

NONLINEAR OSCILLATIONS OF PROTEINS

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ABSTRACT

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Slow modes of proteins exhibit nonlinear characteristics. Large deformations in proteins correspond to collective deformations. These are at relatively low energy compared to $k_B T_{room}$ where T_{room} is approximately $300^0 K$. Therefore the dynamics of slow modes can be understood through Classical Mechanical methods. In this work, we investigate the collective frequencies of proteins to see how big the effects of the nonlinearity on the collective modes of proteins. To do this, we take advantage of Canonical Perturbation Theory. We see that slow mode frequencies deviate from normal frequencies by $(A/R_{ij}^0)^2$ where A is a typical residue fluctuation from its equilibrium position and R_{ij}^0 is the distance between residue pairs at an equilibrium state.

ÖZET

PROTEİNLERDE LİNEER OLMAYAN SALINIMLAR

Proteinlerdeki kollektif salınım hareketleri lineer olmayan özellikler göstermektedir. Proteinlerdeki büyük deformasyonlar aynı zamanda kollektif deformasyonlardır. Bu deformasyonlar oda sıcaklığı enerjisi $k_B T_{oda}$ 'ye göre daha düşük enerjidedirler. Dolayısı ile proteinlerdeki yavaş salınımlar Klasik Mekaniksel metodlar yardımı ile incelenebilir. Bu çalışmamızda lineer olmayan etkilerin sistemin kollektif frekansları üzerindeki etkilerinin büyüklüğünü görebilmek için Hamilton-Jakobi Perturbasyon metodu yardımı ile kollektif frekanslar için genel bir ifade bulmaya çalıştık. Sonuçta kollektif frekansların sistemin harmonik frekanslarından $(A/R_{ij}^0)^2$ ile değiştiğini gördük. Burada A dengeden uzaklaştığında bu iki rezüdü çifti arasındaki mesafeyi, R_{ij}^0 ise iki rezüdü arasındaki denge uzaklığı göstermektedir.

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LIST OF SYMBOLS

| | |
|-------------------|---------------------------------------|
| H | Hamiltonian |
| H_0 | The unperturbed Hamiltonian |
| H_1 | The perturbation Hamiltonian |
| \underline{H} | The perturbed Hamiltonian |
| J | Action variable |
| k_B | Boltzmann constant |
| K | Transformed Hamiltonian |
| q | Mass-weighted coordinates |
| S | Hamiltonian's Principle function |
| W | Hamiltonian's characteristic function |
| Y | Generating function |
| ϵ | Perturbation parameter |
| ν | Harmonic frequency |
| $\underline{\nu}$ | Shifted frequency |
| ω | Angular frequency |
| w | Angle variable |

1. INTRODUCTION

Proteins are essential to the structure and functions of all living systems. They are very high-molecular-weight, organic compounds that are made up of amino acids joined by polypeptide bonds. A polypeptide chain protein consists of a linear sequence of amino acid residues. There are 20 different amino acids, and typically a polypeptide chain contains a few hundreds of these elementary units. Each protein folds into a specific three-dimensional (3D) structure and called the native state, which determines its biological function.

It is known that proteins do not exhibit rigid body motion in their folded state, but instead can execute some structural deformations in the neighborhood of their native states[1]. These can be regarded as fluctuations near equilibrium points, which do not typically exceed several Angstroms.

Details of protein motions are usually investigated through normal mode analysis and molecular dynamics simulations using all-atom empirical potentials developed for proteins[2]. Since most proteins are giant biological systems, the use of atomic approaches becomes computationally inefficient. However, recent studies show that coarse-grain protein models and simplified force fields for describing the vibrational dynamics are suitable for describing collective motions of the large proteins[3].

As a dynamics system, proteins exhibit a spectrum of frequencies from low to high frequencies. The low frequencies usually describe the global motions of proteins, that are important for biological function. The higher ones, on the other hand, are responsible for the more localized motions of proteins, which may be important for protein stability.

An important goal in the investigation of the protein dynamics is the understanding of the role of amplitude-dependent frequencies on the global motion of large proteins. Many examples show that amplitude-dependent frequencies play an impor-

tant role in the global motion of large biological complexes[4].

Global motions of these complexes typically describe the rearrangements of the domains which are essential for the function of proteins. Only such global motions can cause some structural deformation of proteins. Many examples show that the functionally important transition pathways of large proteins follow trajectories along one or more slow modes[5].

One method for the investigation of protein global motions is normal mode analysis[6]. Although normal mode analysis has important limitations such as harmonic approximations, neglect of solvent damping, it still provides important information about proteins dynamics. The identification of the low-frequency domain motions can be regarded as one of the most important contributions of normal mode analysis for the investigation of protein dynamics.

For dynamic systems, large-amplitude low-frequency motions of biomolecules are highly anharmonic because of the the rugged energy landscapes and severe solvent damping[7]. From statistical mechanics, at thermal equilibrium, the square of the average amplitude of thermal fluctuation of any harmonic oscillator is $k_B T / \omega^2$ [8]. In reality, a ligand-induced conformational change can have a much larger amplitude than the equilibrium thermal fluctuation. In other words, the ligand-induced conformational transition is usually an activated not a spontaneous process. Binding of the ligand to the biological complex brings in additional energy to the system. This process is known as *dynamic harmonic deformation* of the proteins. Therefore such a process gives us the liberty to orient each coordinates in the perturbed Hamiltonian along the direction of the corresponding eigenvector which is obtained from normal mode analysis.

Static harmonic deformation is the other kind of deformation which takes place in the dynamics of proteins. In this deformation the system deforms its structure harmonically along their intrinsic normal modes, and then remain statically in this deformed conformation locked by the interactions with their environments[9].

In our work, we are inspired by the ideas regarding the important role of low frequencies on the global motions of proteins claimed by normal mode analysis. Because low frequencies of large proteins are amplitude-dependent, and the square of the average amplitude is proportional to the temperature of the surrounding environment, such global motions have an interaction with the environment of proteins.

Since it is known that the global motion of proteins exhibits some nonlinear characteristics, in our work we tend to keep the cubic terms and neglect the higher ones in the expansion of the potential energy for the forthcoming calculations.

At this point it is worth mentioning some additional characteristics of these large biological systems to decide from which point of view we should see the the dynamics of the system,i.e., classical or quantum mechanical. As stated above, biological systems are extremely large compared to the size of small molecules. Thus atomic details can be neglected and low-resolution, i.e. coarse-grained models, become suitable for these large systems.

Since these collective modes are at relatively lower frequencies compared to typical electronic excitations, their motion has a quasi-classical character. Therefore *canonical perturbation method* can be considered as a suitable method to find the shifted frequencies of the system corresponding to each normal mode.

2. VIBRATIONAL DYNAMICS OF PROTEINS

The investigation of low frequency motions of proteins are of considerable interest. It is known that the collective motions, or low frequency motions, play an important role in the biological function of biomolecular complexes[10].

One of the most important techniques for studying the slow-mode, large-amplitude frequencies and hence the collective motions of proteins is normal mode analysis[11]. Normal mode analysis provides a very detailed description for the vibrational dynamics of the proteins around their minimum energy state.

Although normal mode analysis has some limitations such as harmonic approximation, neglect of solvent damping, and lack of information about the energy barriers, it is still an efficient technique to obtain important information about the collective motions of proteins. For dynamic systems, experiments such as neutron scattering show that large-amplitude, low-frequency motions of proteins are highly *anharmonic* on account of the rugged energy landscape and severe solvent damping[7]. Therefore one might think that normal mode analysis is not a suitable method for investigation of amplitude-dependent motions of proteins.

Actually, there is a difference between the amplitude of the thermal fluctuations of the harmonic oscillator obtained from normal mode analysis and the amplitude of biomolecular deformation that is experimentally observed. At the thermal equilibrium, statistical mechanics states that the square of the average amplitude of thermal fluctuation of any harmonic oscillator is $k_B T / \omega^2$ where k_B is the Boltzmann constant, T is the absolute temperature, and ω is an angular normal mode frequency.

