Published in final edited form as:

Curr Protein Pept Sci. 2004 April ; 5(2): 119–123.

New Advances in Normal Mode Analysis of Supramolecular Complexes and Applications to Structural Refinement

Jianpeng Ma^{1,2,3,*}

¹Graduate Program of Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

²Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

³Department of Bioengineering, Rice University, Houston, TX 77005, USA

Abstract

Normal mode analysis is an effective computational method for studying large-amplitude low-frequency molecular deformations that are ubiquitously involved in the functions of biological macromolecules, especially supramolecular complexes. The recent years have witnessed a substantial advance in methodology development in the field. This review is intended to summarize some of the important advances that enable one to simulate deformations of supramolecular complexes at expended resolution- and length-scales, with particular emphasis on the implications in structural refinement against low- to intermediate-resolution structural data such as those from electron cryomicroscopy and fibre diffraction.

Keywords

Harmonic approximation; Normal mode analysis; Large-scale conformational change; Structural refinement

Introduction

Biomolecules carry out their functions in a dynamic manner. A substantial part of biomolecular functions involves structural motions in a wide range of length scales [1,2], *e.g.*, from vibrations of chemical bonds to global conformational changes of supramolecular complexes. Meanwhile, the available structural data exist in varying resolution scales, *e.g.*, from the high-resolution atomic coordinates provided by x-ray crystallography to low- to intermediate-resolution electron density maps from, for example, electron cryomicroscopy (cryo-EM). Therefore, computational methods of expanded capacity are needed in order to efficiently study biomolecular functions.

Traditionally, an effective way of analyzing molecular motions is modal analyses, which belong to the general category of harmonic approximation. The most commonly used ones are normal mode analysis (NMA) [3], quasi-harmonic analysis [4] and its related version of essential dynamics [5]. Mathematically, all these modal analyses are eigenvalue problems, and for the types of motions where harmonic approximation is appropriate, the methods provide a

*Address correspondence to this author at the One Baylor Plaza, BCM-125, Baylor College of Medicine, Houston, TX 77030, USA; Email: E-mail: jpma@bcm.tmc.edu.

complete basis set, by which any arbitrary molecular deformational motions can be readily expressed as a linear combination. Modal analyses are particularly useful when elastic (harmonic) properties of molecules are concerned. From more than two decades of computational studies [1,2], it is well-established that large-scale elastic deformational motions of biomolecules can be well approximated by low-frequency vibrations of the structures. Therefore, in practice, it is feasible to study biomolecular functions by filtering out the less important high-frequency motions and focusing on those dominating low-frequency components.

In recent years, there has been a significant advance in methodology development of NMA, which provides a substantially broadened toolkit for analyzing biomolecular dynamics. In this review, we briefly summarize some of those new methods that were designed to study motions at multi-length and -resolution scales, with emphasis on their important applications to assisting structural refinement in experimental structural determinations. We will also discuss several important issues in interpreting the results of harmonic modal analysis. This paper is intended to be a concise review of a focused scope and we apologize to those colleagues whose work is not explicitly referred to.

Basic Principles of Normal Mode Analysis

In a standard NMA [1,3,6], the potential surface of a given molecular structure is assumed to be quadratic in the vicinity of a well-defined energy minimum and the motions of the molecule are decomposed into a set of independent harmonic vibrational modes, *i.e.*, normal modes. These normal modes are often regarded as molecular deformational modes, by which the overall molecular motion can then be described as a linear combination. For a system that contains N atoms, the directionality of each mode is provided by a $3N$ -dimensional eigenvector obtained by diagonalizing the second derivative matrix, $\mathbf{H}$, of the total potential function with a matrix transformation, $\mathbf{H} = \mathbf{U}\mathbf{\Lambda}\mathbf{U}^{-1}$, where $\mathbf{U}$ is an orthogonal matrix whose columns represent the eigenvectors of $\mathbf{H}$ and $\mathbf{\Lambda}$ is a diagonal matrix that contains the eigenvalues of $\mathbf{H}$, or force constants of the normal modes. Totally, there are $3N-6$ intrinsic modes. From the normal modes, various dynamic properties of the molecular structure, such as the correlations between atomic fluctuations, can be calculated.

Numerous studies have shown that only a small set of low-frequency modes makes dominant contributions to biologically important large-amplitude concerted motions. A typical example is the study of the molecular chaperonin GroEL [7], an ATP-driven supermolecular motor complex, in which the nature of the *en bloc* domain movements important for its functions was investigated by NMA. Other examples include studies of lysozyme [6,8], myoglobin [9,10], oncogenic protein ras p21 [11], and aspartate transcarbamylase [12-14]. A very important lesson learned from these studies is that many proteins have evolved to utilize their intrinsic structural flexibility, as manifested in normal modes, to facilitate the conformational changes required for functions.

