} bases. The first complete normal mode analysis of a protein using mass-weighted Cartesian coordinates and taking full account of all degrees of freedom was reported by Brooks and Karplus⁴ in 1983. Vibrational analyses of conformations in peptides and polypeptides,^{19,20} as well as DNA²¹⁻²³ and proteins,^{24,25} have been successfully carried out with different emphases and goals. Recently, a normal mode analysis of G-actin²⁶ and of symmetric assemblies have been reported.²⁷

In this article several methods for performing large-scale harmonic analysis are presented. They

include the computational method (which, in turn, includes Hessian generation and storage, full and iterative diagonalization techniques, and the refinement of the results), reduced basis normal mode analysis, quasiharmonic analysis, and reduced basis quasiharmonic analysis. Techniques for the analyses of vibrational frequencies and normal modes are also described. The techniques are embedded in the vibrational facility in the program CHARMM (Chemistry at HARvard Molecular Mechanics).²⁸ These methods are applied in the accompanying articles^{6,7} to bovine pancreatic trypsin inhibitor (BPTI), a 58-residue protein, to provide a direct comparison of methods using a single molecular system and a consistent Hamiltonian. The results are compared in the following article⁷ with an MD simulation of 2 nanoseconds and with several quasiharmonic and reduced basis quasiharmonic analyses of this simulation.

Computational Method

To determine the vibrational motions of a system, the eigenvalues and eigenvectors of a mass-weighted matrix of the second derivatives of potential function are computed. Using the standard normal mode procedure,⁵ the secular equation

$$|\nabla^2 E - \lambda \mathbf{M}| = 0 \quad (1)$$

is solved, where $\nabla^2 E$ is the Hessian and \mathbf{M} is a diagonal mass matrix. In the root-mass-weighted Cartesian displacement coordinate system, which provides a symmetric secular equation, this becomes

$$|\mathbf{M}^{-1/2}(\nabla^2 E)\mathbf{M}^{-1/2} - \lambda \mathbf{I}| = 0 \quad (2)$$

The eigenvalues, λ , which are proportional to the squares of the vibrational frequencies, are determined by a diagonalization procedure. The numerical treatment of the eigenvalue problem can be simplified if the given matrix is first reduced to a diagonal form by means of an orthogonal similarity transformation. The procedure by which the matrix is diagonalized is important not only for the present application but in many applications in physics and engineering.

For large molecular systems, a straightforward diagonalization of the root-mass-weighted second derivative matrix is not always possible because of either resource limitations (computer storage or time requirements) or excessive error accumula-

tion, which affects the stability of the method. Resource limitations cannot be avoided, but techniques have been developed to diagonalize large matrices within the constraints of limited computer memory. The error accumulation which arises due to the large number of operations can be a difficulty for large systems, but there are techniques to deal with it. One method to manage roundoff accumulation is presented in a subsequent section, Refinement of Results.

HESSIAN GENERATION AND STORAGE

One important factor in the use of this technique is the manner in which the root-mass-weighted Hessian matrix of second derivatives is constructed. A second important factor is how the particular matrix is stored. Since the full matrix sometimes cannot reside in rapid memory, efficient processing of the Hessian may require that this matrix be generated in compressed form and then used as is, or transformed to a blocked upper triangular form. These methods do not require that the matrix be constructed directly in a triangular form. For iterative diagonalization techniques^{14,25} and for other types of analysis, such as determination of the Rayleigh quotient, the matrix need never be converted to triangular form. Considering the types of energy contributions found in potential energy functions,²⁸ the elements of the Hessian matrix can be classified as

1. Diagonal interaction (atom with itself)
2. Close interactions, in which no long-range term is computed (atom connected by a bond or angle term)
3. Long-range interactions (van der Waals and electrostatics)
4. Additional close-range interactions, in which a long-range term is also present (1-4 dihedral angles and hydrogen bonding)
5. Zero interactions (atom pairs beyond all long-range cutoffs).

For large systems with a long-range truncation, most elements of the Hessian are zero (item 5 in the preceding list). For a full normal mode analysis, these zeros are not stored initially, but during the tridiagonalization phase of the diagonalization procedure, the transformation matrix that is generated is nonsparse. Thus, full triangular matrix storage and processing is necessary during the diagonalization. For other techniques, such as a reduced

basis normal mode analysis, the zero interactions are never computed or considered, which can provide a significant savings in computational resources.

For matrix storage, the long-range interactions (item 3) are the most abundant and their handling is important. In principle, for any pair interaction, there can be up to nine contributions (dx_i, dy_i, dz_i with any of dx_j, dy_j, dz_j) to the second derivative matrix; and in the general case with nonradial forces, all nine must be calculated and stored. However, in the special case in which the forces are radial, as is the case for typical van der Waals and electrostatic terms used here, these nine elements can be calculated from four values. These four values correspond to two magnitudes ($\partial E/\partial r$ and $\partial^2 E/\partial r^2$) and to two angles which define a direction. The drawback to storing only these four elements is that the transformation to the full matrix is awkward. In practice, since the submatrix is symmetric, six values (the lower triangle) are saved. Also, if single precision is used anywhere throughout the calculation, a case can be made for storing these terms in single precision as well. Each pair is independent and does not constitute an accumulation of terms, and most of them have small values.

Contributions to the diagonal (self-atom) elements (item 1) of the Hessian occur for all energy contributions, because every contribution of the energy has to obey the Newton's third law. Thus the sums of columns and rows of the Hessian must add to zero, and the diagonal elements are sums of many terms. Six values ($\partial^2 E/\partial x^2$, $\partial^2 E/\partial x \partial y$, $\partial^2 E/\partial y^2$, $\partial^2 E/\partial x \partial z$, $\partial^2 E/\partial y \partial z$, $\partial^2 E/\partial z^2$) are needed, and they must be stored in high precision because they represent a sum of many terms.

When atoms are connected by bond or angle terms (item 2), their connectivity is noted in a nonbonded excluded list which indicates that no van der Waals or electrostatic interaction is needed for that pair. The index of this list is useful for pointing to the set of nine matrix elements for each pair. All nine values are needed because these forces are nonradial. Also, several energy terms can contribute to a particular matrix element, so double precision should be used.

The additional close interactions (item 4) are stored in a separate list of nine elements each (again nonradial forces). These interactions consist of 1–4 dihedral interactions and perhaps explicit hydrogen bonding interactions. Contributions to these matrix elements will also be found in

the long-range interaction list. These contributions need not be gathered together to generate a matrix–vector product.

Checking the Hessian by Finite Differences of Forces

All matrix elements are analytically determined, so the method of finite differences is not used in the actual calculation. However, the program was checked by generation of the Hessian using the finite difference method and comparing the results with those derived analytically. The following relation must hold:

$$\begin{aligned} \frac{\partial^2 E}{\partial x_i \partial x_j} &\approx \frac{1}{2} \left[\frac{\partial E(\mathbf{x} + \delta x_j)}{\delta x_i} - \frac{\partial E(\mathbf{x} - \delta x_j)}{\delta x_i} \right] \\ &\approx \frac{1}{2} \left[\frac{\partial E(\mathbf{x} + \delta x_i)}{\delta x_j} - \frac{\partial E(\mathbf{x} - \delta x_i)}{\delta x_j} \right] \quad (3) \end{aligned}$$

Setting up the Diagonalization

This step involves conversion of the second derivative matrix from the compressed form into a form which can be used by the diagonalization method. Also included in this step is the inverse mass-weighting required by the normal coordinate problem.⁵ When the full Hessian is stored in memory, one pass is made through the compressed Hessian, and the elements are added to the correct location of the upper triangular matrix. When the final matrix is to be stored in blocked upper triangular form, one pass through the compressed Hessian will be required for each block. In this way, very large systems may be studied on machines with limited memory.

FULL DIAGONALIZATION TECHNIQUES

The diagonalization method is similar to that used in the QCPE program GIVENS,²⁹ except that the lower triangular storage scheme is wholly inappropriate for a secular problem in which the entire matrix cannot reside in rapid memory. The use of a two-dimensional indexing of the matrix makes GIVENS less efficient than it might be. When the matrix is too large to reside in rapid memory (on a virtual machine, unloaded pages are not considered to be rapid memory), it is necessary to divide the matrix into blocks, each of which contains consecutive rows of the matrix stored in

the upper triangular form. This is an acceptable manner in which to solve the memory storage problem because the computer never needs more than one block in memory at any time; it finishes with one block before going on to the next (on each pass through the tridiagonalization, or for each eigenvector back transformation).

Standard diagonalization techniques are only practical, in general, for relatively small matrices which occur⁵ in the normal mode calculations of small molecules. No general method appears to be known at present which is applicable to randomly sparse (or nonsparse) matrices of order $\geq 10^5$ and which makes possible the facile calculation of many eigenvalues and eigenvectors of such matrices, particularly when the matrices are not diagonally dominant. However, iterative techniques have been successful for generating a limited set of normal mode vectors.^{14,25} These methods make optimal use of the compressed Hessian format. The full diagonalization technique as applied to large matrices to obtain the eigenvalues and eigenvectors is described here. The method employed consists of five steps:

1. Matrix preparation (normalization)
2. Tridiagonalization using the Householder algorithm³⁰
3. Determination of eigenvalues by Sturm bisection³¹
4. Determination of desired eigenvectors of the tridiagonal matrix by inverse iteration³²
5. Back transformation to find eigenvectors of the original matrix.