At the beginning of the conformational transition of proteins, biomolecular deformation has a small amplitude, thus normal mode analysis is a good approximation. It is known that after the conformational transition has been performed by the system, the amplitude of the biomolecular deformation increases. In other words, there

appears to emerge a difference between the amplitude of thermal fluctuations of harmonic oscillators described by normal mode analysis and the amplitude of the molecular deformation that is experimentally observed. So, one may ask the following question.

How far can biomolecules be extended along normal modes in a biologically correct way since large amplitude motions are likely to be anharmonic?

At thermal equilibrium, the square of average amplitude of thermal fluctuation is $k_B T / \omega^2$. For normal mode analysis, $k_B T / \omega^2$ gives a small number at the room temperature[2]. But in reality, the conformational change can have a larger amplitude than thermal fluctuation at thermal equilibrium. This difference stems from the ligand-induced binding energy brought to the system during the ligand-induced conformational change. The term ligand can be, for instance, enzyme substrates, protein interacting partner[9]. Binding of a ligand to a biomolecular system brings an additional energy to the system so that its deformation be stretched along its slow mode. Therefore one can conclude that once we know the direction of the slow modes, the direction of the deformation the system can be predicted.

2.1. Normal Mode Analysis

To carry out normal mode analysis, we need a set of coordinates through which the potential energy can be described. Normal mode analysis starts with the minimization of the potential energy as a function of atomic cartesian coordinates. Then by Taylor expansion[12], the potential energy is expanded at a minimum in terms of mass-weighted coordinates, i.e:

$$q_i = \sqrt{m_j} \Delta x_j, q_{i+1} = \sqrt{m_j} \Delta y_j, q_{i+2} = \sqrt{m_j} \Delta z_j$$

where j and i stands for the N atoms and $3N$ the cartesian coordinates, respectively. After Taylor expansion has been performed, the potential energy becomes

$$V = \frac{1}{2} \sum_{i,j=1}^{3N} \frac{\partial^2 V}{\partial q_i \partial q_j} \Big|_0 q_i q_j + \dots \quad (2.1)$$

where N is the number of the constituent atoms. The first term in the expansion is set to zero because it describes the value of the energy at a minimum. The second term is also zero because it describes the force at the equilibrium. In normal mode analysis, the potential expansion is kept up at the quadratic term and the higher terms are neglected. The second order partial derivatives form a matrix which is called the Hessian. Then the Lagrange equation is given by

$$L = \frac{1}{2} \sum_{i=1}^{3N} \dot{q}_i^2 - \frac{1}{2} \sum_{i,j=1}^{3N} \frac{\partial^2 V}{\partial q_i \partial q_j} \Big|_0 q_i q_j \quad (2.2)$$

or in vector-matrix form

$$L = \frac{1}{2} \dot{q}^T \dot{q} - \frac{1}{2} q^T F q \quad (2.3)$$

where the superscript T denotes the transpose operation and F is the Hessian matrix

$$F = \sum_{i,j=1}^{3N} \frac{\partial^2 V}{\partial q_i \partial q_j} \quad (2.4)$$

Because F is a symmetric matrix, there should be an orthogonal matrix which diagonalizes F , i.e:

$$W^T F W = \Omega \quad (2.5)$$

where Ω is a diagonal matrix, W is a matrix of order $3N$. The equation above can be regarded as an eigenvalue equation, where each element denotes an eigenvalue associated with each column of the matrix W , the eigenvector matrix. Taking advantage of

W , a new set of coordinates can be introduced as follows

$$Q = W^T q \quad (2.6)$$

or

$$Q_i = \sum_{k=1}^{3N} W_{ki} q_k \quad (2.7)$$

where Q_i are normal coordinates. Plugging (2.7) into (2.3) and using Lagrange equation lead to $3N$ decoupled equations with

$$Q_i = A_i \cos(\omega_i t) \quad (2.8)$$

The Equation (2.8) states that each normal mode oscillates with an angular frequency ω_i . Each normal coordinate, therefore, introduce a new set of atomic displacements through the Equation (2.6)

$$q_k = W_{ki} A_i \cos(\omega_i t) \quad (2.9)$$

One can immediately see that in the equation above the normal coordinates are described by the eigenvectors associated with ω_i . Therefore, the normal modes and their frequencies are described by the eigenvector and the eigenvalues of the matrix F . The precise amplitude of a normal coordinate can be found by the initial conditions.

Equipartition theorem, from statistical mechanics, states that for a molecule at a thermal equilibrium,

$$\langle Q_i^2 \rangle = \frac{k_B T}{\omega_i^2} \quad (2.10)$$

or in terms of the mass-weighted coordinates,

$$\langle q_i^2 \rangle = k_B T \sum_{k=1}^{3N-6} \left(\frac{W_{ki}}{\omega_k} \right)^2 \quad (2.11)$$

where k_B is Boltzmann's constant and T the absolute temperature. Scientist in this field use the Equation (2.11) to find the mean-square fluctuation of the atomic B-factors in X-ray crystallography. B-factors are the experimental temperature factors and they have the following relation with the mean square fluctuation

$$B_i = (8\pi^2/3) \langle \Delta R_i^2 \rangle \quad (2.12)$$

Additionally, one can rewrite the Equation (2.11) as follows

$$\left\langle \sum_{i=1}^{3N-6} q_i^2 \right\rangle = \sum_{i=1}^{3N-6} \langle q_i^2 \rangle = k_B T \sum_{k=1}^{3N-6} \frac{1}{\omega_k^2} \quad (2.13)$$

From the Equation (2.13), one can easily see that the as the frequencies decrease the fluctuation increase. In other words, for the lower frequencies, the vibrational motions of proteins become amplitude sensitive.

The correlation function between the cartesian coordinates can be determined from

$$\langle q_i q_j \rangle = k_B T \sum_{k=1}^{3N-6} W_{ik} W_{jk} \frac{1}{\omega_k^2} \quad (2.14)$$

or in matrix notation,

$$U = \langle qq^T \rangle = k_B T W \Omega^{-1} W^T \quad (2.15)$$

where the frequency eigenvalues and eigenvectors are eliminated.

2.2. Elastic Network Model

Actually normal mode analysis is a general method for scrutinizing the low frequency motions of proteins. *Elastic network model* is a low resolution model which examines the global motion of the system without minimization of the potential energy. It predicts the dynamics around the coarse-grained minimum energy conformation with high computational efficiency. In elastic network model all pair of nodes that are within a cutoff distance are considered to be connected by springs. In this model each residue is represented by a node which is located at its α -carbon position[13].

The total potential energy for a system of N residues is the summation over all interactions of (i,j) pairs confined to the cutoff distance, r_c

$$V = (\gamma/2) \sum_i \sum_j h(r_c - R_{ij})(\Delta R_j - \Delta R_i)^2 \quad (2.16)$$

where R_i and ΔR_i predicts the position and the fluctuation vectors of node i , where i changes from 1 to N . The distance between the nodes j and i are given by the parameter R_{ij} in the equation above. Meanwhile, h is a step function. It takes 1 if $r_c \leq R_{ij}$, and zero otherwise. The term γ in the Equation (2.16) is a force constant and is identical for all interactions [14]. Since the formulation of elastic network model is based on the classical normal mode analysis, the Equations (2.11) and (2.12) are also true for this method.

As an example for the global motions of the proteins, it is instructive to mention the collective motions of bacterial RNA polymerases studied by elastic network models[15]. As is well known low frequencies play an important role in the collective dynamics of RNAP. This role can be effectively analyzed by low-resolution model[16].

Collective motions of RNAPs, specifically, take place during the transcription of DNA by RNA polymerases. In 3-D the structure of RNAPs has two main parts, a conserved core around the active site and a multifunctional clamp. As it can be seen from figure 2.1, the clamp is located on one of the pincers. The clamp is known to be a mobile domain, exhibiting opening and closing motion.