Elastic Normal Mode Analysis

In recent years, a different type of NMA, the elastic network model based on C α positions, was introduced to study protein dynamics. There are two classes of elastic network model, Gaussian network model (GNM) [15,16] and anisotropic network model (ANM) [17,18]. GNM is used to estimate the amplitudes of isotropic thermal motions and was demonstrated to be able to reproduce the experimentally measured *B*-factor curves of crystal structures quite accurately. ANM is essentially a standard NMA [3] and was capable of describing the amplitudes and directionality of anisotropic motions. The method is based on a simplified pairwise harmonic potential function [18] described by a single phenomenological force constant γ that can be set

to be the same for all pairs. The absolute value of γ only affects the amplitudes, but not the directionality, of motions. A distinct advantage of ANM is that it treats the initial coordinates as the equilibrium coordinates, therefore it does not require energy minimization that could significantly distort structures as in all-atom based NMA [3]. Despite the drastically simplified potential function, studies have shown that ANM can accurately describe the directionality of low-frequency motions of protein structures [17,19-23]. The latest examples include studies of icosahedral virus shell [24] and 70S complex of ribosome [25].

However, ANM has an inherent weakness, which we call “tip-effects”. In molecules that have certain structural components, the “tips”, sticking out of the main body, *e.g.*, an isolated surface loop, “tip-effects” could lead to pathological behaviors in the modes, the “ghost modes”. In those modes, the magnitudes of motions of the atoms on the tips are much larger than those of the rest of the system. Because the eigenvectors of normal modes are normalized, the abnormally large displacements of the “tips” suck in most of the displacements of the “ghost modes” and make the rest of the system essentially static. Although in a case-by-case situation, one might be able to ease such “tip-effects” by adjusting, for example, the number of points, or the cut-off distance used in the analysis, there has been no effective way to systematically eliminate the “tip-effects”.

Normal Mode Analysis Based on Low-Resolution Density Maps

Very recently, one of the most important advances in NMA is the development of quantized elastic deformational model (QEDM), which was developed by us [26] and by others as well [27]. Its success allows one to calculate normal modes based on low-resolution (as low as 20 ~ 30 Å) density maps without the knowledge of atomic coordinates and amino-acid sequence.

The operation of QEDM relies on an important fact in biomolecular dynamics that the natures of the low-frequency motions of a biomolecule are not sensitive to the detailed structural connectivity, rather they are determined by the overall shape of the molecule, *e.g.*, the mass distribution as depicted by the contours of the low-resolution density maps. The method is performed in such a way that the concept of elastic NMA is applied to a set of points that are chosen to discretize the density maps. Those points can be the grid points used for storing the density maps, or be obtained by vector quantization method [28-30]. The number of points used in QEDM analysis can be chosen at will as long as it is large enough to effectively represent the overall shape of the molecule. This feature tremendously facilitates the calculation of modes for extremely large systems, in which cases a number of points much smaller than that of C α atoms are enough to extract the overall features of low-frequency motions.

It is fair to say that QEDM opens a new horizon for NMA and dramatically extends one's capability of modeling molecular motions of supermolecular complexes to an unprecedented level. For many large systems, the method offers a way to reliably compute the global conformational motions at almost any experimentally accessible resolutions. Since the introduction of the method [26,27], there have been many applications of QEDM on very large molecular complexes [31-34]. Many more applications are expected to come in the near future.

QEDM-Assisted cryo-EM Structural Refinement

Among all the applications of QEDM, a noticeable example is the one on human fatty acid synthase (FAS) [31], which is a huge enzymatic complex with extraordinarily large structural flexibility. For FAS, the application of QEDM to the 19-Å cryo-EM density maps [35] clearly revealed the likely deformational modes of the structure. Far more importantly, for the first time the QEDM-predicted conformers were confirmed in experimental observation [36]. This was achieved in a spirit somewhat similar to multiple-copy x-ray crystallographic refinement

[37] in which a simultaneous multi-reference refinement was performed to search for the presence of structural variations in the cryo-EM images of FAS using QEDM-predicted conformers as initial models [36]. The converged models obtained from such a refinement procedure were evidently in accord with the QEDM-predicted conformers.