The matrix preparation step requires that each element is divided by the root sum square of all of the elements in the matrix. Thus, the matrix was scaled so that the sum of all the elements squared is unity.

The computational effort involved in most diagonalization methods is at worst proportional to n^3 , where n is the order of the matrix ($n = 3N$ for a full normal mode calculation in the Cartesian basis). In this process the most time-consuming steps are step 2—transforming the matrix to the tridiagonal form, which is proportional to n^3 —and potentially step 5—finding the eigenvectors of the original matrix, which is proportional to n^2m , where m is the number of roots needed. When all roots are desired, step 5 requires twice the effort of step 2, but when only a few roots are desired, step 2 is dominant. By comparison, the remaining steps

take a minor amount of time. If the entire storage requirement exceeds the memory of the machine, then the tridiagonalization step requires a partial pass through the upper triangular matrix for each element in the secular equation (each successive pass requires less data). This step is modified so that necessary portions of the matrix are brought into rapid memory at the appropriate time by means of either a direct I/O call or the paging system of the virtual memory operating system. Step 5 requires one full pass for each eigenvector and must also be modified in a similar manner. During step 2 of the tridiagonalization phase, the $n(n+1)/2$ storage locations required for the matrix are slowly converted to storage for the tridiagonal transformation unitary matrix. During step 5, the back transformation phase, this nonsparse transformation matrix is used to convert eigenvectors of the tridiagonal matrix to those of the original matrix (which is no longer present in the memory).

For large systems, it may not be practical to complete the calculation in one run, or it might be desirable to generate additional eigenvectors beyond those originally requested without repeating steps 1 through 3. For these reasons, the method can be restarted at step 2 and 5, with all data being saved externally. This is also useful in the event of a machine failure. For example, with a 3000-atom system, a full diagonalization will require approximately 2.1×10^{12} floating point operations and will require one gigabyte of virtual memory storage. On a machine capable of sustaining 20 megaflops, this operation will require about 30 hours of CPU time. Several modern machines can easily handle systems of this size, but when slower machines are used, it is useful to compute this calculation with several shorter runs.

To solve the problem of error accumulation, the calculation should be carried out with as great a precision as practical, typically double precision. The greatest errors are incurred in steps 2 and 5 in the preceding list, but there is little that can be done to eliminate this aspect of the problem directly, except to use higher precision floating point arithmetic. A correction procedure is presented in the section titled Refinement of Results.

ITERATIVE DIAGONALIZATION METHOD

For very large molecular systems in which straightforward diagonalization is not feasible, it is still possible to extract information concerning normal modes from the structure by using an iterative

eigenvector extraction procedure to obtain a small number of modes of interest.^{14,25} The main difference in this type of approach is that only a few selected modes are studied, and one must guess the nature of the mode in question. The other major issue concerning very large molecules is the manner and the efficiency in which the mass-weighted second derivative matrices are stored. For very large molecular systems, the compressed Hessian storage technique (see Hessian Generation and Storage) significantly reduces the computation effort for this method, because zeros in the Hessian are not considered.

Iterative eigenvector extraction techniques have, for many years now, been an invaluable tool in the area of configuration interaction (CI) calculations.³³ The method presented here differs from CI iterative eigenvector extraction techniques in that many of the commonly used assumptions are no longer valid. Among these assumptions are that the matrix is diagonally dominant; the roots are well separated; and the lowest root or few roots are desired. None of these are true for molecular vibrations. The procedure presented here may also provide a benefit to electronic structure calculations in which convergence is less than optimum.

Basic Method (Order n^2 per Root)

Although there are several distinct methods for iterative diagonalization schemes, those involving relaxation methods^{33,34} require that the method can be approximated through perturbation theory and that the eigenvalues are well separated. The method derived here is based on Davidson's modification of Lanczos's algorithm.³⁵ It overcomes to a large degree the problem of nearly degenerate roots by using an increasingly orthonormal subspace. Consider the secular equation

$$\mathbf{F}\mathbf{y}_i \approx \lambda_i \mathbf{y}_i \quad (4)$$

with

$$\mathbf{F} = \mathbf{M}^{-1/2}(\nabla^2 E)\mathbf{M}^{-1/2} \quad (5)$$

where \mathbf{M} is the diagonal mass matrix and E is the energy. The associated Raleigh quotient is given by

$$\rho(\mathbf{y}) = \frac{\mathbf{y}^\dagger \mathbf{F} \mathbf{y}}{\mathbf{y}^\dagger \mathbf{y}} \quad (6)$$

for any arbitrary value of \mathbf{y} . The stationary points on the surface of $\rho(\mathbf{y})$ correspond to eigenvectors

of \mathbf{F} , and $\rho(\mathbf{y})$ corresponds to associated eigenvalues. If only methods for the lowest root are considered, a global minimization on the surface of $\rho(\mathbf{y})$ is required. The gradient of $\rho(\mathbf{y})$ is given as

$$\nabla \rho(\mathbf{y}) = \frac{2q(\mathbf{y})}{\mathbf{y}^\dagger \mathbf{y}} \quad (7)$$

where $q(\mathbf{y})$ is the residual vector given by

$$q(\mathbf{y}) = (\mathbf{F} - \rho(\mathbf{y}))\mathbf{y} \quad (8)$$

For any vector \mathbf{y} , the Raleigh quotient $\rho(\mathbf{y})$ and its gradient can be found with an order n^2 operation of a vector on a matrix. One could easily implement a steepest descent or conjugate gradient type of minimization procedure to find the lowest root, but due to the cost per iteration, rapid convergence is desirable.

Consider an orthonormal subspace which is made up of vectors $\mathbf{b}_1, \mathbf{b}_2, \dots, \mathbf{b}_k$. The lowest eigenvector of \mathbf{F} can be approximated by

$$\mathbf{y}_k = \sum_{i=1}^k c_i \mathbf{b}_i \quad (9)$$

where \mathbf{c} is the lowest eigenvector of the symmetric matrix \mathbf{W}' such that

$$\mathbf{W}'_{ij} = \mathbf{b}_i^\dagger \mathbf{F} \mathbf{b}_j \quad (10)$$

(Note that the Raleigh quotient is the lowest eigenvalue of \mathbf{W}' .) The essential concept of the Davidson algorithm is to add an orthonormal correction vector \mathbf{b}_{k+1} into the existing subspace such that the eigenvector approximation \mathbf{y}_{k+1} is closer to the true eigenvector. The actual form of the correction vector suggested by Davidson is

$$\mathbf{b}_{k+1}'' = \frac{(\mathbf{F}\mathbf{y}_k)_p}{\rho(\mathbf{y}_k)} \quad (11)$$

where \mathbf{b}'' is the "raw" vector which must be orthogonalized to the remaining subspace

$$\mathbf{b}_{k+1}' = \mathbf{b}_{k+1}'' - \sum_{i=1}^k \mathbf{b}_i \mathbf{b}_i^\dagger \mathbf{b}_{k+1}'' \quad (12)$$

and then normalized

$$\mathbf{b}_{k+1} = \mathbf{b}_{k+1}' (\mathbf{b}_{k+1}'^\dagger \mathbf{b}_{k+1}')^{-1/2} \quad (13)$$

One concept used in extending this method to molecular vibrations is that \mathbf{b}_{k+1}'' can be expressed as any arbitrary function of \mathbf{y}_k . The optimum

(one-step convergence) function is given by

$$\mathbf{b}_{k+1}'' = (\mathbf{F} - \lambda)^{-1} \mathbf{F} \mathbf{y}_k \quad (14)$$

Since it is not practical to compute $(\mathbf{F} - \lambda)^{-1}$ directly, the essence of this method is to choose a functional form that gives a reasonable approximation to $(\mathbf{F} - \lambda)^{-1}$ with the restriction that the operation remain, at most, of order n^2 . The choice of denominator $[\rho(\mathbf{y}_k)]$ in eq. (11) is optimum when the matrix is diagonally dominant (i.e., the matrix can be approximated by first-order perturbation theory).

The Positive Semidefinite Case

In the case of molecular vibrations, a particular mode of interest (the hinge bending mode in lysozyme, for example)²⁵ does not necessarily correspond to the lowest eigenvalue of \mathbf{F} . Convergence of the iterative method can be improved considerably if the desired root is at an eigenvalue extreme and the roots are well separated. A method for this is to replace the matrix \mathbf{F} by $(\mathbf{F} - \gamma \mathbf{I})^2$, where γ is an arbitrary value. The eigenvectors remain unchanged by this replacement and the eigenvalues are shifted by $\lambda_i' = (\lambda_i - \gamma)^2$. If γ is chosen as the current "best guess" eigenvalue of the desired root of \mathbf{F} , this root becomes the lowest root $(\mathbf{F} - \gamma \mathbf{I})^2$ and will have an eigenvalue of zero.