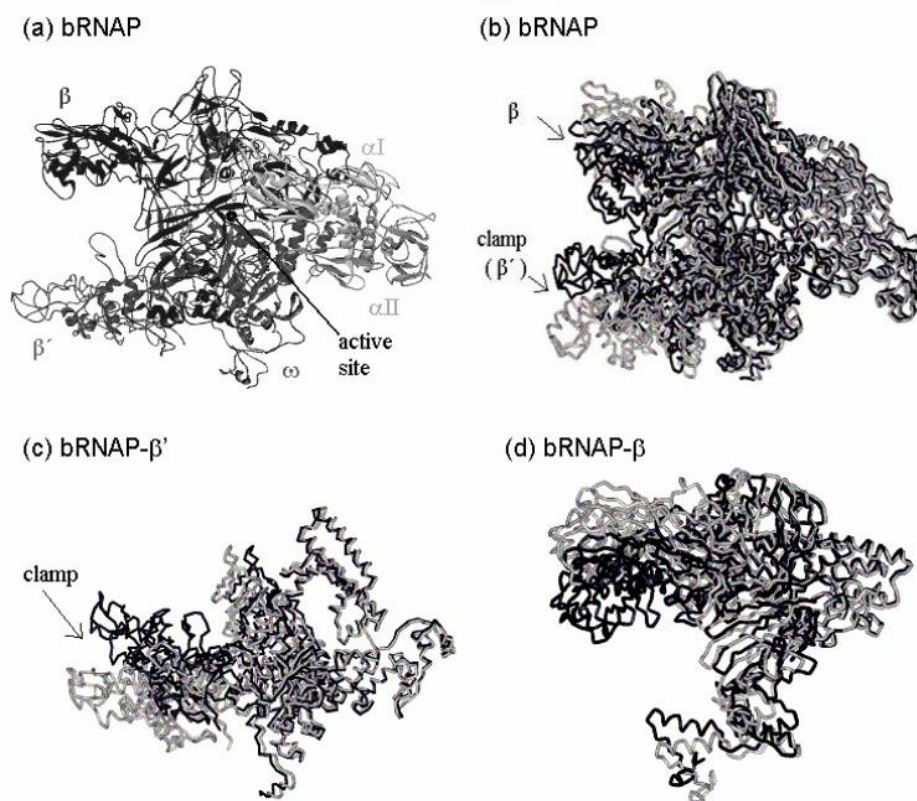


Figure 2.1. (a) X-ray structure of bacterial RNA polymerase (bRNAP) (b) Deformation of clamp and upper pincer (c) deformation of clamp (d) Deformation of upper pincer

Figure(2.1) shows the collective motion of bRNAP. In part(a), the observed x-ray structure of bRNAP is shown[17]. In part(b), the black and gray colors show the positive and negative deformations of bRNAP around native structure. Part(c) and (d) are the exaggerated images of clamp(β' subunit) and upper pincer (β subunit) after the molecular deformation takes place. Thus, experimentally observed opening and closing motions of the clamp can be obtained successfully by elastic network model.

2.3. Frequency of Nonlinear Periodic Systems

In this part we are going to see that there exist amplitude-dependent frequencies if the potential of the system contains a nonlinear term[18]. A general form of a potential energy after Taylor expansion has been performed can be given by

$$V = \frac{1}{2}\alpha x^2 - \frac{1}{3}\beta x^3 + \frac{1}{4}\gamma x^4 + \dots \quad (2.17)$$

where α , β , and γ are constants. The minus sign here included for the convenience. So the equation of motion can be written as

$$\ddot{x} + \alpha x - \beta x^2 + \gamma x^3 = 0 \quad (2.18)$$

Taking advantage of Fourier series

$$x = a_0 + b_1 \cos(\omega t) + b_2 \cos(2\omega t) + \dots \quad (2.19)$$

One of the fourier coefficient, a_0 , can be found as follows

$$a_0 = \frac{1}{2\pi\alpha} \int_0^{2\pi} (\beta x^2 - \gamma x^3) d(\omega t) \quad (2.20)$$

After all the coefficients has been determined, x becomes

$$x = \frac{\beta b_1^2}{2\alpha} + b_1 \cos(\omega t) - \frac{\beta b_1^2}{6\alpha} \cos(2\omega t) \quad (2.21)$$

Replacing the equation above into (2.18), one can find the frequency of the anharmonic system as

$$\omega^2 = \alpha + \frac{3}{4}\gamma b_1^2 - \frac{5}{6} \frac{\beta^2 b_1^2}{\alpha} \quad (2.22)$$

One can realize that the frequency of this nonlinear system is really amplitude dependent. This method appears to be useful in solving all the nonlinear systems but in reality it is not. As it will be seen in the following part, this method is not suitable to the nonlinear system in several degrees of freedom.

2.4. Periodic Solutions of Nonlinear Systems

Given the following nonlinear differential equation in one freedom[19]

$$\ddot{x} + \omega^2 x = \epsilon f(x) \quad (2.23)$$

we can try to find a solution for this system in the form of

$$x(t) = x_0(t) + \epsilon x_1(t) + \epsilon^2 x_2(t) + \dots \quad (2.24)$$

In the case of autonomous systems, a complication emerge because the period of the system is as follows

$$T' = T + \tau(\epsilon) \quad (2.25)$$

Therefore, the equation for the periodicity should satisfy

$$x\left(t + \frac{2\pi}{\omega} + \tau\right) = x_0\left(t + \frac{2\pi}{\omega} + \tau\right) + \epsilon x_1\left(t + \frac{2\pi}{\omega} + \tau\right) + \epsilon^2 x_2\left(t + \frac{2\pi}{\omega} + \tau\right) + \dots \quad (2.26)$$

Thus one can not states that

$$x\left(t + \frac{2\pi}{\omega} + \tau\right) = x(t) \quad (2.27)$$

because the parameter τ is dependent on ϵ . According to Krylov-Bogolibov , this difficulty can be removed by setting

$$T' = \frac{2\pi}{\omega} + \tau = \frac{2\pi}{\omega}(1 + h_1\epsilon + h_2\epsilon^2 + \dots) \quad (2.28)$$

where T' is the period of the solution of the Equation (2.23) by means of the substitution

$$t = \frac{\tau}{\omega}(1 + h_1\epsilon + h_2\epsilon^2 + \dots) \quad (2.29)$$

Rewriting the Equation (2.23) in terms of the Equation (2.29) gives

$$\frac{d^2x}{d\tau^2} + x(1 + h_1\epsilon + h_2\epsilon^2 + \dots)^2 = \frac{\epsilon}{\omega^2}f(x)(1 + h_1\epsilon + h_2\epsilon^2 + \dots)^2 \quad (2.30)$$

whose period is 2π .

We can now investigate the periodic solution of the Equation (2.30) in a perturbation form

$$x(t) = x^{(0)}(\tau) + \epsilon x^{(1)}(\tau) + \epsilon^2 x^{(2)}(\tau) + \dots \quad (2.31)$$

Since the period is 2π and is independent of the parameter ϵ , the constants in front of the expansion of x are periodic functions with 2π . Plugging the Equation (2.31) into (2.30) produces an equation of motion corresponding to each degree of freedom. Obviously, the first differential equation is

$$\ddot{x}^{(0)} + x^{(0)} = 0 \quad (2.32)$$

The solution of this equation of motion is clearly

$$x^{(0)} = M_0 \cos \tau \quad (2.33)$$

where M_0 is an arbitrary constant. The second differential equation is

$$\ddot{x}^{(1)} + x^{(1)} = -2h_1 M_0 \cos \tau + \frac{1}{\omega^2} f(M_0 \cos \tau) \quad (2.34)$$

This differential equation has periodic solution if

$$\int_0^{2\pi} f(M_0 \cos \tau) \sin \tau d\tau = 0 \quad (2.35)$$

and

$$-2h_1 M_0 + \frac{1}{\pi \omega^2} \int_0^{2\pi} f(M_0 \cos \tau) \cos \tau d\tau = 0 \quad (2.36)$$

Observe the first differential equation determines M_0 , and the second one, on the other hand, determines h_1 . Therefore, we have the following solution for x_1

$$x_1 = \varphi_1 + M_1 \cos \tau + N_1 \sin \tau \quad (2.37)$$

where φ_1 is a certain periodic function of τ with period 2π , and M_1 and N_1 are arbitrary constants. So far we have explained Pioncare-Lindstedt method for a system in one freedom.