A common, yet natural, concern in the single-particle analysis is the potential model bias [38]. To examine the authenticity of the observed structural variations, the authors performed several robust tests [36]. One of the tests was to perform multi-reference refinement in which, instead of using QEDM-predicted conformers as initial models, several nearly identical and featureless models derived from a single averaged structure seeded with low-levels of noise were used. The refinement indeed reproduced structural variations very similar to QEDM-predicted conformers, but took, much longer time to converge.

Like in x-ray crystallography and many other structural determination methods, the conformational heterogeneity of biomolecules imposes severe limitations on structural determination to higher resolution by single-particle cryo-EM. Although currently this problem is only manifested in extraordinarily flexible systems such as FAS, given the dynamic nature of biological systems, it is expected to become a common issue in many systems once the resolutions of cryo-EM are high enough. The success of the initial study of QEDM-assisted refinement procedure [36] demonstrates the potential of improving the resolution of the final reconstruction in single-particle cryo-EM by dividing the particle images into more homogeneous particle subsets in terms of molecular conformations. And, QEDM-predicted conformers provide reasonable initial models to start the refinement, thereby significantly reducing the computational expense needed for multi-reference refinement, especially for extremely large systems and/or at high resolutions [36]. Thus, QEDM-assisted cryo-EM structural refinement provides a practical venue for effectively dealing with structural flexibility and obtaining better structures.

Certainly, there is still a long way to go before QEDM-assisted cryo-EM refinement can be integrated into routine structural determination procedures. A number of technical challenges need to be dealt with. For example, as the raw particle images are subgrouped in terms of different molecular conformational states, it naturally happens that fewer particles are available for each subgroup, which itself limits the ability to improve the resolutions. Therefore, a larger set of particle images is needed. Moreover, the optimal ways of combining different modes remain unsolved. Though in a case-by-case situation, the modal combination issue may be circumvented, in general, this is a challenging problem as combinations of even a small set of modes can quickly lead to a very large number of conformers for the program to search. When there are only a limited number of particle images, over-inflation of initial searching conformers could impose severe problems. Finally, the amplitudes of modes in generating the initial searching conformers need to be determined. Theoretically, the molecule can have a continuous distribution of deformational amplitude even along a single normal mode. Picking a few extreme snapshots along the normal mode displacement vector as representative searching conformers is just an approximation.

Despite these difficulties, QEDM-assisted cryo-EM refinement is expected to facilitate structural determination by cryo-EM to high-resolutions. It is certainly foreseeable that there will be more new methodology development along this line of research in the future.

Normal Mode Analysis in Length Scales of Several Microns

In cells, there are many long filamentous systems that are important for cellular functions. An example of these systems is F-actin that attains several microns. A new method, called substructure synthesis method (SSM) [39], for determining modes at very long length scales

has also been developed recently. The idea of SSM is to treat a given structure as an assemblage of substructures. The choices of substructures can be quite natural, such as domains, subunits, or large segments of biomolecules. Methods such as NMA are first employed to obtain a set of substructure modes. Then, a number of substructures are linked together to generate the final structure of desired length using a set of constraints to enforce geometric compatibility at the interfaces of neighboring substructures. The vibrational modes of the final structure are calculated from substructure modes using the Rayleigh-Ritz principle [40]. For periodically repeating systems, a hierarchical synthesis scheme (HSS) can be used in conjunction with SSM to rapidly achieve the desired final structure. It has been shown that, by SSM-HSS, one can determine the normal modes of a filamentous system with almost any length [41]. Computationally, SSM gains efficiency by only dealing with an eigenvalue problem for the much smaller substructures.

Fibre Diffraction Refinement Based on Long-Range Normal Modes

An extremely important application of methods such as SSM that determine the long-range normal modes for biological fibres is to the refinement of structural models against fibre diffraction data [42]. It has long been suspected in the field of fibre diffraction that long-range filamentous deformations may have significant contributions to the errors of the refined models. In the application of SSM to fibre diffraction refinement of F-actin, the atomic model of F-actin was refined against fibre diffraction data using long-range normal modes as adjustable parameters to account for the collective long-range filament deformations. In order to isolate the contributions of long-range deformations, each of the four domains of monomeric G-actin was treated as rigid-body and the improvement in refinement was indicated by the drop in R - and R_{free} -factors, using individual or combinations of modes calculated by SSM for the fibre. It was found that among all the modes, the bending modes make the most significant contributions to the refinement. Inclusion of only 7 ~ 9 bending modes as adjustable parameters resulted in a decrease of 2.4% in R -factor. The results clearly demonstrate that, for any fibre diffraction data, a substantial portion of refinement errors is due to long-range deformations, in particular bending, of the filaments. The effects of these long-range deformations must be properly accounted for in the refinement in order to minimize the refinement errors in structure determinations of fibre diffraction. SSM-based normal mode refinement has the advantage of using a small set of long-range modes as adjustable parameters to achieve a good fit, thus preventing the potential overfitting problem.