The magnitude of

$$\mathbf{y}_k^\dagger (\mathbf{F} - \rho(\mathbf{y}_k) \mathbf{I})^2 \mathbf{y}_k \quad (15)$$

is one indication of convergence. Since the matrix \mathbf{F} can be very large, a direct determination of \mathbf{F}^2 is not feasible. For the method to work with the matrix squared, matrix elements of $\mathbf{b}_i^\dagger \mathbf{F}^2 \mathbf{b}_j$, $\mathbf{b}_i \mathbf{F} \mathbf{b}_j$, and $\mathbf{b}_i \mathbf{I} \mathbf{b}_j = \delta_{ij}$ are needed in the orthonormal basis. This is done by storing the intermediate vectors

$$\mathbf{a}_i = \mathbf{F} \mathbf{b}_i \quad (16)$$

and expressing these results as

$$V_{ij} = \mathbf{b}_i^\dagger \mathbf{F}^2 \mathbf{b}_j = \mathbf{a}_i^\dagger \mathbf{a}_j \quad (17)$$

$$W_{ij} = \mathbf{b}_i^\dagger \mathbf{F} \mathbf{b}_j = \mathbf{b}_i^\dagger \mathbf{a}_j \quad (\text{or as } \mathbf{a}_i^\dagger \mathbf{b}_j) \quad (18)$$

If W_{ij}' is replaced in eq. (10) by

$$W_{ij}' = V_{ij} - 2\rho(\mathbf{y}_k)W_{ij} + \rho(\mathbf{y}_k)^2 \delta_{ij} \quad (19)$$

the remaining steps discussed in the preceding section (Basic Method (order n^2 per root)) remain unchanged [except that $(\mathbf{F} - \rho)^2$ replaces \mathbf{F}].

Improving Convergence

As pointed out in the preceding section, the approximation of \mathbf{F}^{-1} or $(\mathbf{F} - \rho)^2$ is essential for the success of this method. Consider a transformation \mathbf{U} such that $\mathbf{U}^\dagger \mathbf{F} \mathbf{U}$ is diagonally dominant. Given that such a transformation is known, \mathbf{F}^{-1} can then be approximated by

$$\mathbf{F}_{\text{app}}^{-1} = \mathbf{U} \left(\frac{\delta_{ij}}{(\mathbf{U}^\dagger \mathbf{F} \mathbf{U})_{ij}} \right) \mathbf{U}^\dagger \quad (20)$$

The transformation \mathbf{U} must be sparse for this approximation to be effective. The matrix \mathbf{F}_{app} is never stored, but its contribution in eq. (14) is computed as

$$\mathbf{b}_{k+1}'' = \mathbf{U} \left(\frac{\delta_{ij}}{(\mathbf{U}^\dagger \mathbf{F} \mathbf{U})_{ij}} (\mathbf{U}^\dagger \mathbf{y}_k) \right) \quad (21)$$

with three successive operations on a single vector. Since \mathbf{b}'' is not constrained to be any particular type of vector, it is not even required that \mathbf{U} be a unitary transformation.

A simple choice for the transformation \mathbf{U} lies in the principal axis transformation required to diagonalize the (3×3) matrix for each atom's self-terms. Using this transformation, bond stretching motion is separated from angle bending and other low-frequency motion and makes the matrix more diagonally dominant and separable (i.e., more like a block diagonal matrix). In this case, the \mathbf{U} matrix is simply a series of (3×3) transformation matrices which span the number of atoms. Another choice is to include all bonding and angle (and perhaps dihedral and hydrogen bonding) terms. In this case it is necessary for \mathbf{U} to be approximately a sparse transformation, since the actual transformation matrix would be dense and difficult to compute.

In practice, it is best not to use any single \mathbf{F}^{-1} approximation, since each approximation is likely to be deficient in some respect. For the initial stages of the calculation, a straightforward "conjugate gradient" step can be used. One chooses

$$\mathbf{b}_{k+1}'' = \mathbf{F} \mathbf{y}_k \quad (22)$$

and advances to more rapid convergence by

$$\mathbf{b}_{k+1}'' = (\mathbf{F}_{\text{app}}^{-1}) \mathbf{F} \mathbf{y}_k \quad (23)$$

once the vector approaches the converged result.

Removal of Constrained Motion or Other Eigenvectors

Once a particular eigenvector is known and additional eigenvectors are desired, it is useful to remove the components of the known eigenvectors from the calculation. It is also helpful to remove unwanted motion from the calculation using constraints. This is most useful in removing the effects of net translation and rotation. This gains increasing importance when the desired mode has a very low frequency.

The procedure requires that the orthonormal expansion of $\mathbf{b}_1, \mathbf{b}_2, \dots, \mathbf{b}_k$ remain orthogonal to the known or constrained vectors (i.e., motion that is to be removed from the calculation). This prevents any contribution of these unwanted vectors to the guess vectors. In the event that subsequent convergence fails because contributions of the unwanted vectors appear in the residue vectors, it may be necessary to relax the constraints. This can occur with the rotation mode if the molecule is not at a stationary point on the energy surface (e.g., Coriolis coupling).³⁶ For this reason, it is best to carry out this type of calculation only when the molecule is at a stationary point (minimum). If it is not feasible to find such a minimum, the "root shifting method"³³ to apply a restraint (instead of a constraint) can be used by adding $\alpha_i(\mathbf{c}_i \mathbf{c}_i^\dagger) \mathbf{b}_j$ to the vector $\mathbf{F} \mathbf{b}_j$, where \mathbf{c}_i is the constraint vector corresponding to rotational motion and α_i is an arbitrary positive restraint constant.

REFINEMENT OF RESULTS (CORRECTION PROCEDURE)

Once the approximated eigenvectors and eigenvalues have been obtained, it is possible to refine these eigenvectors to a higher level of accuracy by operating with them on the original matrix followed by an additional diagonalization in a smaller representation. This is useful for both full diagonalization method and the iterative method. Errors accrue in the full diagonalization method due to the vast number (order n^3) of numerical operations. This problem becomes more severe as the size of the secular problem increases. For single precision methods, the order of the secular problem should not exceed 100 (i.e., about 30 atoms); consequently, all normal mode calculations for macromolecules should be carried out at high precision. The upper limit of n for an accurate analysis using IEEE double floating point operations precision (52 bits at mantissa) is not established. It

was not necessary to use a correction procedure for a problem with 2500 atoms (unpublished results from full basis phenylalanine tRNA).

Consider the case in which the 100 lowest normal modes are required in a system with 1000 atoms (a total of 3000 possible modes). The refinement procedure is as follows:

1. Set up the inverse mass-weighted energy second derivative matrix

$$\mathbf{F} = \mathbf{M}^{-1/2} (\nabla^2 E) \mathbf{M}^{-1/2} \quad (24)$$

2. Diagonalize the matrix \mathbf{F} and obtain the first 200 approximate eigenvectors such that

$$\mathbf{F} \mathbf{y}_i \approx \lambda_i \mathbf{y}_i \quad (25)$$

and the Rayleigh quotient is equal to the corresponding²⁵ eigenvalue

$$\lambda_i = \frac{\mathbf{y}_i^\dagger \mathbf{F} \mathbf{y}_i}{\mathbf{y}_i^\dagger \mathbf{y}_i} \quad (26)$$

3. Construct the Hermitian matrix with elements $H_{ij} = \mathbf{y}_i^\dagger \mathbf{F} \mathbf{y}_j$ with the approximate vectors. Note that \mathbf{H} will be a diagonal (200×200) matrix and contain the eigenvalues if the \mathbf{y} 's are true eigenvectors.
4. Diagonalize \mathbf{H} by the orthogonal transformation matrix \mathbf{U} into the similar diagonal matrix $\mathbf{\Lambda}$ —that is, $\mathbf{U}^\dagger \mathbf{H} \mathbf{U} = \mathbf{\Lambda}$. The columns of \mathbf{U} are equal to the eigenvectors of \mathbf{H} , and the diagonal elements of $\mathbf{\Lambda}$ are the eigenvalues of \mathbf{H} .
5. Choose the final refined eigenvectors as a linear combination of the original lowest 100 roots of \mathbf{H} as

$$\mathbf{y}'_i = \sum_j U_{ij} \mathbf{y}_j \quad (27)$$

6. Choose the final eigenvalues from the diagonal elements of $\mathbf{\Lambda}$.

The assumption here is that most of the contamination in the modes of lowest frequencies will come from other low-frequency modes. The second diagonalization [in this case, (200×200)] has a much lower error than the original (3000×3000) calculation. Out of the (200×200) calculation, the first 100 modes should be sufficiently accurate. This refinement could be performed on any subset of modes.

Other methods can be used in conjunction with this procedure. One simple approach is to reduce the size of a large calculation by removing irrelevant components. Consider the case in which the molecule contains a number of hydrogens. If modes involving the motion of hydrogens are not needed but the hydrogen atoms are explicitly included, these atoms can be grouped with heavy atoms to which they are bonded and the whole treated as a rigid body. Other types of reduction may also be acceptable. For example, if one is studying the interface region between two large molecules (a macromolecular dimer, for example), it may be possible to treat large sections of the structure as having no internal degrees of freedom, thus substantially reducing the overall size of the secular equation. This is done by using the approximate eigenvectors as input to the reduced basis method presented in the next section.

Even for cases in which the refinement step appears to be unnecessary, as is the case of BPTI, the construction of the partial basis matrix is a valuable check of the method. If the matrix \mathbf{H} is diagonal, then the diagonalization procedure will be accurate and no refinement will be necessary.

Reduced Basis Normal Mode Analysis

For a typical macromolecular system, the problem may be intractable and it becomes desirable to reduce the computation cost of a harmonic analysis by making some approximations concerning the nature of the motions. One such approximation involves the reduction of the size of the secular equation by partitioning the Hessian matrix into relevant and irrelevant parts through the use of an appropriate unitary transformation, which approximately block diagonalizes the full $(3N \times 3N)$ Hessian matrix. The irrelevant part is subsequently ignored, and the relevant part is represented by a smaller $(m \times m)$ matrix.