Now we are going to discuss results we have obtained after this method has been implemented to the system we are interested in. We see that the equation of the motion for our system can be easily derived from the Hamiltonian given in AppendixA

$$\ddot{q}_i + \omega_i^2 q_i + \epsilon \Gamma_{ijk} q_j q_k = 0 \quad (2.38)$$

where ϵ is a small parameter and q_i are normal coordinates. Imposing the following

expansion,

$$t = \frac{\tau_i}{\Omega_i} = \frac{\tau_i}{\omega_i} (1 + h_i^{(1)} \epsilon + h_2^{(2)} \epsilon^2 + \dots) \quad (2.39)$$

then the equation of motion becomes

$$\ddot{q}_i + q_i(1 + 2\epsilon h_i^{(1)} + 2\epsilon^2 h_i^{(2)} + \dots) = -\epsilon \frac{\Gamma_{ijk}^2}{\omega_i} q_j q_k (1 + 2\epsilon h_i^{(1)} + 2\epsilon^2 h_i^{(2)} + \dots) \quad (2.40)$$

Similarly, if we expand the coordinates as well

$$q_i(\tau_i) = M_i \cos \tau_i + \epsilon q_i^{(1)}(\tau_i) + \epsilon^2 q_i^{(2)}(\tau_i) + \dots \quad (2.41)$$

and after solving the equation of motion we have obtained we will find some dangerous factors in the solution of these equations of motion. At least giving the following part of the solution may recover us from not using this method for the further calculations, i.e:

$$q_i^{(1)} = A_i^{(1)} + A_i^{(2)} \cos 2\tau_i + A_i^{(3)} \cos \alpha_{jk} \tau_i + A_i^{(4)} \cos \beta_{jk} \tau_i \quad (2.42)$$

where

$$A_i^{(1)} = -\frac{\Gamma_{iii} M_i^2}{2\omega_i^2} \quad (2.43)$$

$$A_i^{(2)} = \frac{\Gamma_{iii} M_i^2}{6\omega_i^2} \quad (2.44)$$

$$A_i^{(3)} = -\sum_{j,k(j,k \neq i)} \frac{\Gamma_{ijk} M_j M_k}{2\omega_i^2 (1 - \alpha_{jk}^2)} \quad (2.45)$$

where

$$\alpha_{jk} = \frac{\omega_j + \omega_k}{\omega_i} \quad (2.46)$$

and finally

$$A_i^{(4)} = - \sum_{j,k(j,k \neq i)} \frac{\Gamma_{ijk} M_j M_k}{2\omega_i^2 (1 - \beta_{jk}^2)} \quad (2.47)$$

where

$$\beta_{jk} = \frac{\omega_j - \omega_k}{\omega_i} \quad (2.48)$$

where M_i are constants. Observe that if , by an accident, there appear some resonance frequencies, and of course there will appear because the biological systems have finitely many frequencies, $q_i^{(1)}$ will blow up.

It can show the following quantities have been obtained from the solution of this quasi-harmonic equation

$$h_i^{(1)} = 0 \quad (2.49)$$

and

$$h_i^{(2)} = \sum_i \frac{5}{12} \frac{(\Gamma_{iii})^2 M_i^2}{\omega_i^4} \quad (2.50)$$

thus t becomes

$$t = \sum_i \frac{\tau_i}{\omega_i} \left(1 + \epsilon^2 \frac{5}{12} \frac{(\Gamma_{iii})^2 M_i^2}{\omega_i^4} \right) \quad (2.51)$$

and from the Equation (2.39), the normal frequency corresponding to i^{th} mode is

$$\Omega_i = \sum_i \omega_i \left(1 - \epsilon^2 \frac{5}{12} \frac{(\Gamma_{iii})^2 M_i^2}{\omega_i^4} \right) \quad (2.52)$$

Please notice that in the equation above, the shifted frequencies are amplitude dependent which this coincides with the prediction made by normal mode analysis.

3. CANONICAL PERTURBATION THEORY

3.0.1. Introduction

Many problems in classical mechanics do not have exact solutions. In such cases, we are trying to find approximate solutions for these problems. Even if solving such problems looks quite complicated, some properties of these problems provide relatively easy ways towards the solution. For instance, usually in a physical problem that can not be solved directly, the Hamiltonian contains two main parts, a leading part and a perturbation. The leading part, the *unperturbed Hamiltonian*, is assumed to have an exact solution. The *perturbation Hamiltonian* part, on the other hand, is only a slight deviation from the unperturbed Hamiltonian. Addition of unperturbed Hamiltonian and perturbation Hamiltonian is called the *perturbed Hamiltonian*. The way in which perturbation theory approaches to such problems depends on the form of the potential energy of the system. There are two main approaches in classical perturbation theory, time-dependent perturbation method and time-independent perturbation method. Time-dependent perturbation method is useful if the Hamiltonian of the system is velocity dependent, time-independent method, on the other hand, can be taken into account if the Hamiltonian is time-independent. Because the Hamiltonian of our system is time-independent, we should explain this method briefly.

3.0.2. Canonical Transformations

It is known that canonical transformations may be introduced to rewrite a given Hamiltonian in terms of new coordinates and momenta so that the problem takes a simpler form[20]. Canonical transformations provide us a possibility to write the Hamiltonian in a cyclic form. Unfortunately, we can not always express the Hamiltonian in such a way that all the coordinates are cyclic. Once we obtain the cyclic form of the Hamiltonian in coordinates, we can introduce conjugate momenta such as

$$p_i = \alpha_i \tag{3.1}$$

which are constant. Since the Hamiltonian can not be an explicit function of neither the time nor the cyclic coordinates, it may be rewritten as

$$H = H(\alpha_1 \dots \alpha_n) \quad (3.2)$$

So, from the Hamilton's equations, for \dot{q}_i

$$\dot{q}_i = \frac{\partial H}{\partial \alpha_i} = \omega_i \quad (3.3)$$

where the ω_i are functions of the α_i 's only and therefore are constant in time, so we may write

$$q_i = \omega_i t + \beta_i \quad (3.4)$$

where β_i 's are constant of the integration and can be determined by the initial conditions.

As it was mentioned above, canonical transformation help us construct a transformed Hamiltonian in terms of a new set of coordinates and momenta (Q_i, P_i) . In other words, the transformation we are looking for, converts (q_i, p_i) to (Q_i, P_i) with invertible equation for the transformation, i.e :

$$Q_i = Q_i(q_i, p_i, t) \quad (3.5)$$

$$P_i = P_i(q_i, p_i, t) \quad (3.6)$$

In developing Hamiltonian mechanics, only those transformations can be of interest for which the new set of (Q, P) is canonical coordinates. Possibility to write the

transformed Hamiltonian as $K(Q, P, t)$ and the equations of the motion as

$$\dot{Q}_i = \frac{\partial K}{\partial P_i} \quad (3.7)$$

$$\dot{P}_i = -\frac{\partial K}{\partial Q_i} \quad (3.8)$$

are necessary conditions for a new set of coordinates (Q_i, P_i) to be canonical coordinates.

The function K plays the role of the Hamiltonian, and therefore it can be called *transformed Hamiltonian*. It is important to keep in mind that such transformations are Hamiltonian independent transformations. In other words, what we are doing here is nothing more than constructing the Hamiltonian under the change of the variables in which the Hamiltonian's equations of motion remain the same. Therefore such a transformation is called *canonical transformation*.

