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Review Article

Normal mode analysis and beyond

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Normal mode analysis provides a powerful tool in biophysical computations. Particularly, we shed light on its application to protein properties because they directly lead to biological functions. As a result of normal mode analysis, the protein motion is represented as a linear combination of mutually independent normal mode vectors. It has been widely accepted that the large amplitude motions throughout the entire protein molecule can be well described with a few low-frequency normal modes. Furthermore, it is possible to represent the effect of external perturbations, *e.g.*, ligand binding, hydrostatic pressure, as the shifts of normal mode variables. Making use of this advantage, we are able to explore mechanical properties of proteins such as Young's modulus and compressibility. Within thermally fluctuating protein molecules under physiological conditions, tightly packed amino acid residues interact with each other through heat and energy exchanges. Since the structure and dynamics of protein molecules are highly anisotropic, the flow of energy and heat should also be anisotropic. Based on the harmonic approximation of the heat current operator, it is possible to analyze the communication map of a protein molecule. By using this method, the energy transfer pathways of photoactive yellow protein were calculated. It turned out that these pathways are similar to those obtained via the Green-Kubo formalism with equilibrium molecular dynamics simulations, indicating that normal mode analysis captures the intrinsic nature of the transport properties of proteins.

**Key words:** mechanical property, transport property, allostery

In 1983, three research groups reported their pioneering studies on the protein dynamics based on normal mode analysis (NMA) [1–3]. Ever since, NMA has kept its influence, directly or indirectly, on the trend of biophysical computations. This review article highlights three aspects of NMA (Fig. 1), i.e., *computational method*, *data analysis*, and *property analysis*. We especially shed light on the last aspect because *properties* directly lead to protein biological functions, while the first two aspects are briefly summarized in the subsequent sections as they have been extensively reviewed elsewhere (see for instance [4]).

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◄ *Significance* ►

Normal mode analysis provides a powerful tool in biophysical computations. Particularly, we shed light on its application to protein properties because they directly lead to biological functions. For instance, the effect of external perturbations such as ligand binding and hydrostatic pressure on protein structures can be represented as the shifts of normal mode variables. Normal mode analysis is also applicable to transport properties of proteins. Since the structure and dynamics of protein molecules are highly anisotropic, the flow of energy and heat should also be anisotropic. Based on the harmonic approximation of the heat current operator, it is possible to analyze the communication map of a protein molecule.
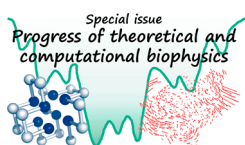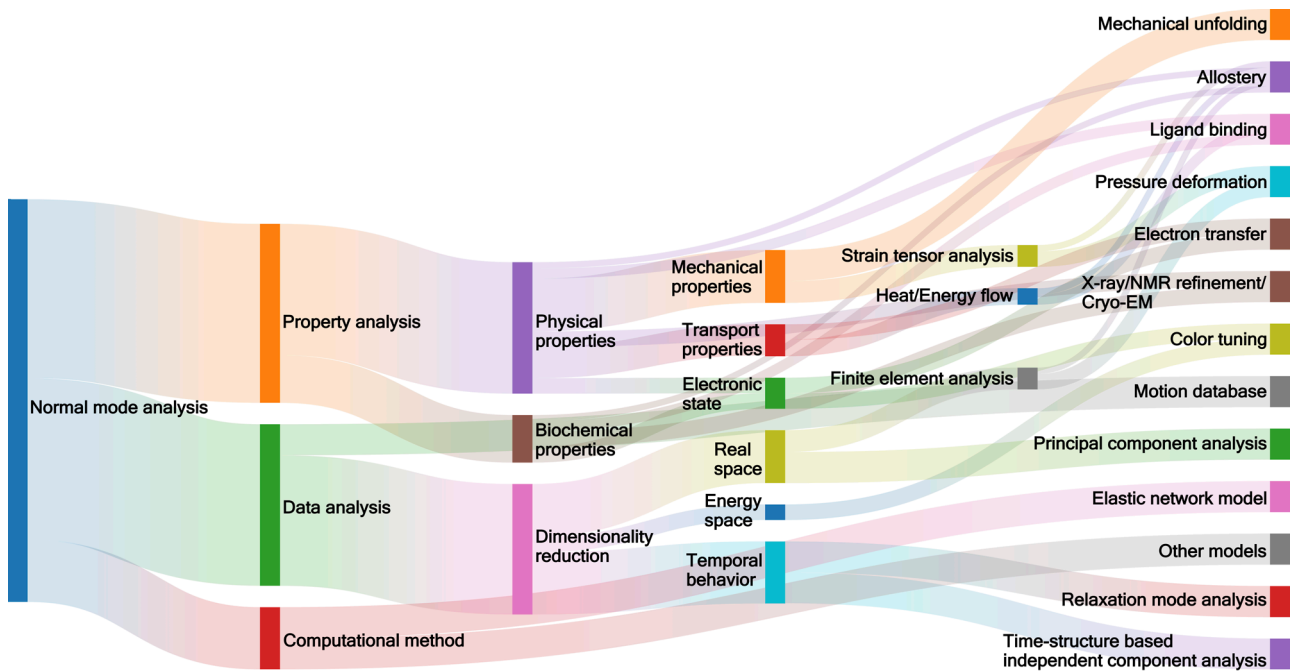
Figure 1 Normal mode analysis and its application to biophysically interesting properties. Various computational methods, analyses, and biophysically interesting properties are shown here at a glance, together with other related computational methods. The link widths are normalized so that the widths of the links that are connected to the terminal (rightmost) nodes become all equal. The links and nodes are colored so that they are clearly distinguished from one to another, where the color codes have no meaning themselves.