OVERVIEW OF REDUCED BASIS METHODS

The torsion basis methods developed by Gō² and Levitt³ represent a particular implementation of the present method in which the torsional motion is defined as the relevant part of the Hessian, and all other parts are removed. Though the dihedral basis is an obvious choice, it may be useful to argument this basis with low-frequency spherical harmonic motion and/or the six rotational and

translational degrees of freedom for subunits or small molecules. These account for some important types of motion absent from the torsional basis. For example, in the X-ray structure of BPTI there are four internal water molecules. Carrying out a reduced basis calculation without deleting these water atoms and without allowing them any freedom will significantly alter the nature of the low-frequency vibrational spectrum of BPTI. This is analogous to performing an MD simulation with these atoms fixed in space. One method to allow these waters to move would be to add some additional torsion angles to the water from nearby residues. A more straightforward technique used here is to include the six translational and rotational degrees of freedom for each water molecule as a part of the set of reduced basis vectors.

The method presented here can exactly reproduce the results of the torsion methods. The advantages or features of this method are as follows:

1. The method is analytical. The reduced basis Hessian is obtained by direct transformation of the nonzero elements of the compressed Hessian in the full Cartesian basis (not determined by finite differences). This is accurate and can be implemented efficiently.
2. Any types of motion may be included or rejected from the calculation. Thus, the method is not restricted to torsion angles.
3. The use of Cartesian coordinates to represent an orthonormal subbase avoids the complexity of a nondiagonal kinetic energy matrix. This retains the simplicity of the secular equation (i.e., it remains Hermitian), which requires the solution of an ordinary eigenvalue problem, as opposed to the generalized eigenvalue problem arising from the use of more general coordinates.
4. The generation procedure for the orthonormal basis using the Gram-Schmidt orthogonalization procedure allows linear dependencies to be easily removed by the rejection of any basis vector in which the residual norm is less than a specified tolerance. Linear dependencies are found in loop structures, when removing net translational and rotational components of motion, and when too many torsion angles or bond angles are included in the set of basis vectors.
5. A back transformation of the reduced basis results into the full basis allows for easy analysis of the results and allows comparison

with those from the full basis set. Such a detailed analysis is presented in the following articles.^{6,7}

6. The reduction of the Hessian is performed efficiently in a two-step procedure [the first step is at most of order $(3N)^2 m$, and the second is of order $3Nm^2$] rather than by a direct transformation, which requires $(3Nm)^2$ operations, and the first step involves only the nonzero components of Hessian.

Reduced basis normal mode analysis techniques allow the study of motions of interest of the desired systems in the harmonic limit. The reduction of the problem can be viewed as either the removal of unwanted motion through the use of constraints or as the inclusion of desired motion.

REDUCED BASIS NORMAL MODES PROCEDURE

The procedure for calculating the reduced basis normal modes is as follows:

1. Generation of the Hessian in the full basis in compressed form
2. Inverse mass-weighting the Hessian
3. Generation of the reduced basis transformation vectors
4. Generation of the reduced Hessian
5. Diagonalization of the reduced Hessian
6. Back transformation of the reduced basis normal modes into the full basis.

Generation of the Hessian

The structure, the energy (preferably a local minimum), and the Hessian need to be determined using the full Cartesian coordinate basis. This means that the structure, the energy, the forces, and the Hessian are known in the full conformational space. If the forces are nonzero, coupling between rotational and internal motion should be considered.

Inverse Mass-Weighting the Hessian

The inverse mass-weighted energy second derivative matrix

$$\mathbf{F} = \mathbf{M}^{-1/2}(\nabla^2 E)\mathbf{M}^{-1/2} \quad (28)$$

is set up and only nonzero elements are stored (see Hessian Generation and Storage). This means that internal motions are defined for all $3N$ degrees of freedom of the system, and \mathbf{F} is a compressed matrix of dimension $(3N \times 3N)$ in the root-mass-weighted Cartesian basis.

Generation of Reduced Basis Transformation Matrices

The reduced basis transformation is the transformation which approximately block diagonalizes \mathbf{F} into relevant and irrelevant portions. An example of this is in the separation of the low-frequency dihedral angle motion from the other higher frequency (bond stretching and bending) internal degrees of freedom. The success of this approach depends on the extent of coupling between the relevant (calculated part) and the irrelevant (discarded part) of \mathbf{F} .

To carry out a reduced basis calculation, an orthonormal basis set (i.e., the basis set in which vectors are mutually orthogonal and are all of length 1) is generated. The basis spans an orthonormal subspace S_1 of the full root-mass-weighted Cartesian basis. The orthogonal subspace complementary to S_1 is S_2 , where S_1 represents the relevant motion to be analyzed and S_2 represents the portion to be neglected. This becomes a set of constraints on motion of the system. The direct sum ($S_1 \oplus S_2$) of both the orthonormal subspaces is the complete square root-mass-weighted Cartesian space.

The question is how to choose the best basis set. One possibility is to try and fit the 0 to 100 cm^{-1} region accurately. Generation of an orthonormal basis set is determined by the way the constraints are applied to the system or the system's internal motions of interest are described. The procedure for basis set generation is as follows:

Definition of the Motions of Interest (Relevant and Irrelevant Motions). To include or to constrain a particular motion, it must be represented as a normalized vector in the root-mass-weighted Cartesian bases. This is done by defining a particular motion in terms of Cartesian displacements or derivatives, where the displacements (such as dihedral motion) depend on the position of the atom. Thus

$$\delta \mathbf{x}_i = \mathbf{O}_i(\mathbf{x}) \quad (29)$$

where \mathbf{O}_i is the operator which defines a particular motion i . The commonly used operators are as

follows:

- translation (all or subset of motion): $\delta \mathbf{x}_i = \mathbf{I}_i$
- rotation (all or subset of motion): $\delta \mathbf{x}_i = \mathbf{R}_i(\mathbf{x} - \mathbf{x}_{cm})$
- free torsion (dihedral motion) and other torsion (improper dihedrals and double bond torsion): $\delta \mathbf{x}_i = (\partial \mathbf{x} / \partial \phi_i)|_{\text{all other motions fixed}}$
- spherical harmonic motion: $\delta \mathbf{x}_i = \mathbf{L}_i(\mathbf{x})$
- angles: $\delta \mathbf{x}_i = (\partial \mathbf{x} / \partial \theta_i)|_{\text{all other motions fixed}}$
- improper dihedral:
 $\delta \mathbf{x}_i = (\partial \mathbf{x} / \partial \omega_i)|_{\text{all other motions fixed}}$
- bonds: $\delta \mathbf{x}_i = (\partial \mathbf{x} / \partial r_i)|_{\text{all other motions fixed}}$
- other: force vectors— $\delta \mathbf{x}_i = \nabla E$; coordinates— $\delta \mathbf{x}_i = (\mathbf{x} - \mathbf{x}^0)$; velocities— $\delta \mathbf{x}_i = (\partial \mathbf{x} / \partial t)$; primary principal axis motion; ring pucker phase; ring pucker amplitude and so on.

The basis vectors can also be obtained from other sources, such as the approximate eigenvectors, an iterative procedure, a harmonic analysis of a slightly different coordinate set, or a motion of interest such as a predicted hinge mode.

Removal of Irrelevant Degrees of Freedom. The irrelevant motions are usually the net translation, net rotation, bond stretching, and angle bending. One needs to define constraints (i.e., define the vectors describing irrelevant motions). In this way one reduces the number of degrees of freedom for a particular system of interest. Let

$$\Psi_i'' = \frac{\mathbf{M}^{1/2} \delta \mathbf{x}_i}{\|\mathbf{M}^{1/2} \delta \mathbf{x}_i\|} \quad (30)$$

define the vector describing particular unwanted or constrained motion, where $\| \cdot \|$ is the norm operator. Then add each such vector to the orthonormal basis set consisting of orthonormal vectors of constrained motions by

$$\Psi_i' = \Psi_i'' - \sum_{j < i} \Psi_j(\Psi_j, \Psi_i'') \quad (31)$$

where

$$\Psi_i = \frac{\Psi_i'}{\|\Psi_i'\|} \quad (32)$$

is the desired orthonormal vector. This process of constructing orthogonalized vectors is the Gram-Schmidt method of constructing a sequence of orthogonal vectors out of any arbitrary set of vec-

tors. If $\|\Psi_i'\|$ is greater than a specified tolerance (with double precision calculations 10^{-10} is appropriate), then the vector Ψ_i describing certain irrelevant motion is added to the orthonormal basis set consisting of the constrained motion. With this procedure the linear dependencies are removed, and p vectors corresponding to the constraint motion are obtained.

Definition of Retained Motions. This step is concerned with the generation of the orthonormal basis set of unconstrained motion. This corresponds to a subspace of full Cartesian space and includes all the degrees of freedom of the motion of interest. Let

$$\Phi_i'' = \frac{\mathbf{M}^{1/2} \delta \mathbf{x}_i}{\|\mathbf{M}^{1/2} \delta \mathbf{x}_i\|} \quad (33)$$

define the vector describing the relevant motion. Apply the orthogonalization procedure

$$\Phi_i' = \Phi_i'' - \sum_{j < i} \Phi_j(\Phi_j, \Phi_i'') - \sum_{j=1}^p \Psi_j(\Psi_j, \Phi_i'') \quad (34)$$

where

$$\Phi_i = \frac{\Phi_i'}{\|\Phi_i'\|} \quad (35)$$

is the orthonormal vector. If the $\|\Phi_i'\|$ exceeds the tolerance, as in the previous case, then we add the vector Φ_i describing certain relevant motion to the orthonormal basis set consisting of unconstrained motion. This procedure removes the linear dependencies. Thus the number of included motions, m , is defined.