If (Q_i, P_i) are canonical coordinates, they must satisfy the Hamilton's Principle, that is

$$\delta \int_{t_1}^{t_2} (P_i \dot{Q}_i - K(Q, P, t)) dt = 0 \quad (3.9)$$

This principle must also hold for the old coordinates

$$\delta \int_{t_1}^{t_2} (p_i \dot{q}_i - H(q, p, t)) dt = 0 \quad (3.10)$$

The simultaneous validity of Equations (3.9) and (3.10) does not mean that the integrand must be equal to each other, but they could differ by an exact differential. Therefore, one can conclude that there must be a generating function F , which converts old generalized coordinates and momenta (q_i, p_i) to new generalized coordinates

and momenta (Q_i, P_i) . Thus,

$$p_i \dot{q}_i - H(q, p, t) = P_i \dot{Q}_i - K(Q, P, t) + \frac{dF}{dt} \quad (3.11)$$

Dependence of F on the coordinates introduces specific canonical transformations. In other words, the type of the problem determines naturally the appropriate generating function. Since we will use specifically a generating function of the $F = F_2(q, P, t)$, we should better find the canonical transformation equations generated by F_2 . This can be accomplished by plugging the following generating function F_2 into Equation (3.11)

$$F = F_2(q, P, t) - Q_i P_i \quad (3.12)$$

Thus, plugging the Equation (3.12) into Equation (3.11) leads to

$$p_i \dot{q}_i - H = P_i \dot{Q}_i - K + \frac{d}{dt} F_2(q, P, t) \quad (3.13)$$

$$p_i \dot{q}_i - H = P_i \dot{Q}_i - K + \frac{\partial F_2}{\partial t} + \frac{\partial F_2}{\partial q_i} \dot{q}_i + \frac{\partial F_2}{\partial P_i} \dot{P}_i - \dot{Q}_i P_i - \dot{P}_i Q_i \quad (3.14)$$

Collecting the coefficients of the right side of Equation (3.14) leads to the following equations

$$p_i = \frac{\partial F_2}{\partial q_i}, \quad (3.15)$$

$$Q_i = \frac{\partial F_2}{\partial P_i} \quad (3.16)$$

with

$$K = H + \frac{\partial F_2}{\partial t} \quad (3.17)$$

Equation(3.15) are to be solved for P_i as a function of q_j , p_j , and t to correspond to second half of the transformation Equations (3.5) and (3.6). The remaining half of the transformation equations is then provided by (3.6).

3.0.3. Hamilton-Jacobi Theory

The new variables Q_i and P_i are chosen to be constant in time by requiring that the transformed Hamiltonian, K , should be identically zero, therefore equations of motion are[20]

$$\frac{\partial K}{\partial P_i} = \dot{Q}_i = 0 \quad (3.18)$$

$$-\frac{\partial K}{\partial Q_i} = \dot{P}_i = 0 \quad (3.19)$$

Thus, K must be related to the old Hamiltonian and to the generating function by the equation

$$K = H + \frac{\partial F}{\partial t} \quad (3.20)$$

and hence will be zero if F satisfies the equation

$$H(q, p, t) + \frac{\partial F}{\partial t} = 0 \quad (3.21)$$

If F is described in terms of old coordinates, q_i , and the new momenta, P_i , then one of the equation of the transformation is

$$p_i = \frac{\partial F_2}{\partial q_i} \quad (3.22)$$

where $F = F_2(q, P, t)$. Consequently, Equation (3.21) becomes

$$H(q_1, \dots, q_n; \frac{\partial F_2}{\partial q_1}, \dots, \frac{\partial F_2}{\partial q_n}; t) + \frac{\partial F_2}{\partial t} = 0 \quad (3.23)$$

The Equation (3.23) is called Hamilton-Jacobi equation. Obviously, it contains a partial differential equation in $n+1$ variables, q_1, q_2, \dots, q_n , and t . The solution of (3.23) is conventionally denoted by S , which is known as *Hamiltonian's principle function*. Because the integration of Equation (3.23) provides the dependence on old coordinates and time, it gives us a chance to choose specific momenta suitable to the system we are interested in. Equation (3.23) has a form of the first-order partial differential equation in $n+1$ variables. Suppose that the solution of Equation (3.23) is denoted by

$$F_2 = S(q_1, \dots, q_n; \alpha_1, \dots, \alpha_{n+1}; t) \quad (3.24)$$

where the quantities $\alpha_1, \dots, \alpha_{n+1}$ are $n+1$ independent constants of integration. One of the constants of integration, however, is in fact irrelevant to the solution, for it will be noted that S itself does not appear in equation (3.23); only its partial derivatives with respect to q or t are involved. Hence, if S is some solution of the differential equations, then $S + \alpha$, where α is any constant, must also be a solution. Because the additive constant does not have any importance on solving the Hamilton-Jacobi equation, the complete solution to Equation (3.23) must be of the form

$$S = S(q_1, \dots, q_n; \alpha_1, \dots, \alpha_n; t) \quad (3.25)$$

Since we are free to take n constants of integration to be the new momenta we can set

$$P_i = \alpha_i \quad (3.26)$$

the transformation equations can now be written as

$$p_i = \frac{\partial S(q, \alpha, t)}{\partial q_i} \quad (3.27)$$

where q_i and α are the complete set of quantities. The other half of the equations of transformation, which provide the new coordinates, appear to be in the form of

$$Q_i = \beta_i = \frac{\partial S(q, \alpha, t)}{\partial \alpha_i} \quad (3.28)$$

Equation (3.28) can be rewritten as

$$q_j = q_j(\alpha, \beta, t) \quad (3.29)$$

which solves the problem of giving the coordinates as functions of time and β . After differentiation in Equation (3.27) has been performed, Equation (3.29) may be substituted for the q 's, thus giving the momenta p_i as functions of α , β , and t

$$p_j = p_j(\alpha, \beta, t) \quad (3.30)$$

Equations (3.29), and (3.30) contain the desired complete solution of the Hamilton 's equations of motion.

To understand the physical meaning of S , let us take the total derivative of S with respect to time

$$\frac{dS}{dt} = \frac{\partial S}{\partial q_i} \dot{q}_i + \frac{\partial S}{\partial t} \quad (3.31)$$

since P_i 's are constant in time. By the equations 3.27 and (3.23) this relation can also be written as

$$\frac{dS}{dt} = p_i \dot{q}_i - H = L \quad (3.32)$$

so that

$$S = \int L dt + constant \quad (3.33)$$

Unlike Hamilton's principle, which is a statement about the definite integral of L , Equation 3.33 is an indefinite integral form of the Lagrangian and it is solving the same problem in completely different way.

3.0.4. Action-Angle Variables

There are periodic motions in many branch of physics. Usually, we are not interested in the details of the orbit as in the frequency of the motion. A very effective method for solving such systems is provided by Hamilton-Jacobi procedure. In this technique, the integration constants α_i appearing directly in the solution of Hamilton-Jacobi equation are not themselves chosen to be the new momenta. Instead, new constants, J_i , are introduced to handle the problem. These new constants are known as *action variables*[20].

For simplicity we shall first consider action-angle variables in one degree of freedom. It is assumed that the system is conservative so that the Hamiltonian can be written as

$$H(q, p) = E \tag{3.34}$$

where E is a constant. Solving for the momentum, we find that

$$p = p(q, E) \tag{3.35}$$

equation (3.34) in general describe the orbit traced out by the system point in two-dimensional phase space when the Hamiltonian is a constant of motion. What is meant by the periodic motion is determined by the characteristics of the phase space orbit. There are two kinds of periodic motions:

1. In the first type, the orbit is *closed* and the system point retraces its steps periodically. Both q and p are then periodic functions of time with the same frequency.

2. In the second type of periodic motion, the orbit in the phase space is such that p is some periodic function of q , with period q_0 . Equivalently, this kind of motion implies that when q is increased by q_0 , the configuration of the system remains essentially the same.

For either type of motion, one can introduce a new variable J designated to replace E . The so-called action variable is defined as

$$J = \oint pdq \quad (3.36)$$

where the integration is to be carried over one complete cycle. Eq.(3.34) also implies that

$$E \equiv H = H(J) \quad (3.37)$$

Therefore, one can compute the Hamilton's characteristic function as follows

$$W = W(q, J). \quad (3.38)$$

The generalized coordinate conjugate to J , the *angle variable* w , is defined by the transformation equation

$$w = \frac{\partial W}{\partial J} \quad (3.39)$$

Correspondingly, the equation of the motion for w is

$$\dot{w} = \frac{\partial H(J)}{\partial J} = \nu(J) \quad (3.40)$$

where ν is a constant function of J only. Equation (3.40) has the solution

$$w = \nu t + \beta \quad (3.41)$$

so that w is a linear function of time.