Computational method

NMA is formulated based on the harmonic approximation of the potential energy surface (Fig. 2). After energy minimization, the Hessian matrix is obtained and normal mode vectors and frequencies are evaluated. As a result, the internal motion of a protein is represented as a linear combination of independent normal mode vectors. This model provides useful descriptions of a complex protein molecule, and a large number of computational biophysics researchers have used this model to demonstrate not only protein dynamics but also various molecular properties such as ligand binding, volume fluctuation, heat and energy transfer, allosteric transition and so forth. Later in 1996, Tirion proposed a model called elastic network model (ENM) with simplified force field functions [5], and Sanejouand and coworkers developed the elNémo program based on ENM [6,7]. Note that their methods require no energy minimization and also they can perform coarse grained NMA. Recently, Holger, F., *et al.* reviewed the application of ENM to the mechanical properties of proteins [8].

Data analysis

Besides the aforementioned aspect, data analysis is another important point. Within thermally fluctuating protein molecules under physiological conditions in the cell, a numerous number of tightly packed atoms are interacting with each other, and their movements are governed by the equations of motions for multibody systems. Proteins are
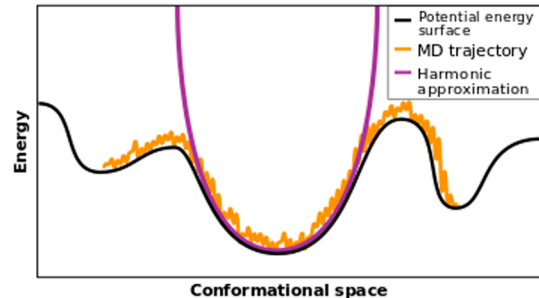

Figure 2 Energy landscape and harmonic approximation. A naturally occurring polypeptide chain has a complex potential energy surface and its conformational space can be explored by using molecular dynamics simulations. At an energy minimum point corresponding to the native state on such potential surface, the harmonic approximation is applicable and thereby the potential energy is represented as a parabolic surface in multidimensions. Based on this approximation, NMA is performed to study the dynamics of proteins and their properties.

unique in that they often exhibit a broad range of spatiotemporal dynamics, and a variety of molecular functions are realized by such molecules. Due to the complexity of proteins, however, biophysicists often encounter difficulties in interpreting experimental and computational data to discover underlying molecular mechanisms of protein functions. As such, NMA provides a powerful tool for computational biophysicists with which many degrees of freedom are separated into mutually independent normal modes.

In particular, characteristic behaviors of concerted motions throughout the entire molecule are well represented with only a few low-frequency normal modes (Dimensionality reduction). Although molecular movements associated with low-frequency normal modes should be significantly affected under the presence of solvent molecules and exhibit diffusion, these normal modes provide important "basis set" that spans the functionally important conformational space of proteins. Horiuchi and Go performed molecular dynamics (MD) and Monte Carlo (MC) simulations of human lysozyme and projected these trajectories onto few normal mode axes, and demonstrated that few normal modes determine the characteristic features of protein dynamics [9]. Kidera, A., *et al.* formulated their original method for anisotropic refinement of x-ray structure factors based on NMA, and successfully separated the internal motions and external motions of human lysozyme [10]. NMA was applied to NMR refinement [11,12] as well as x-ray refinement. Recent developments in cryo-electron microscopy (cryo-EM) combined with NMA opened up new possibilities for the study of macromolecular structures [13,14]. Wako and coworkers provided a database of protein motions, ProMode, so that normal mode vectors are visualized on their website [15].