Definition of Other Motions. All other motions ($3N - p - m$) can be considered as added to the basis of irrelevant motions, which then becomes the constraints of motion.

Generation of the Reduced Hessian

Once the reduced basis transformation matrix for an relevant motion is generated (i.e., all the degrees of freedom of the system of interest are defined), the upper triangular, inverse mass-weighted energy second derivatives matrix \mathbf{F} has to be transformed to the reduced basis by $\mathbf{H} = \Phi^\dagger \mathbf{F} \Phi$. This can be carried out using either the direct method, which needs $(3Nm)^2$ operations, or a two-step procedure, which efficiently reduces the number of operations at most to $((3N)^2 m +$

$3Nm^2$). The two-step procedure used is as follows:
Let

$$W_{aj} = \sum_{b=1}^{3N} F_{ab} \phi_j \quad a = 1, 3N \text{ and } j = 1, m \quad (36)$$

where ϕ is the vector describing the relevant motion and F is the full inverse mass-weighted Hessian in which only nonzero elements of the full Hessian are stored. The number of operations to accomplish this step is at most $(3N)^2 m$. In the next step the reduced Hessian is generated

$$H_{ij} = \sum_{a=1}^{3N} \phi_i^* W_{aj} \quad \text{and } i, j = 1, m \quad (37)$$

with the number of operations being $3Nm^2$.

Diagonalization of the Reduced Hessian

To obtain the frequencies and normal mode vectors of the reduced motion, the eigenvalue problem for H has to be solved (i.e., the matrix H must be diagonalized). There exists a unitary matrix U such that $U^\dagger H U = (\phi U)^\dagger F (\phi U) = \Lambda$ is a diagonal matrix of dimension $(m \times m)$ and similar to F . The problem of finding the eigenvalues of the full matrix F is reduced to the eigenvalue problem for the $(m \times m)$ Hermitian matrix H . The eigenvalues of H are bounded by the eigenvalues of F , which means that the lowest eigenvalue of H is greater than the lowest eigenvalue of F , and the highest eigenvalue of H is less than the highest eigenvalue of F . It can be seen that the eigenvalue problem for F with constraints can be interpreted as an unconstrained problem for a leading principal submatrix of a matrix similar to F .

The Resulting Normal Modes

The resulting normal mode vectors are the eigenvectors which correspond to the original (full) basis eigenvectors. The approximate eigenvectors of the full basis can be computed by means of inverse vector transformation

$$y_a = \sum_{j=1}^m \phi_j U_{ja} \quad a = 1, 3N \quad (38)$$

and y 's are the resulting approximate normal modes in the full root-mass-weighted Cartesian basis.

Quasiharmonic Analysis

Quasiharmonic analysis is a method for obtaining effective modes of vibration from fluctuations calculated by an MD simulation. The underlying principle is that from atomic fluctuations, an effective force field can be calculated relative to the average dynamic structure that yields the same fluctuation matrix as that obtained from a normal mode calculation. Since the fluctuation matrix is inversely proportional to the effective force constant matrix, they have common eigenvectors which correspond to the quasiharmonic modes of vibration. They may or may not be similar to the normal modes since anharmonic effects are implicitly included. Quasiharmonic modes can be analyzed in the same way as normal modes, and comparison of the results with those from harmonic approximation calculations for the same system is straightforward. The approach will provide similar results to a normal mode calculation if the dynamics correspond to anharmonic motion in a single minimum. If multiple minima are present for the atomic motions, the quasiharmonic treatment will yield a softer effective force matrix (lower frequencies), corresponding to the increased motion.

There are two ways of performing the quasiharmonic analysis. The first is to compute the kinetic energy matrix directly from the velocities of the simulation and the fluctuation matrix when short simulations are performed. Subsequently, the generalized eigenvalue problem has to be solved (i.e., the two matrices have to be diagonalized simultaneously; method not presented). The alternative is the method presented here. This approach assumes that the kinetic energy matrix is diagonal. For long simulations, in which the approximation of equipartition of energy is good, the kinetic energy covariance matrix need not be accumulated during the simulation. This implies that only the fluctuation matrix has to be diagonalized, which allows the ordinary eigenvalue problem to be solved. For longer simulations, the approach presented here should be superior and less expensive to compute. In addition, this approach gives exactly the same atomic mean square fluctuations obtained from MD simulation as those computed from quasiharmonic modes each populated with $k_B T$ of energy. This equality of fluctuations is an advantage, because with the method presented here the correct magnitude of the fluctuation is

retained even if transitions between substates are an important component of the motion.

From an MD simulation, coordinates which define the position of all atoms as a function of time are saved. From these data, both the average position and the covariance matrix of fluctuations about the average position can be calculated. Let $\mathbf{x}(t)$ be the coordinates from MD simulation of the system at equilibrium and $\langle \mathbf{x} \rangle$ denote the average with respect to the probability distribution $P(\mathbf{x})$ according to which $\mathbf{x}(t)$ is distributed. Let $P(\mathbf{x})$ be a normalized multivariate Gaussian distribution function³⁷

$$P(\mathbf{x}) = \frac{1}{(2\pi)^{n/2}} |\boldsymbol{\sigma}|^{1/2} \times \exp \left[-\frac{1}{2} (\mathbf{x} - \langle \mathbf{x} \rangle)^T \boldsymbol{\sigma}^{-1} (\mathbf{x} - \langle \mathbf{x} \rangle) \right] \quad (39)$$

where $n = 3N$ is the number of the degrees of freedom of the system, $\langle \mathbf{x} \rangle$ is the vector of average coordinates, $|\boldsymbol{\sigma}|$ is the determinant of the matrix $\boldsymbol{\sigma}$, and

$$\sigma_{ij} = \langle (x_i - \langle x_i \rangle)(x_j - \langle x_j \rangle) \rangle \quad (40)$$

is a covariance (fluctuation) matrix with variances (time dependent) as the diagonal elements (a measure of the spread of the distribution) and with covariances as the off-diagonal elements. Since the potential determines the configurational partition function, we have

$$P(\mathbf{x}) = \frac{1}{(2\pi)^{n/2}} |\boldsymbol{\sigma}|^{1/2} \exp(-E(\mathbf{x})/k_B T) \quad (41)$$

In the quasiharmonic model³⁸

$$E(\mathbf{x}) = \frac{1}{2} \mathbf{x}^T \mathbf{F}_{\text{eff}} \mathbf{x} \quad (42)$$

where \mathbf{F}_{eff} , the effective force constant matrix, is

$$\mathbf{F}_{\text{eff}} = k_B T \boldsymbol{\sigma}^{-1} \quad (43)$$

at temperature T , k_B is the Boltzmann constant and $\boldsymbol{\sigma}^{-1}$ is the inverse of $\boldsymbol{\sigma}$. As pointed out earlier, \mathbf{F} and $\boldsymbol{\sigma}$ have common eigenvectors. Thus, it is sufficient to construct the fluctuation matrix $\boldsymbol{\sigma}$ by calculating all the elements σ_{ij} and solving the eigenvalue problem for $\boldsymbol{\sigma}$ to obtain quasiharmonic modes of vibration and the corresponding quasiharmonic frequencies, as in a standard normal mode analysis. The matrix \mathbf{F}_{eff} need not be computed.

Using the standard normal mode procedure and eq. (43), the set of simultaneous homogeneous linear equations which result from the equations of motion is

$$(k_B T \boldsymbol{\sigma}^{-1} - \lambda \mathbf{M}) \Delta \mathbf{x} = 0 \quad (44)$$

where \mathbf{M} is the kinetic energy diagonal matrix. This equation can be cast to

$$[\boldsymbol{\sigma} - (k_B T / \lambda) \mathbf{M}^{-1}] \Delta \mathbf{x} = 0 \quad (45)$$

and by matrix algebra

$$[(\mathbf{M}^{1/2} \boldsymbol{\sigma} \mathbf{M}^{1/2}) \mathbf{M}^{1/2} - (k_B T / \lambda) \mathbf{M}^{1/2}] \Delta \mathbf{x} = 0 \quad (46)$$

Substituting $\boldsymbol{\sigma}' = \mathbf{M}^{1/2} \boldsymbol{\sigma} \mathbf{M}^{1/2}$, $\lambda' = k_B T / \lambda$, and $\boldsymbol{\eta} = \mathbf{M}^{1/2} \Delta \mathbf{x}$, the system of algebraic equations can be written as

$$(\boldsymbol{\sigma}' - \lambda' \mathbf{I}) \boldsymbol{\eta} = 0 \quad (47)$$

and the corresponding characteristic (secular) equation is

$$|\boldsymbol{\sigma}' - \lambda' \mathbf{I}| = 0 \quad (48)$$

By the diagonalization of $\boldsymbol{\sigma}'$, the eigenvalues λ' result where $\lambda = k_B T / \lambda'$. The eigenvectors are the solution of the equation

$$(\mathbf{M}^{1/2} \boldsymbol{\sigma} \mathbf{M}^{1/2} - k_B T / \lambda) \boldsymbol{\eta} = 0 \quad (49)$$

Back transformation to the Cartesian displacements basis results in the corresponding quasiharmonic modes of vibration

$$\Delta \mathbf{x} = \mathbf{M}^{-1/2} \boldsymbol{\eta} \quad (50)$$

and the quasiharmonic frequencies are given by $(k_B T / \lambda')^{1/2}$.