So far the action-angle variables are considered to be the transformed coordinates and momenta to which the Hamilton-Jacobi equation follows.

Now consider the change in w as q goes over one complete cycle:

$$\Delta w = \oint \frac{\partial w}{\partial q} dq. \quad (3.42)$$

By the Equation (3.39) this can also be written

$$\Delta w = \oint \frac{\partial^2 W}{\partial q \partial J} dq. \quad (3.43)$$

Because J is independent of q , the derivative of it with respect to J can be taken outside the integral

$$\Delta w = \frac{d}{dJ} \oint \frac{\partial W}{\partial J} dq = \frac{d}{dJ} \oint pdq = 1 \quad (3.44)$$

Equation (3.44) states that w changes by unity as q makes a complete tour through one cycle.

To grasp the physical meaning of ν , which is defined in Equation (3.40), let τ be the period for a complete cycle of q , then

$$\Delta w = 1 = \nu \tau \quad (3.45)$$

Hence the constant ν can be identified as the reciprocal of the period,

$$\nu = \frac{1}{\tau} \quad (3.46)$$

and is therefore *the frequency associated with the periodic motion of q* . The use of action-angle variables thus provides a powerful technique for obtaining the frequency

of the periodic motion *without a complete solution to the motion of the system.*

3.0.5. Time-independent Perturbation Method

Time-independent perturbation method is looking for an approximate solution of the Hamiltonian given in the form[20]

$$H = H_0 + \epsilon H_1 + \epsilon^2 H_2 + \dots \quad (3.47)$$

where the H_0 is called the unperturbed Hamiltonian and we assume that H_0 has an exact solution, whereas ϵH_1 ,and $\epsilon^2 H_2$ are only slight shifts from the unperturbed Hamiltonian. Time-independent perturbation method is looking for the frequencies of the perturbed Hamiltonian H . Additionally, time-independent perturbation method can be applied only to conservative systems in which the potential energy is velocity independent. To find the frequencies of the perturbed Hamiltonian , H , one should first express the unperturbed Hamiltonian in terms of the action-angle variables determined from unperturbed Hamiltonian. Once we obtain the unperturbed Hamiltonian in terms of action-angle variables, we can write the perturbed Hamiltonian H in terms of new action-angle variables. This process is nothing more than a canonical transformation from the set (w, J) to $(\underline{w}, \underline{J})$ where \underline{w} and \underline{J} are new action-angle variables or transformed action-angle variables. Since the transformation from (w, J) to $(\underline{w}, \underline{J})$ is a canonical transformation, it must satisfy the properties of a canonical transformation which are mentioned previously. At the same time, one must keep in mind that the transformed Hamiltonian, \underline{H} , must be cyclic in the new angle variables, \underline{w} .

Now, we assume a set of action-angle variables (w, J) for the unperturbed Hamiltonian H_0 , so we now write $H_0(J)$. The unperturbed frequency is given by

$$\nu = \frac{\partial H_0}{\partial J} \quad (3.48)$$

with

$$w = \nu t + \beta \quad (3.49)$$

Because H_0 is periodic in w with period 1, from Equation (3.45), the coordinate and momenta can be expanded in Fourier series in the form

$$q = \sum_{\kappa=-\infty}^{+\infty} A_{\kappa}(J) \exp(2\pi i \kappa \cdot w) \quad (3.50)$$

with the similar expression for p . Now suppose that our perturbation Hamiltonian contains the small change from the unperturbed Hamiltonian H_0 up to the order ϵ , i.e:

$$H = H_0 + \epsilon H_1 \quad (3.51)$$

where ϵ is some small parameter that can be varied continuously from 0. Since the perturbed system, H , is still periodic, for sufficiently small values of ϵ , there must be a new set of action-angle variables $(\underline{w}, \underline{J})$ suitable to the system, with $H = H(\underline{J})$. Since such a transformation is a canonical transformation, the transformed action variables \underline{J} must be constant and the transformed angle variables \underline{w} must be a linear function of time:

$$\underline{w} = \underline{\nu} t + \underline{\beta} \quad (3.52)$$

with the new frequency $\underline{\nu}$ to be obtained from $H(\underline{J})$. Nonetheless w, J remain canonical variables in the perturbed system. In other words, if we assume that $H(\underline{J})$ is integrable and hence has its action angle variables $(\underline{w}, \underline{J})$, there must exist canonical transformations from the set (q, p) to both (w, J) and $(\underline{w}, \underline{J})$. As has been emphasized previously, the canonical property of a transformation, and therefore such a set of variables, is independent of the specific form of the Hamiltonian. But when we transform J to \underline{J} , in the transformed perturbation Hamiltonian, \underline{J} does not remain constant and hence \underline{w} is not a linear function of time. At this point, we should notice that the coordinates and momenta q and p , are still periodic function of w and from

Equation (3.45) both w and \underline{w} increase by unity as q goes through one complete period of motion. The two sets of canonical variables, (w, J) and $(\underline{w}, \underline{J})$, must be related by a canonical transformation with some generating function Y . It is worth using the second type of generating function which is a function of old angle variables w_i and new action variables \underline{J} in this canonical transformation. Since our Hamiltonian deviates from unperturbed Hamiltonian H_0 slightly, we can use a generating function such that it deviates slightly from the identity transformation, i.e:

$$Y(w, \underline{J}) = w\underline{J} + \epsilon Y_1(w, \underline{J}) + \epsilon^2 Y_2(w, \underline{J}) + \dots \quad (3.53)$$

We are looking for the functional dependence of H on \underline{J} so that the perturbed frequency ν can be found. If the functional dependence of perturbation Hamiltonian H on \underline{J} , and ϵ is given by $\underline{H}(\underline{J}, \epsilon)$, one can expand $\underline{H}(\underline{J}, \epsilon)$ as

$$\underline{H}(\underline{J}, \epsilon) = \underline{H}_0(\underline{J}) + \epsilon \underline{H}_1(\underline{J}) + \epsilon^2 \underline{H}_2(\underline{J}) + \dots \quad (3.54)$$

Hence Hamilton-Jacobi equation can be expressed as

$$H(w, J) = H\left(w, \frac{\partial Y}{\partial w}\right) = \underline{H}(\underline{J}, \epsilon). \quad (3.55)$$

One can write Hamilton-Jacobi equation to second order in ϵ^2 as

$$H_0\left(\frac{\partial Y}{\partial w}\right) + \epsilon H_1\left(w, \frac{\partial Y}{\partial w}\right) + \epsilon^2 H_2\left(w, \frac{\partial Y}{\partial w}\right) = \underline{H}_0(\underline{J}) + \epsilon \underline{H}_1(\underline{J}) + \epsilon^2 \underline{H}_2(\underline{J}). \quad (3.56)$$

Meanwhile, notice that the equation linking \underline{J} to J is

$$J = \frac{\partial Y}{\partial w} = \underline{J} + \epsilon \frac{\partial Y_1}{\partial w} + \epsilon^2 \frac{\partial Y_2}{\partial w} \quad (3.57)$$

We again expand the terms H_0, H_1, H_2 in Taylor series around $J = \underline{J}$, including the

terms ϵ^2 in H_0 up to terms of ϵ in H_1 , and with J_i replaced directly by \underline{J} in H_2 . So the expansions have been performed, H 's up to terms of order ϵ^2 takes the following form

$$\begin{aligned} H_0 \left(\underline{J} + \epsilon \frac{\partial Y_1}{\partial w} + \epsilon^2 \frac{\partial Y_2}{\partial w} + \dots \right) &= H_0(\underline{J}) + \left(\epsilon \frac{\partial Y_1}{\partial w} + \epsilon^2 \frac{\partial Y_2}{\partial w} \right) \frac{\partial H_0}{\partial \underline{J}} + \\ &+ \frac{1}{2} \left(\epsilon \frac{\partial Y_1}{\partial w} \right)^2 \frac{\partial^2 H_0}{\partial \underline{J}^2} \end{aligned} \quad (3.58)$$

$$\begin{aligned} H_1 \left(w, \frac{\partial Y}{\partial w} \right) &= H_1 \left(w, \underline{J} + \epsilon \frac{\partial Y_1}{\partial w} + \dots \right) \\ &= H_1(w, \underline{J}) + \epsilon \frac{\partial Y_1}{\partial w} \frac{\partial H_1}{\partial \underline{J}} \end{aligned} \quad (3.59)$$

$$H_2 \left(w, \frac{\partial Y}{\partial w} \right) = H_2(w, \underline{J}) \quad (3.60)$$