Further efforts have been continued to extract important degrees of freedom in proteins from molecular dynamics trajectories. Later, several groups proposed to use the principal component analysis (PCA) to characterized the protein conformational fluctuations [16,17]. In PCA, the large amplitude motions throughout the entire molecule are well represented by few principal components (PC), like those associated with few low-frequency normal modes in NMA. In PCA, the conformational dynamics of proteins is separated into different PCs according to each fluctuation amplitude. Other research groups, on the other hand, focused their attentions on the temporal behavior of protein dynamics and separated the protein movements into different components according to their characteristic times. Takano and coworkers proposed relaxation mode analysis (RMA) [18] and Naritomi & Fuchigami. performed time-structure independent component analysis (tICA) [19]. In 2010, Watanabe, H. C., *et al.* performed extended PCA study on the color tuning mechanism of rhodopsin [20]. They added an electronic excitation energy as an additional "coordinate", and successfully illustrated the molecular mechanism of the shift of optical absorption maximum of conger rhodopsin.

Physical and Biochemical Properties of Proteins

Mechanical properties

NMA particularly highlights the solid state nature of proteins. In 1987, Nishikawa, T., *et al.* performed NMA of bovine pancreatic trypsin inhibitor, obtained the density of states as a function of frequency, and evaluated Young's modulus of the protein [21]. This study opened up new pathways towards continuum mechanical representations of

complex biomacromolecules.

Later, Go and coworkers applied NMA to investigate volume fluctuations and pressure deformation of proteins [22,23]. Note that NMA was also applied to the studies of ligand binding [24,25] and to the pressure dependence of electron transfer reactions in proteins [26]. Furthermore, Mimura, S., *et al.* investigated the relationships between the isothermal compressibility and the molecular evolution of lysozyme [27].

The potential energy of a protein molecule in the neighborhood of an minimum energy point in the conformational space $\{\theta_1, \theta_2, \cdots, \theta_N\}$ is expressed as

$$E = \frac{1}{2} \sum_{i,j} f_{ij} \theta_i \theta_j, \tag{1}$$

where matrix $\{\mathbf{F}\}_{ij} = f_{ij}$ is called F-matrix and N is the total number of degrees of freedom in the molecule. We define the conformational variables so that $\theta_i = 0$ ($i = 1, 2, \ldots N$) at the minimum energy point. The kinetic energy is given by

$$T = \frac{1}{2} \sum_{i,j} h_{ij} \dot{\theta}_i \dot{\theta}_j, h_{ij} = \sum_k m_k \left(\frac{\partial \mathbf{r}_k}{\partial \theta_i} \right) \cdot \left(\frac{\partial \mathbf{r}_k}{\partial \theta_j} \right), \tag{2}$$

where m_k, $\mathbf{r}_k$ respectively are the mass and position vector of the k-th atom, and the matrix $\mathbf{H}$ whose (i, j) element equals h_{ij} is called H-matrix. It is possible to find a linear transformation, $\theta_i = \sum_k U_{ij} \sigma_j$, $(\{\mathbf{U}\}_{ij} \equiv U_{ij})$, that simultaneously diagonalizes $\mathbf{F}$ and $\mathbf{H}$, i.e., $\mathbf{U}^t \mathbf{H} \mathbf{U} = \mathbf{I}, \mathbf{U}^t \mathbf{F} \mathbf{U} = \Omega$, where $\mathbf{I}$ is the identity matrix and the (i, i) element of the diagonal matrix Ω provides the square of the angular frequency ω_i of the i-th normal mode variable σ_i ($i = 1, 2, \ldots, N$). Next, we assume that the volume changes linearly near the minimum energy point, i.e.,

$$\Delta V = \sum_i v_i \sigma_i. \tag{3}$$

As a result, the average shift of each normal mode variable under hydrostatic pressure, P, becomes

$$\sigma_i(P) = -P \frac{v_i}{\omega_i^2}, \tag{4}$$

which means that the pressure induced shift occurs in such a way that the volume decreases (energy increases) as much (small) as possible. Also, the isothermal compressibility β_T is calculated as

$$\beta_T = \frac{1}{<V>} \sum_i \frac{v_i^2}{\omega_i^2}. \tag{5}$$

Strain tensor analysis

In contrast to isotropic systems such as, for instance, liquid argon, protein structures are highly anisotropic and inhomogeneous. Accordingly, the pressure induced deformation of proteins should be also non-uniform. To analyze such non-uniform conformational changes, we represented the protein structure as an assembly of Delaunay tetrahedra

consisting of nearby heavy atoms, and then calculated strain tensor for each tetrahedron [22,28]. As a result, we demonstrated that the interhelical regions in myoglobin were considerably more compressible than the individual helices, indicating that the experimentally observed large compressibilities [29] of α helical proteins are, possibly, accounted for by such large interhelical compressibilities. Note that this method was also applied to illustrate the strain tensor field along the ligand migration pathways in myoglobin [30].