The computational steps required for the quasiharmonic modes of vibration and the quasiharmonic frequencies can now be outlined as follows:

1. From the MD simulation, obtain the average root-mass-weighted Cartesian displacement coordinates $\langle \mathbf{x} \rangle$.
2. Generate the $\boldsymbol{\sigma}$ matrix using eq. (40).
3. Square root-mass-weight the $\boldsymbol{\sigma}$ matrix, $\boldsymbol{\sigma}' = \mathbf{M}^{1/2} \boldsymbol{\sigma} \mathbf{M}^{1/2}$.
4. Diagonalize the $\boldsymbol{\sigma}'$ matrix to obtain the eigenvalues λ' and the eigenvectors $\boldsymbol{\eta}$.

5. Calculate the quasiharmonic frequencies $\omega = (k_B T / \lambda')^{1/2}$.
6. Calculate the quasiharmonic modes of vibration $\Delta \mathbf{x} = \mathbf{M}^{-1/2} \boldsymbol{\eta}$.

Reduced Basis Quasiharmonic Analysis

Reduced basis quasiharmonic analysis represents a way of studying modes of vibration from dynamic fluctuations for problems of a large size. Various degrees of freedom (e.g., bond stretching) are removed from the full basis in order to reduce the matrix size for the computation. The method presented here is similar to reduced basis normal mode analysis described in detail in the previous section, with the important difference that in the reduced basis quasiharmonic calculation there is no Hessian; instead there is a fluctuation (covariance) matrix. The method is analytical (i.e., the reduced covariance matrix is obtained by direct transformation of the fluctuation matrix). The method is capable of treating any type of motion of the system. Linear dependencies are easily removed using the Gram-Schmidt orthogonalization procedure. A back transformation of the reduced basis quasiharmonic analysis results into the full basis enables easy comparison with quasiharmonic or normal modes of vibration. The reduction of the covariance matrix is performed using the two-step procedure in which the total number of operations is $((3N)^2 m + 3Nm^2)$.

The procedure for quasiharmonic reduced basis analysis is as follows:

1. From an MD simulation the average coordinates $\langle \mathbf{x} \rangle$ are calculated.
2. Generate the fluctuation matrix $\boldsymbol{\sigma}$ in the full basis using eq. (40).
3. Square root-mass-weight the $\boldsymbol{\sigma}$ matrix to obtain $\boldsymbol{\sigma}' = \mathbf{M}^{1/2} \boldsymbol{\sigma} \mathbf{M}^{1/2}$.
4. Generate the reduced basis transformation matrices. This procedure is identical to that presented for reduced basis normal mode.
5. Generate the reduced basis covariance matrix $\boldsymbol{\Theta} = \boldsymbol{\Phi}^\dagger \boldsymbol{\sigma}' \boldsymbol{\Phi}$. This is carried out in the two-step procedure which is as follows. Let

$$Y_{aj} = \sum_{b=1}^{3N} \sigma'_{ab} \phi_j \quad a = 1, 3N \text{ and } j = 1, m \quad (51)$$

where $\boldsymbol{\sigma}'$ is the root-mass-weighted fluctuation matrix in full basis. Then

$$\Theta_{ij} = \sum_{a=1}^{3N} \phi_i^\dagger Y_{aj} \quad i, j = 1, m \quad (52)$$

is the fluctuation matrix in the reduced basis (i.e., the reduced basis fluctuation matrix).

6. To obtain the quasiharmonic frequencies and the quasiharmonic modes of vibration of the reduced motion, the reduced fluctuation matrix has to be diagonalized. There exists a unitary matrix \mathbf{U} such that $\mathbf{U}^\dagger \boldsymbol{\Theta} \mathbf{U} = (\boldsymbol{\Phi} \mathbf{U})^\dagger \boldsymbol{\sigma}' (\boldsymbol{\Phi} \mathbf{U}) = \boldsymbol{\Lambda}$ is a diagonal matrix of dimension $(m \times m)$ and similar to $\boldsymbol{\sigma}'$. The diagonal elements of $\boldsymbol{\Lambda}$ provide the approximate λ' values.
7. The resulting reduced basis quasiharmonic eigenvectors approximate eigenvectors of the original basis, can be computed by means of the inverse vector transformation, and are

$$\eta_a = \sum_{j=1}^m \phi_j U_{ja} \quad a = 1, 3N \quad (53)$$

8. The last step of the procedure requires the calculation of the quasiharmonic frequencies by

$$\omega = (k_B T / \lambda')^{1/2} \quad (54)$$

Analysis of Normal Modes and Frequencies

Once the normal modes have been obtained, a great variety of analyses can be performed. The analysis tool and methods are included in the CHARMM²⁸ vibrational analysis facility (VIBRAN). Examples of some of the techniques are presented in the following articles.^{6,7}

NORMAL MODE PROPERTIES

The easiest properties to examine relate directly to the eigenvectors and eigenvalues. These include the frequency; the percent of translation-rotation content, $100 \sum_{j=1}^6 (\mathbf{y}_i^\dagger \cdot \boldsymbol{\Phi}_j)^2$ (where $\boldsymbol{\Phi}_j$ is the subbase for translation-rotation motion; see Reduced Basis Normal Modes Procedure); dot products between normal vectors for different calculations (dot products for single calculation reproduce the identity matrix); and others.

If a vacuum structure is not at a stationary point (minimum, maximum, or saddle), then the rotational motion mixes with the internal degrees of freedom. This mixing can be avoided by extensive minimization or by the removal of the rotational degrees of freedom from the calculation (see Iterative Diagonalization Method).

Imaginary frequencies may be obtained when the square root-mass-weighted Hessian has negative eigenvalues. For structures at a stationary point, this indicates the presence of a saddle point (or maximum) and that one or more degrees of freedom have an energy at a maximum value. The order of the saddle point is equal to the number of negative eigenvalues. For structures not at a stationary point, this indicates that some degrees of freedom have a negative curvature. The imaginary frequencies were present in previous work⁴ because the structure was only partially minimized due to the use of harmonic restraints designed to keep the structure close to the X-ray coordinates. It was felt at that time that a structure near the X-ray coordinates was more important than being at a stationary point. When using frequencies to estimate thermodynamics, the imaginary frequencies may be ignored, especially if they are local in character (see Local versus Global Characteristics, later in this article), or the frequencies may be adjusted based on the best fit of a parabola through the energy data points (see Frequency Adjustment, later in this article) generated from an exploration of a particular mode. In the Results presented in the following articles,^{6,7} all minimizations are exhaustive and there are no negative eigenvalues.

CARTESIAN DERIVATIVES AND DISPLACEMENTS

The Cartesian displacement is given by $\delta \mathbf{x} = \alpha_i \mathbf{M}^{-1/2} \mathbf{y}_i$, where α_i , the amplitude, can be determined in several ways:

- $\alpha_i = \text{constant}$: simple step for all modes
- $\alpha_i = L(\text{tr}(\mathbf{M}))^{1/2}$: where L is the desired mass-weighted rms deformation defined by $\delta \mathbf{x}$
- $\alpha_i = (2E/\lambda)^{1/2}$: where E is the desired vibrational energy assigned on a per normal mode basis (assuming classical oscillators)
- $\alpha_i = (2k_B T/\lambda)^{1/2}$: where T is the desired absolute temperature applied on a per normal mode basis

- $\alpha_i = L(N^{1/2}(\mathbf{y}^\dagger \mathbf{M}^{-1} \mathbf{y})^{-1/2})$: where L is the desired root mean square (rms) deformation defined by $\delta \mathbf{x}$.

The choice of method used to generate α_i factors depends on what subsequent analysis is intended for the $\delta \mathbf{x}$ vectors. The step length involving energy or temperature should be used if relative properties, such as fluctuation or correlation functions, are to be analyzed. This approach may utilize a low-frequency cutoff tolerance so that negative λ values are ignored, and very large step sizes are not used for translational and rotational degrees of freedom. From a knowledge of the appropriately weighted Cartesian derivatives, $\delta \mathbf{x}$, other terms, such as the energy derivatives $\nabla E \cdot \delta \mathbf{x}$, the dipole derivative $(\partial \boldsymbol{\mu} / \partial \mathbf{x}) \delta \mathbf{x}$, and normal mode displacement vectors $\Delta \mathbf{x} = \delta \mathbf{x}$, can easily be determined. The $\delta \mathbf{x}_i$ vectors span neither a normal nor an orthogonal space.

There are several other possible expressions for α_i , such as those which reproduce the displacement that includes quantum effects. The quantum scale factor is given by $[(q/2)(e^q + 1)/(e^q - 1)]^{1/2}$, where $q = \hbar \omega / k_B T$.

INTERNAL COORDINATE (DIHEDRAL) DERIVATIVES

These are used to determine which dihedral angles contribute to a particular mode of vibration. Internal coordinate derivatives may be determined by $\delta \Phi_a = \sum_i B_{ai} \delta \mathbf{x}_i$; where \mathbf{B} is Wilson's \mathbf{B} matrix,⁵ which defines the transformation from Cartesian to internal coordinates, and

$$B_{ai} = \left(\frac{\partial \Phi_a}{\partial x_i} \right)_{\text{all other } x_{j \neq i} \text{ fixed}}$$

$$a = 1, \dots, m, \text{ and } i = 1, \dots, 3N \quad (55)$$

are the constants determined by the geometry of the molecule.