As was emphasized before, since unperturbed Hamiltonian, H_0 , has its action-angle variables (w, J) , the transformed unperturbed Hamiltonian should be of the form

$$H_0 = \nu J \quad (3.61)$$

which will be clarified in the next subsection. Thus,

$$\frac{\partial H_0}{\partial J} = \nu \quad (3.62)$$

So, Equation (3.58) becomes

$$\begin{aligned} H_0 \left(\underline{J} + \epsilon \frac{\partial Y_1}{\partial w} + \epsilon^2 \frac{\partial Y_2}{\partial w} + \dots \right) &= H_0(\underline{J}) + \left(\epsilon \frac{\partial Y_1}{\partial w} + \epsilon^2 \frac{\partial Y_2}{\partial w} \right) \nu + \\ &+ \frac{1}{2} \left(\epsilon \frac{\partial Y_1}{\partial w} \right)^2 \frac{\partial^2 H_0}{\partial \underline{J}^2} \end{aligned} \quad (3.63)$$

Furthermore, Equation (3.61) says H_0 is linear in J , so the last term of Equation (3.63) drops out

$$\frac{\partial^2 H_0}{\partial \underline{J}^2} = 0. \quad (3.64)$$

Therefore, Equation (3.63) becomes

$$H_0 \left(\underline{J} + \epsilon \frac{\partial Y_1}{\partial w} + \epsilon^2 \frac{\partial Y_2}{\partial w} + \dots \right) = H_0(\underline{J}) + \left(\epsilon \frac{\partial Y_1}{\partial w} + \epsilon^2 \frac{\partial Y_2}{\partial w} \right) \nu \quad (3.65)$$

Collecting the powers of ϵ in Equation (3.56) leads to the following expressions for \underline{H} :

$$\underline{H}_0(\underline{J}) = H_0(\underline{J}), \quad (3.66)$$

$$\underline{H}_1(\underline{J}) = \nu \frac{\partial Y_1}{\partial w} + H_1(w, \underline{J}), \quad (3.67)$$

$$\underline{H}_2(\underline{J}) = \nu \frac{\partial Y_2}{\partial w} + \frac{\partial Y_1}{\partial w} \frac{\partial H_1}{\partial \underline{J}} + H_2(w, \underline{J}) \quad (3.68)$$

Now, the transformation of \underline{w} to w is given by

$$\underline{w} = \frac{\partial Y}{\partial \underline{J}} = w + \epsilon \frac{\partial Y_1}{\partial \underline{J}} + \epsilon^2 \frac{\partial Y_2}{\partial \underline{J}} + \dots \quad (3.69)$$

In order for (q, p) set to be periodic in both w and \underline{w} with period 1, all the terms in the perturbation part of the generating function Y must be periodic functions of w , that is

$$Y_n(w, \underline{J}) = \sum_{\kappa} B_{\kappa}^n(\underline{J}) \exp(2\pi i \kappa . w) \quad (3.70)$$

Because the average of the first terms in Equations (3.67) and (3.68) over one complete cycle is zero, taking the average of the Equations (3.66), (3.67), (3.68) over the period

of w reduces these equations to

$$\underline{H}_0(\underline{J}) = H_0(\underline{J}) \quad (3.71)$$

$$\underline{H}_1(\underline{J}) = \langle H_1(w, \underline{J}) \rangle \quad (3.72)$$

$$\underline{H}_2(\underline{J}) = \left\langle \frac{\partial Y_1}{\partial w} \frac{\partial H_1}{\partial \underline{J}} \right\rangle + \langle H_2(w, \underline{J}) \rangle \quad (3.73)$$

Please notice that the first part of equation Equation (3.73) does not have to vanish. It is true that the average of derivatives of Y_n with respect to (w) is zero, but it is not guaranteed that the average of the product vanishes. Hence, to find the second-order correction to frequency of the Hamiltonian, one needs the first-order canonical transformation. Now it is time to apply all the theory we have reviewed so far to a non-linear differential equation in many degrees of freedom.

3.0.6. A General Periodic Solution for Nonlinear Bimolecular Systems

In this part we are investigating the temperature dependent frequencies of the collective motions of the proteins. We first consider protein to have N residues and these residues are connected by springs. Because we have N residues and the motions take place in 3-dimensions, there must be $3N - 6$ degrees of freedom for vibrational motion of the proteins.

To determine the Hamiltonian of the system, we first compute the potential energy of system. After we have obtained the Hamiltonian of this system, we took advantage of Taylor expansion to expand the potential energy of the system around equilibrium points. The potential energy which we have obtained after the expansion contains the coupled quadratic, the cubic terms, and the other higher order terms. But for convenience, we keep system in the 3rd order.

After normal mode analysis has been implemented, the Hamiltonian of the pro-

teins become(See Appendix A)

$$H = \sum_i \frac{p_i^2}{2} + \frac{\Omega_i^2}{2} q_i^2 + \epsilon \sum_{i,j,k} \Gamma_{ijk} q_i q_j q_k \quad (3.74)$$

where $\epsilon \ll 1$ and q_i are normal coordinates. Here ϵ is put by hand to keep track of perturbation expansion. The small term are already in Γ_{ijk} . Γ_{ijk} are constants coming naturally in front of the cubic terms in the Hamiltonian after the normal mode analysis has been performed. From the form of the Hamiltonian, we see that Γ_{ijk} could be totally symmetrized in (i, j, k) indices. Now we can start to implement time-independent perturbation method. Remember that time-independent perturbation method is looking for a solution for Hamiltonian of the form

$$H = H_0 + \epsilon H_1 + \epsilon^2 H_2 + \dots \quad (3.75)$$

where H_0 is the unperturbed Hamiltonian, and the other parts, ϵH_1 , $\epsilon^2 H_2$ are small deviations from the unperturbed Hamiltonian, H_0 . One must keep in mind that to take the further steps toward the solution of the perturbed Hamiltonian, the unperturbed part should have an exact solution.

Fortunately, the equation we are interested in resembles to the Equation (3.75) and immediately one can see that

$$\begin{aligned} H_0 &= \sum_i \frac{p_i^2}{2} + \frac{\Omega_i^2}{2} q_i^2 \\ H_1 &= \sum_{i,j,k} \Gamma_{ijk} q_i q_j q_k \\ H_2 &= 0 \end{aligned} \quad (3.76)$$

Now, let us investigate the exact solution of the unperturbed Hamiltonian, H_0 . We know that the unperturbed Hamiltonian is an integrable system and hence it has its action-angle variables, which can be denoted by the set (w_i, J_i) . To find the action angle-variables of the unperturbed Hamiltonian, H_0 , one must construct the Hamilton-

Jacobi equation for the system. So, from Equation (3.23) one can write Hamilton-Jacobi equation for the unperturbed Hamiltonian as

$$H_0 \left(q_i, \frac{\partial S}{\partial q_i} \right) + \frac{\partial S}{\partial t} = 0 \quad (3.77)$$

where we set p_i equal to

$$p_i = \frac{\partial S}{\partial q_i} \quad (3.78)$$

and S is Hamilton's principle function which is to be determined later. Since the Hamiltonian is separable, we look for a separable solution in which

$$S = \sum_i^N S_i(q_i) \quad (3.79)$$

So, Hamilton-Jacobi equation for unperturbed Hamiltonian becomes

$$\frac{1}{2} \left[\left(\frac{\partial S_i}{\partial q_i} \right)^2 + \Omega_i^2 q_i^2 \right] + \frac{\partial S_i}{\partial t} = 0 \quad (3.80)$$