Some Important Issues in Normal Mode Analysis

Numerous studies [6-14,24,25,43] have clearly demonstrated that biomolecules often follow the trajectories of one or a few low-frequency normal modes to achieve their functionally important conformational transitions. However, a question commonly asked is how NMA, as a harmonic approximation that presumably cannot overcome the energy barriers separating two states, is capable of revealing the trajectories of the transition? The answer to it lies in the fact that NMA performed on one stable state *only* provides a set of modes that are likely to be followed in the transition based on the inherent structural flexibility of the molecule. Walking along the modes away from the equilibrium conformation does cause the energy to increase. Thus, the actual transition from one state to another will happen only upon external perturbations, such as ligand binding, that provide the energy needed for the transitions, thereby shifting the equilibrium toward the new conformational state. For the same reason, the amplitude of conformational transition along a normal mode, upon ligand-binding, can be much larger than that allowed by equilibrium thermal fluctuations ($k_B T/k$, where k_B is the Boltzmann constant, T is temperature and k is the effective force constant for a harmonic oscillator). Any ligand-induced conformational transition is an activation process, not an equilibrium process. In other words, the second ligand-bound conformational state is inherently unstable in the first

ligand-free system. A simple example is the hinge-bending motion of lysozyme that can be effectively described by NMA [6,8,44]. In the absence of the ligand, the open conformation of lysozyme can be bent, along a single normal mode, to a state very similar to the ligand-bound close state with an elevated energy. However, in reality, *only* upon ligand binding can the conformational transition to the closed state actually take place. Moreover, once the ligand is bound, the structure will be locked in the new state until the ligand dissociates from it. Of course, NMA only provides the overall trends of motions that a structure is likely to make upon external perturbations. Certain small structural adjustments, or induced-fits, upon the ligand binding that serve as the final “catch” of the structure, can not be revealed by the method. Furthermore, it is commonly perceived that the energy of the total system (for example, protein plus ligand) is higher at the partially engaged transition state, forming the traditional energy barrier for bi-molecular association reaction. However, in many cases of conformational transitions such as those in ATP-driven molecular motors [7,45,46], the heights of barriers could be significantly lowered by continuing engagement of favorable protein-ligand interactions in the process of binding. The energy landscape for transition could thus be fairly smooth. A distinct feature of conformational transitions in those proteins is *en bloc* domain movements around some well-evolved structural hinges for maximal efficiency of binding. In these cases, NMA becomes a particularly effective tool in analyzing the conformational changes. While in others such as p21^{ras} [11], when more localized movements are involved, the normal modes can only be used to study the initial stages of the transitions.

It is well-established from experimental observations, such as neutron scattering [47], that low-frequency motions of biomolecules are highly *anharmonic* because of severe solvent damping [48,49], which also dramatically slows down the rates of motions relative to those of vacuum normal modes. Then, the question is how *harmonic* normal modes can approximate those *anharmonic* biomolecular motions? The explanation to this dilemma is that many large-scale conformational changes follow effective harmonic trajectories (only in an overall sense) in space as described by normal modes, but do not follow the timescales of free harmonic oscillators. In some extreme cases, the equilibrium conformational distribution along a particular mode may also deviate from the equilibrium Gaussian distribution of a harmonic oscillator [50]. A good example is the distribution of conformers identified by QEDM-assisted cryo-EM refinement for FAS [36]. Although the initial searching models were generated along a harmonic mode, the populations of conformers classified based on those searching models were nearly equal, which indicates that the motion along that particular normal mode for FAS has a significantly altered distribution function. This could be attributed to environmental influences on the structural dynamics that altered the residing times of different conformers relative to those in vacuum due to trapping effects, thereby the relative populations of those conformers along a harmonic trajectory. Therefore, in general, in analyzing the conformational transitions of supermolecular complexes, the directionality of low-frequency motions determined from eigenvectors is much more useful for interpreting the functionally relevant motions than the timescales and amplitudes of the motions derived from eigenvalues. This is also the reason that the elastic NMA is a valid approach because the highly simplified potential function it uses only affects the absolute values of eigenvalues, but not the directionality of eigenvectors.