LOCAL VERSUS GLOBAL CHARACTERISTICS

Normal modes of vibration can involve all the atoms of the system, as in the case of low-frequency global deformation motions, or be localized in one particular part of the macromolecule. It is useful to have indicators which define the degree of global character or local character for particular modes of vibration. Since the eigenvectors form an orthonormal basis $\sum_{j=1}^N y_{ji}^2 = 1$, a local character indicator is

given by

$$\sum_{j=1}^{3N} y_{ji}^4 \quad (56)$$

which is large for modes (i) with significant local character. The global character indicator is given by

$$\left(\frac{\sum_{j=1}^{3N} |y_{ji}|}{(3N)^{1/2}} \right)^{-4} \quad (57)$$

which is large for modes without significant global character. These indicators are not invariant with the orientation of the system and are only qualitative indicators of character because only the sum of the squares of the components of the vector for each atom is invariant to rotation. If the motion is dominated by a single component of a single atom, then y_i will be close to unity for that element and zero for all others. This results in the maximum possible value for the local indicator of 1 and a maximum for the lack of global character indicator of $9N^2$. The other extreme is represented by a net translation of all atoms in the (1,1,1) direction, where all elements are the same with a value of $(3N)^{-1/2}$. In this case, the local indicator has a minimum value of $(3N)^{-1}$ and the lack of global indicator will have a minimum value of 1.

Modes with significant mixing of global and local character may have a large local indicator and a small lack of global character indicator; thus two indicators are needed to evaluate the character of the motion.

PROJECTION OF NORMAL MODES ONTO INTERNAL COORDINATES OR OTHER MOTION OF INTEREST

To determine which mode contributes to a motion of interest, such as a particular deformation or angle deformation, the normalized vector

$$\mathbf{w} = \left\| \mathbf{M}^{1/2} \left(\frac{\partial \mathbf{x}}{\partial \phi} \right)_{\text{all other fixed}} \right\| \quad (58)$$

is generated and projected on the normal mode basis, to determine the overlap $\mathbf{w}^\dagger \mathbf{y}_i$ (see Comparison of Normal Modes, later in this article).

PROJECTION OF COORDINATE DIFFERENCES ONTO NORMAL MODES

To determine which modes of vibration contribute to a particular coordinate difference, the

normalized vector is given by

$$\mathbf{w} = \|\mathbf{M}^{1/2}(\mathbf{x} - \mathbf{x}_{\text{ref}})\| \quad (59)$$

and the overlap is again computed as $\mathbf{w}^\dagger \mathbf{y}_i$ (see Comparison of Normal Modes).

ACCUMULATION OF STATISTICS FOR GROUPS OF ATOMS

Analysis of atomic group behavior can be performed by a detailed analysis of the $\delta \mathbf{x}$ vector for a particular normal mode of vibration. A set of six normalized ϕ vectors can be generated (see Reduced Basis Normal Mode Analysis) corresponding to the rotation and translation of the subset of atoms of interest and can then be projected onto the full normal mode basis.

ENERGY SURFACE EXPLORATION

Surface exploration involves computation of the energy (or other properties) for a series of structures generated by systematically deforming the reference structure along one or more modes of interest (usually those lowest in frequency). As an energy analysis, this is often done to obtain anharmonic correction to the frequencies or to obtain details regarding the energetics of the motions of the interest. For this analysis the actual Cartesian displacement may be a simple step, $\Delta \mathbf{x} = \delta \mathbf{x}$; a constraint step, $\Delta \mathbf{x} = \text{SHAKE}(\delta \mathbf{x}, \mathbf{x}_{\text{ref}})$, where stretching is eliminated; or an internal coordinate deformation, $\Delta \mathbf{x} = \mathbf{x}_{\text{pert}} - \mathbf{x}_{\text{ref}}$, where \mathbf{x}_{pert} is the best fit mass-weighted rms fit structure which best satisfies the internal coordinate geometry given by $\Theta_{\text{pert}} = \Theta_{\text{ref}} + (\partial \Theta / \partial \mathbf{x})_{\text{ref}} \delta \mathbf{x}$ using a nonlinear least squares procedure.

The Cartesian displacement is best used when examining global motion or motion involving van der Waals interactions. The internal coordinate deformation step is best used when analyzing internal degrees of freedom, because this avoids the linearization of motion as in a simple Cartesian displacement. For example, large-amplitude angle bending is nonlinear in that the bond length does not deform as is required with the linear approximation.

FREQUENCY ADJUSTMENT

Frequency adjustment involves modification of the predicted frequency based on an exploration of the energy surface. By searching regions of phase

space near the coordinate used in the calculation, some anharmonic effects can be accounted for. One reason for frequency adjustment is to modify the frequency of the negative modes (those with imaginary eigenvalues) so that they fall in the real realm (see Accumulation of Statistics for Groups of Atoms). Another reason is to incorporate the anharmonic effects of some rather high-frequency motions that are disguised as low-frequency modes due to the nonharmonic character of the energy surface. Also, the anharmonic character of lowest frequency modes may be of interest.² The steps of the frequency adjustment are as follows:

1. Construct a set of coordinates corresponding to a linear projection of a normal mode in Cartesian space.
2. Optimally adjust these coordinate sets using SHAKE³⁹ so that there is no change in any bond length.
3. Compute the energy for each coordinate set.
4. Fit a parabola through the energy values using a least squares fitting procedure and a Boltzmann weighting factor for each data point.
5. From the curvature of the parabola, reassign the frequency.

The first step is straightforward, but since a linear interpolation of a rotation tends to stretch bonds and introduce other distortions, a further correction is needed. Since the bond stretching frequencies are high, their involvement in any low-frequency mode is expected to be small and can be neglected.⁴⁰ For this reason, the bond deformations are usually removed using SHAKE. The effect of this correction is to move some of the distortion into bond angle deformation, but this effect is expected to be almost constant for most low-frequency modes. Once energy values have been obtained, a least squares parabola fitting procedure is used. The reason that a Boltzmann weighting factor is introduced is that regions of the energy surface that make only a small contribution are given little or no weight.

There are some drawbacks to this simple approach to frequency adjustment. The most serious is the artificial angle deformations that are introduced as a result of SHAKE. For every case (including the "modes" involved with rotating the molecule), there is an excessive increase of the frequency. However, every mode seems to be nearly equally affected, and thus a small constant

shift in all the adjusted eigenvalues may be all that is necessary. A second deficiency is that no anharmonic mixing of modes is considered. Such anharmonic mixing would be present in a dynamic simulation, but to incorporate these terms a multi-dimensional search of the energy surface would be required.

ATOMIC FLUCTUATIONS

The atomic fluctuations from a harmonic analysis in the classical limit are given by

$$\langle \Delta x_k^2 \rangle = k_B T \sum_{i=1}^{3N-6} \frac{|y_{ki}|^2}{M_k \omega_i^2} \quad (60)$$

where i spans the modes of interest and k corresponds to the component of motion of an atom in the x , y , or z direction. Treating the normal mode as a collection of quantum mechanical oscillators, the expression for the atomic fluctuations is given by

$$\langle \Delta x_k^2 \rangle = \frac{\hbar}{2} \sum_{i=1}^{3N-6} \frac{|y_{ki}|^2}{M_k \omega_i} \coth \left(\frac{\hbar \omega_i}{2k_B T} \right) \quad (61)$$

where $\hbar = h/2\pi$ and h is Planck's constant.

INTERNAL COORDINATE FLUCTUATIONS

The internal coordinate fluctuations are given by

$$\langle \Delta \phi_j^2 \rangle = \sum_{i=1}^{3N-6} \frac{k_B T}{\omega_i^2} \sum_{k=1}^{3N} |B_{jk} M_k^{-1/2} y_{ki}|^2 \quad (62)$$

in the classical limit, where \mathbf{B} is Wilson's \mathbf{B} matrix.⁵ The corresponding quantum mechanical result for the internal coordinate fluctuations is given by

$$\langle \Delta \phi_j^2 \rangle = \sum_{i=1}^{3N-6} \frac{\hbar}{2 \omega_i} \coth \left(\frac{\hbar \omega_i}{2k_B T} \right) \sum_{k=1}^{3N} |B_{jk} M_k^{-1/2} y_{ki}|^2 \quad (63)$$

CORRELATION OF ATOMIC MOTION

The correlation of atomic motions from a normal mode analysis is given by

$$\langle \delta x_k \delta x_j \rangle = \sum_{i=1}^{3N-6} \frac{k_B T}{\omega_i^2} (M_j^{-1/2} M_k^{-1/2} y_{ki} y_{ji}) \quad (64)$$

For internal coordinates or other types of motion, the correlation of motions is given by

$$\langle \phi_k \phi_j \rangle = \sum_{i=1}^{3N-6} \frac{k_B T}{\omega_i^2} (\mathbf{y}_i^\dagger \mathbf{M}^{-1/2} \mathbf{B}_k^\dagger \mathbf{B}_j \mathbf{M}^{-1/2} \mathbf{y}_i) \quad (65)$$

These correlation values are often normalized by the geometric mean of the fluctuation values for the individual degrees of freedom (e.g., ϕ_k and ϕ_j).