A brief comment on the characteristic nature of this strain tensor analysis is in order. One of the standard method to compare a pair of different protein structures is to calculate the root-mean-square displacement of atoms. To perform this analysis, the center of gravity of the second structure is translated to that of the first structure. Then, the second structure is rotated around the center of gravity so that the average displacement of atoms is minimized. Accordingly, the magnitude of atomic displacement becomes dependent on the distance between the atom and the center of gravity, which is inconvenient because we are usually interested in the "local" flexibility around each atom.

Recently, Leibler and coworkers applied the strain tensor analysis to investigate allosteric coupling between the active site and the regulation site in proteins, and successfully identified allosteric signaling pathways in adenylate kinase [31]. Note that they pointed out the importance of shear strain in the allosteric signaling. A possible reason for this may be explained like this: Within a protein molecule under physiological conditions, the protein atoms are densely packed because internal cavity is unfavorable in free energy. Therefore, any conformational change would occur in such a way that the local volume expansion, which is related with the bulk strain, is minimized.

Mechanical unfolding

NMA has been applied not only to the native fluctuations of proteins but also to the characterization of the energy landscape related with mechanical unfolding processes [32]. Single-molecule force spectroscopy by using atomic force microscopy opened up new pathways to get insights into the mechanical unfolding of membrane proteins. Such experiments typically exhibit characteristic saw-like pattern of the force distance (*FD*) curves. Since each pattern depend on the protein species, it serves as the unique fingerprint of each molecule. However, the origin of such patterns has not been fully understood yet. Recently, we have developed our original program, ppfc (PolyPeptide chain Force-distance Curve simulator), to study the forced unfolding mechanism of membrane proteins [33,34], and successfully reproduced experimentally observed *FD* curves. The program and user's manual are available on the web site [35].

Transport Properties

Heat and energy flow

Within thermally fluctuating protein molecules under physiological conditions, tightly packed amino acid residues interact with each other by exchanging heat and energy between them. Since the structure and dynamics of protein molecules are highly anisotropic, the flow of energy and heat should also be anisotropic. By using biophysical computation methods, several groups have been engaged in the characterization of such energy transport processes in proteins [36–51]. We are particularly interested in how the energy relaxation process intervene in proteins control of their biological functions such as allostery.

In 2000, Kidera and coworkers examined the role of the anharmonic coupling between different normal modes in the vibrational energy relaxation in myoglobin [36]. Based on the calculation of heat current operator with the harmonic approximation, Leitner proposed a method called frequency resolved communication map, and investigated anisotropic energy transport in proteins [38,44,51] such as hemoglobin and photoactive yellow protein (PYP). Importantly, energy transport pathways observed in PYP by the communication map analysis were in good agreement with those obtained via the Green–Kubo formalism with equilibrium MD simulation [45]. This observation indicates that NMA captures essential feature of transport properties such as energy and heat flow in proteins. To explore the vibrational energy transport pathways in proteins, we have developed a computer program, CURP (CURrent calculations for Proteins) [52]. By using this program, we further demonstrated characteristic features of "hidden dynamic allostery" in PDZ domain [47] and allosteric transition in the oxygen sensor domain of FixL [48].

Summary and Outlook

In this article, we briefly reviewed the development of normal mode analysis and its application to biophysically interesting properties. We highlighted its three different aspects, i.e., *Computational method*, *Data analysis*, and *Property analysis*. The third aspect was further classified into *Mechanical properties* and *Transport properties*, and was discussed in depth.

The strain tensor analysis of proteins was turned to be useful in the characterization of local mechanical properties. In particular, this method was successfully applied to identify allosteric signaling pathways between the active site and the regulation site in adenylate kinase.

Transport properties play important roles in the biological functions of proteins. The concept of communication map was developed based on normal mode analysis. The application of this method identified energy transfer pathways of a photoreceptor protein, and these pathways were similar to those obtained via Green-Kubo formalism with equilibrium molecular dynamics simulations. The role of these energy

transfer pathway in allosteric signaling, which is an important issue to be addressed and has not been fully understood yet.

Acknowledgments

Conflicts of Interest

T. Y. and O. L. declare that we have no conflict of interest.

Author Contribution

T. Y. and O. L. jointly wrote the paper.

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