As opposed to free harmonic oscillators, harmonic deformations in proteins can also occur in a static fashion. For example, a particular structural component in a complex, such as a subunit or a secondary structural element, can deform along its own intrinsic normal modes (which can be determined when the structural element is in isolation) in a harmonic fashion, but gets locked in its deformed conformation by surrounding interactions statically in the complex. A good example [51] is provided by a principal-component analysis of the flexibility of α -helices in all coiled-coil proteins in the SCOP database [52]. Although every α -helix in these systems is in a permanently deformed state, it was found that the principal modes (bending and twisting

modes) determined from the ensemble of all static snapshots are in extremely good agreement with the dynamic normal modes. Each of the statically deformed conformer is like a frozen harmonic wave. Such a result once again reinforces the suggestion that protein structures have been evolved to utilize their intrinsic structural flexibility, as manifested in normal modes, for the functionally important conformational variations, both statically and dynamically. Moreover, one must note that it is easy to misinterpret the hidden harmonic nature of the molecular deformations depending on how the analysis is carried out. If one analyzes the system including the interested structural components plus their interacting neighbors, it may very well appear to be that the conformational changes are not harmonic as in the cases of those permanently deformed α -helices [51]. But if one separates the interested structural components from the surrounding locking interactions, the harmonic nature of the conformational changes could become evident.

Finally, there is a question on how to identify the modes that are functionally relevant in NMA. Normal modes, as an orthonormal basis set, only suggest how the structure could move. They do not *per se* tell how the structure actually moves in a particular protein. In other words, given a set of modes calculated on a structure at whatever resolution, one usually cannot tell which mode is (or modes are) functionally relevant unless additional experimental data are available. However, conversely, it is almost always true that the functionally relevant modes, once identified, are always one or several of the low-frequency modes contained in the normal mode basis set because they represent the transition pathways with the minimal energy cost.

Acknowledgments

The author acknowledges support from the American Heart Association (AHA-TX0160107Y), the Robert A. Welch Foundation (Q-1512), the National Institutes of Health (R01-GM067801), and the National Science Foundation Career Award (MCB-0237796). The author is also a recipient of the Award for Distinguished Young Scholars Abroad from National Natural Science Foundation of China.

References

1. Brooks CL III, Karplus M, Pettitt BM. Adv Chem Phys 1988;71:1.
2. McCammon, JA.; Harvey, S. Dynamics of Proteins and Nucleic Acids. Cambridge University Press; Cambridge: 1987.
3. Brooks BR, Janezic D, Karplus M. J Compt Chem 1995;16:1522.
4. Janezic D, Venable RM, Brooks BR. J Comput Chem 1995;16:1554.
5. Amadei A, Linssen ABM, Berendsen HJC. Proteins 1993;17:412. [PubMed: 8108382]
6. Levitt M, Sander C, Stern PS. J Mol Biol 1985;181:423. [PubMed: 2580101]
7. Ma J, Karplus M. Proc Natl Acad Sci USA 1998;95:8502. [PubMed: 9671707]
8. Brooks B, Karplus M. Proc Natl Acad Sci USA 1985;82:4995. [PubMed: 3860838]
9. Seno Y, Go N. j Mol Biol 1990;216:111. [PubMed: 2231726]
10. Seno Y, Go N. j Mol Biol 1990;216:95. [PubMed: 2231732]
11. Ma J, Karplus M. J Mol Biol 1997;274:114. [PubMed: 9398520]
12. Thomas A, Field MJ, Perahia D. j Mol Biol 1996;261:490. [PubMed: 8780788]
13. Thomas A, Field MJ, Mouawad L, Perahia D. j Mol Biol 1996;257:1070. [PubMed: 8632469]
14. Thomas A, Hinsen K, Field MJ, Perahia D. Proteins 1999;34:96. [PubMed: 10336386]
15. Bahar I, Atilgan AR, Erman B. Fold Des 1997;2:173. [PubMed: 9218955]
16. Haliloglu T, Bahar I, Erman B. Phys Rev Lett 1997;79:3090.
17. Atilgan AR, Durell SR, Jernigan RL, Demirel MC, Keskin O, Bahar I. Biophys J 2001;80:505. [PubMed: 11159421]
18. Tirion MM. Phys Rev Lett 1996;77:1905. [PubMed: 10063201]
19. Keskin O, Bahar I, Flatow D, Covell DG, Jernigan RL. Biochemistry 2002;41:491. [PubMed: 11781087]