PROJECTION OF MOLECULAR DYNAMICS TRAJECTORIES ONTO NORMAL MODES

It is useful to be able to compare normal modes of vibration (or other type of motion) with the MD simulation. This allows examination of the amplitudes of this type of motion and the time scales. This is a straightforward procedure in which the effects of solvent damping may be explored for the case in which the MD simulation is carried out with explicit solvent even if the normal mode calculation is carried out in vacuum. The procedure is to generate a time series of the projection of the difference of trajectory position and the average position into a mode of interest given by

$$A_a(t) = \sum_{i=1}^{3N} y_{ai} M_i^{1/2} (x_i(t) - \langle x_i \rangle) \quad (66)$$

This time series can be evaluated with standard procedures to determine correlation functions and spectra. It is also useful in analyzing the results of a quasiharmonic analysis to determine which modes are predominantly vibrational in character and which arise mainly from jumping between energy substates. The results of this analysis are reported in the second following article.⁷

DISPLAYING NORMAL MODE MOVIES

One of the best ways to understand a normal mode of vibration is through visual examination. To do this, a "trajectory" must be created which depicts the molecular system as a function of time from a given starting configuration. For the Cartesian basis, this is given by

$$\mathbf{x}(t) = \langle \mathbf{x} \rangle + \sum_{\text{modes to view}} \alpha_i \mathbf{M}^{-1/2} \mathbf{y}_i \cos(\omega_i t + \delta_i) \quad (67)$$

where α_i is the desired amplitude of the motion (see Cartesian Derivatives and Displacements) and

δ_i is an arbitrary phase used to determine the initial state for each mode. It is also possible to generate the trajectory file in the internal coordinate basis.²⁴

POTENTIAL ENERGY DISTRIBUTION

The potential energy distribution procedure⁴¹ is used to identify which internal coordinates participate in the energetics of a particular normal mode of vibration. The energy associated with a particular internal coordinate is given by

$$E_{ji} = \frac{k_B T}{\omega_i^2} (\mathbf{y}_i^\dagger \mathbf{M}^{-1/2} \mathbf{B}_j^\dagger) \left(\frac{\partial^2 E}{\partial \phi_j^2} \right) (\mathbf{B}_j \mathbf{M}^{-1/2} \mathbf{y}_i) \quad (68)$$

where j refers to a particular internal coordinate and i to a particular normal mode of vibration. A list of internal coordinates with large E_{ji} values can be obtained as well as overall statistics regarding what percent of the energy is due to bond stretching, bond bending, and dihedral and improper torsion motion.

ENERGETIC DISTRIBUTION OF MOTION

One of the most important aspects of harmonic analysis is the ability to analyze in detail the energetics of the motion of a particular normal mode of vibration. This is done by formally partitioning the energy of a macromolecular system into atomic contributions, such that

$$E(\mathbf{x}) = \sum_{i=1}^n \varepsilon_i(\mathbf{x}) \quad (69)$$

where ε_i is the portion of the total energy attributed to atom i . This is accomplished by dividing each energy contribution evenly among at the atoms contributing to the terms. The energetic derivative for a given atom i and a given mode of vibration j is given by

$$\delta \varepsilon_{ij} = \nabla \varepsilon_i^\dagger (\alpha_j \mathbf{M}^{-1/2} \mathbf{y}_j) \quad (70)$$

Because of the complexity in the computational storage of $\nabla \varepsilon_i$, this calculation is usually performed by finite differences, where

$$\delta \varepsilon_{ij} = \frac{\varepsilon_i(\mathbf{x} + \delta \mathbf{x}_j) - \varepsilon_i(\mathbf{x} - \delta \mathbf{x}_j)}{2} \quad (71)$$

and $\delta \mathbf{x}_j$ is given by $(\alpha_j \mathbf{M}^{-1/2} \mathbf{y}_j)$.

The $\delta \varepsilon$ values may be accumulated and analyzed in a number of ways. The example of the use

of this method was presented in Figure 5 of Brooks and Karplus,²⁵ in which the energetics of the hinge motion of hen egg white lysozyme were determined on a residue-by-residue basis. It was seen that two residues dominate the closing motion of the active site cleft and several dominate in the opening motion. The largest contribution comes from the conserved Trp 28. This analysis suggested that this tryptophan play a dominant role in keeping the active site cleft in lysozyme open and that its mutation would result in a structure that would open with greater difficulty.

STRESS POINT ANALYSIS

The term *stress point analysis* is used to describe a systematic analysis of the energetics of a collection of important modes of vibration and other interesting motions. This analysis is intended to provide a practical tool for predicting the structural effects of some types of protein mutations, such as those which affect thermostability. This analysis allows potentially interesting residues to be identified and free-energy differences to be approximated at far less cost and complexity than with direct simulation techniques. This method is analogous to the finite element analysis technique commonly used in engineering. Examples of this analysis and further details of the method will be presented in a subsequent article.

VIBRATIONAL CONTRIBUTION TO THERMODYNAMIC PROPERTIES

Assuming that the normal modes at a macromolecular system may be treated as a collection of quantum mechanical oscillators, the vibrational contributions to thermodynamic properties may be easily determined from the frequencies.⁴² These include the classical free energy, the frequency, the energy, the entropy, the heat capacity, and the zero point correction, given by

$$A_{\text{classical}} = k_B T \ln(U) \quad (72)$$

$$A_{\text{vib}} = -k_B T \ln(Q) \quad (73)$$

$$E_{\text{vib}} = k_B T \frac{U}{e^U - 1} \quad (74)$$

$$S_{\text{vib}} = \frac{E_{\text{vib}} - A_{\text{vib}}}{T} \quad (75)$$

$$C_{V_{\text{vib}}} = k_B U^2 \frac{e^U}{(e^U - 1)^2} \quad (76)$$

where Q , the partition function, is given by

$$Q = (1 - e^{-U})^{-1} \quad (77)$$

and U is given by

$$U = \frac{h \omega_i c}{k_B T} \quad (78)$$

where h is Planck's constant, c is the speed of light, and ω_i is the frequency in cm^{-1} . Some of these quantities are sensitive to the frequencies of the lowest few modes of vibration. Thus approximations which modify these frequencies should not be used when analyzing thermodynamic properties. This includes the reduced basis normal mode procedure when only torsional degrees of freedom are included and used with an energy potential not specifically designed for torsional basis. This problem also is present when long-range energy cutoffs, such as a switching function, that significantly alter the lowest frequencies are used (see the Results section in the following articles^{6,7}).

DETERMINATION OF NEUTRON SCATTERING SPECTRA

Time of flight neutron scattering spectra have been obtained⁴³⁻⁴⁵ using normal mode analysis and have been extensively studied.

COMPARISON OF NORMAL MODES

One procedure for comparing normal modes obtained from different simulations is by computing an inner product of the two modes. If the structures are not identical, the structures must be rms best fit to minimize the atom position differences and to align the centers of mass. The normal mode vectors must be rotated during the best fit procedure. When the number of atoms is different, the homology must be specified and the comparison becomes more difficult depending on the nature of the mode of interest and on the type of homology being studied. In this article a simple overlap scheme has been developed to determine the similarity between the lowest five modes of two different normal mode calculations given by

$$\text{Overlap } IJ = \frac{1}{6} \sum_{i=1}^5 \sum_{j=1}^3 (\mathbf{y}_{iI}^\dagger \mathbf{y}_{jI} + \mathbf{y}_{jI}^\dagger \mathbf{y}_{iI}) \quad (79)$$

where \mathbf{y}_{iI} is the i th eigenvector of calculation I . The overlap will be 1.0 if the two sets of modes are

identical and 0.0 if they are orthogonal. Although the first five modes contribute to this expression, the first three modes dominate in the overlap. This scheme was developed mainly to examine the lowest three modes of vibration. However, since eigenvector ordering can change between calculations, including the five lowest modes in the cross comparison gives a result that is less subject to large changes in the frequency spectrum. Without a two-tier approach, there is a lot of random noise in the overlap results. This overlap value is used for low-frequency eigenvectors comparison.^{6,7}

When comparing modes using different coordinate sets, the coordinate sets are best fit using mass weighting so that net rotation is removed from the comparison. When comparing results in which the number of atoms differs, such as in comparison of the polar hydrogen atoms calculation with the all-atom calculation, the dimensionality of the larger set is reduced by averaging the motion of the hydrogen atoms with the associated heavy atom, with the appropriate mass weighting, to preserve the net motion of the cluster of atoms while removing any internal motion. The modes with internal motion removed are reorthonormalized before computing overlap values.

Conclusions

The present work provides a variety of methods for the normal mode calculation of large systems, such as biological macromolecules, when all degrees of freedom are taken into account (i.e., for full conformational space) as well as for reduced conformational space (i.e., reduced basis normal mode calculation), quasiharmonic analysis, and reduced basis quasiharmonic analysis. If the same macromolecular system employing the same potential function is used in all of the calculations, then a direct comparison of all the methods is possible.^{6,7} Through the combination of methods presented, insight can be gained into the dynamics and flexibility of large molecular systems.

Because of the large size of the systems, normal mode analysis of macromolecules performed in full conformational space often requires development of special diagonalization techniques to calculate the vibrational frequencies and normal modes. The diagonalization technique described here can be applied to any large matrix to obtain the eigenvalues and eigenvectors. If a subset of normal modes in full conformational space is of

interest, an iterative diagonalization method may apply. When the harmonic analysis of the reduced conformational space is suitable for study, the reduced basis normal mode calculation is the method of choice. Quasiharmonic analysis, an alternative to harmonic analysis, can incorporate some effects due to the anharmonic nature of macromolecules. Once determined, normal modes and quasiharmonic modes can be analyzed using a variety of techniques.

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