20. Doruker P, Jernigan RL, Bahar I. J Comput Chem 2002;23:119. [PubMed: 11913377]
21. Tama F, Sanejouand YH. Protein Eng 2001;14:1. [PubMed: 11287673]
22. Tama F, Gadea FX, Marques O, Sanejouand YH. Proteins 2000;41:1. [PubMed: 10944387]
23. Hinsen K. Proteins 1998;33:417. [PubMed: 9829700]
24. Tama F, Brooks CL III. J Mol Biol 2002;318:733. [PubMed: 12054819]
25. Tama F, Valle M, Frank J, Brooks CL III. Proc Natl Acad Sci U S A 2003;100:9319. [PubMed: 12878726]
26. Ming D, Kong Y, Lambert M, Huang Z, Ma J. Proc Natl Acad Sci USA 2002;99:8620. [PubMed: 12084922]
27. Tama F, Wriggers W, Brooks CL. J Mol Biol 2002;321:297. [PubMed: 12144786]
28. Gray RM. Vector Quantization. IEEE ASSP Mag 1984;1:4.
29. Martinetz TM, Berkovich SG, Schulten KJ. IEEE Trans Neural Networks 1993;4:558.
30. Makhoul J, Roucos S, Gish H. Proc IEEE 1985;73:1551.
31. Ming D, Kong Y, Wakil SJ, Brink J, Ma J. Proc Natl Acad Sci USA 2002;99:7895. [PubMed: 12060737]
32. Kong Y, Ming D, Wu Y, Stoops JK, Zhou ZH, Ma J. J Mol Biol 2003;330:129. [PubMed: 12818207]
33. Beuron F, Flynn TC, Ma J, Kondo H, Zhang X, Freemont PS. J Mol Biol 2003;327:619. [PubMed: 12634057]
34. Chacon P, Tama F, Wriggers W. J Mol Biol 2003;326:485. [PubMed: 12559916]
35. Brink J, Ludtke SJ, Yang CY, Gu ZW, Wakil SJ, Chin W. Proc Natl Acad Sci U S A 2002;99:138. [PubMed: 11756679]
36. Brink J, Ludtke S, Kong Y, Wakil SJ, Ma J, Chiu W. Structure. 2004In Press
37. Gros P, van Gunsteren WF, Hol WG. Science 1990;249:1149. [PubMed: 2396108]
38. Grigorieff N. Acta Crystallogr D 2000;56:1270. [PubMed: 10998623]
39. Ming D, Kong Y, Wu Y, Ma J. Proc Natl Acad Sci USA 2003;100:104.
40. Temple, G.; Bickley, WG. Rayleigh's principle and Its Applications to Engineering. Dover, New York: 1956.
41. Ming D, Kong Y, Wu Y, Ma J. Biophys J 2003;85:27. [PubMed: 12829461]
42. Wu Y, Ma J. Biophys J 2004;86:116–124. [PubMed: 14695255]
43. Li G, Cui Q. Biophys J 2002;83:2457. [PubMed: 12414680]
44. Bruccoleri RE, Karplus M, McCammon JA. Biopolymers 1986;25:1767. [PubMed: 3768485]
45. Yang W, Gao YQ, Cui Q, Ma J, Karplus M. Proc Natl Acad Sci U S A 2003;100:874. [PubMed: 12552084]
46. Ma J, Flynn TC, Cui Q, Leslie A, Walker JE, Karplus MA. Structure 2002;10:921. [PubMed: 12121647]
47. Cusack S, Smith J, Finney J, Tidor B, Karplus M. J Mol Biol 1988;202:903. [PubMed: 2459399]
48. Lamm G, Szabo A. J Chem Phys 1986;85:7334.
49. Kottalam J, Case DA. Biopolymers 1990;29:1409. [PubMed: 2361153]
50. McQuarrie, DA. Statistical Mechanics. Harper & Row; 1976.
51. Emberly EG, Mukhopadhyay R, Wingreen NS, Tang C. J Mol Biol 2003;327:229. [PubMed: 12614621]
52. Murzin AG, Brenner SE, Hubbard T, Chothia C. J Mol Biol 1995;247:536. [PubMed: 7723011]

Abbreviations

NMA

Normal Mode Analysis

GNM

Gaussian Network Model

ANM

	Anisotropic Network Model
SSM	Substructure Synthesis Method
QEDM	Quantized Elastic Deformational Model
VQ	Vector Quantization
cryo-EM	Electron Cryomicroscopy
FAS	Fatty Acid Synthase

Biography