Since the explicit dependence of S on t is involved only in the last term, a solution for (3.80) can be found in the form

$$S_i(q_i, E_i, t) = W_i(q_i, E_i) - E_i t \quad (3.81)$$

where E_i is a constant. We see that the constants E_i are nothing more than the energy corresponding to each normal mode. As for W_i , it is a characteristic function which separates the coordinates and momenta from the time variable. Also notice that in Equation (3.81), since unperturbed Hamiltonian is cyclic in time parameter, the term E_i enters into Equation (3.81) as a constant parameter corresponding to time variable. Having proposed a type of function for the solution of Hamilton-Jacobi equation, one

can reorganize that Equation (3.80) becomes

$$\frac{1}{2} \left[\left(\frac{\partial W_i}{\partial q_i} \right)^2 + \Omega_i^2 q_i^2 \right] = E_i \quad (3.82)$$

Now one can integrate Equation (3.82) immediately to

$$W_i(q_i, E_i) = \int dq_i \sqrt{2E_i - \Omega_i^2 q_i^2}$$

or after taking Ω_i out of the square root one can write the equation above as follows

$$W_i(q_i, E_i) = \int dq_i \Omega_i \sqrt{\frac{2E_i}{\Omega_i^2} - q_i^2} \quad (3.83)$$

Plugging Equation (3.83) into Equation (3.81), immediately one can see that the Hamilton's Principle function is :

$$S_i(q_i, E_i, t) = \int dq_i \Omega_i \sqrt{\frac{2E_i}{\Omega_i^2} - q_i^2} - E_i t \quad (3.84)$$

Due to the fact that the integral is an indefinite one , we do not have to carry integral out for the future use. Let's now turn our attention to the calculation of the action variables , J_i , for the unperturbed Hamiltonian, H_0 . Similar to action variable which is defined in Equation (3.36), the action variables in many degrees of freedom can be expressed as follows

$$J_i = \oint p_i dq_i \quad (3.85)$$

To find the integrand p_i in Equation (3.85), let's find p_i from the following equation

$$\frac{1}{2} (p_i^2 + \Omega_i^2 q_i^2) = E_i \quad (3.86)$$

One can immediately find p_i as a function of E_i and q_i from the equation above, i.e :

$$p_i = \sqrt{2E_i - \Omega_i^2 q_i^2} \quad (3.87)$$

Thus, the Equation (3.85) becomes

$$J_i = \oint dq_i \sqrt{2E_i - \Omega_i^2 q_i^2} \quad (3.88)$$

Unlike the indefinite integral in the Equation (3.84), the definite integral in the Equation (3.88) can be found immediately. To determine the boundaries of the closed integral in Equation (3.88), one must find the critical points of the integrand. To do so, we set the integrand equal to zero

$$\sqrt{2E_i - \Omega_i^2 q_i^2} = 0$$

Thus, we have two critical points which determine the boundaries of the integral, i.e :

$$q_i = \mp \sqrt{\frac{2E_i}{\Omega_i^2}}$$

So, the definite integral (3.88) with the boundaries becomes

$$J_i = 2 \int_{-\sqrt{\frac{2E_i}{\Omega_i^2}}}^{+\sqrt{\frac{2E_i}{\Omega_i^2}}} dq_i \Omega_i \sqrt{\frac{2E_i}{\Omega_i} - q_i^2} \quad (3.89)$$

the factor 2 in the equation above enters into the calculation of the definite integral due to the fact that the orbit of the harmonic part of the Hamiltonian is closed. Setting q_i as

$$q_i = \sqrt{\frac{2E_i}{\Omega_i}} \sin \theta_i$$

and hence

$$dq_i = \sqrt{\frac{2E_i^2}{\Omega_i}} \cos \theta_i dq_i$$

results in

$$\begin{aligned} J_i &= 2 \int_{-\frac{\pi}{2}}^{+\frac{\pi}{2}} d\theta_i \Omega_i \frac{2E_i}{\Omega_i^2} \cos^2 \theta_i \\ &= \frac{4E_i}{\Omega_i} \int_{-\frac{\pi}{2}}^{+\frac{\pi}{2}} d\theta_i \cos^2 \theta_i \end{aligned}$$

Finally one gets immediately the action variables from the calculations above:

$$J_i = \frac{2\pi E_i}{\Omega_i} \quad (3.90)$$

Equation (3.83) is also true for the other indexed variables. It is worth mentioning Ω_i here. This is angular frequency corresponding to i^{th} mode and it was obtained from normal analysis. Now, let's turn our attention to find the angle variables w_i of the integrable Hamiltonian, H_0 . Similar to equation (3.39) the generalized coordinate conjugate to J_i , the angle variables w_i is introduced through the transformation equation given below

$$w_i = \frac{\partial W_i}{\partial J_i} \quad (3.91)$$

From equation(3.90) one can conclude that

$$E_i = \frac{\Omega_i J_i}{2\pi}$$

therefore equation(3.83) becomes

$$W_i = \int dq_i \Omega_i \sqrt{\frac{J_i}{\pi \Omega_i} - q_i^2} \quad (3.92)$$

Replacing equation(3.92) into equation(3.92), one can find the following indefinite integral

$$w_i = \frac{\partial}{\partial J_i} \int dq_i \Omega_i \sqrt{\frac{J_i}{\pi \Omega_i} - q_i^2} \quad (3.93)$$

Since the J_i is independent of q_i , after the canonical transformation has been performed, the derivative can be taken inside integral. Taking the derivative of Equation (3.93) produces

$$w_i = \frac{1}{2\pi} \int \frac{dq_i}{\sqrt{\frac{J_i}{\pi \Omega_i} - q_i^2}}$$

The solution of such an indefinite integral is well known and it is

$$w_i = \frac{1}{2\pi} \arcsin \frac{q_i}{\sqrt{\frac{J_i}{\pi \Omega_i}}} \quad (3.94)$$

The Equation (3.94) can be immediately inverted to find q_i as a function of angle variable w_i . Thus,

$$q_i = \sqrt{\frac{J_i}{\pi \Omega_i}} \sin 2\pi w_i \quad (3.95)$$

Replacing the following canonical transformation we have obtained before into the unperturbed Hamiltonian, H_0 , we can write

$$p_i = \sqrt{\frac{J_i \Omega_i}{\pi}} \cos 2\pi w_i, \quad q_i = \sqrt{\frac{J_i}{\pi \Omega_i}} \sin 2\pi w_i$$

So one can obtain

$$H_0 = \frac{\Omega_i J_i}{2\pi}$$

or

$$H_0(J_i) = \frac{\Omega_i J_i}{2\pi} = E_i \quad (3.96)$$

The oscillation frequencies or the equation of motion for angle variables can be obtained from the Equation (3.40) as follows

$$\dot{w}_i = \frac{\partial H_0(J_i)}{\partial J_i} = \frac{\Omega_i}{2\pi} = \nu_i \quad (3.97)$$

where ν_i are the frequencies of the oscillations and Ω_i are the eigenvalues of the matrix

$$M^{-\frac{1}{2}} K M^{-\frac{1}{2}}$$

Therefore, having found the action-angle variables of the integrable unperturbed Hamiltonian, one can express the perturbed Hamiltonian, H , in terms of these action-angle variables, i.e:

$$H(w_i, J_i) = \sum_i H_0(J_i) + \epsilon H_1(w_i, J_i) \quad (3.98)$$

where

$$H_0(J_i) = \sum_i \nu_i J_i \quad (3.99)$$

$$H_1(w_i, J_i) = \sum_{i,j,k} \Gamma_{ijk} \left(\frac{J_i J_j J_k}{\pi^3 \Omega_i \Omega_j \Omega_k} \right)^{\frac{1}{2}} \sin 2\pi w_i \cdot \sin 2\pi w_j \cdot \sin 2\pi w_k \quad (3.100)$$

As was explained before, J_i are the action variables of the unperturbed Hamiltonian, whereas w_i are the generalized coordinates corresponding to the angle variables, or simply, they are action variables which are derived from the unperturbed Hamiltonian, $H_0(J_i)$. Meanwhile, Ω_i are constants corresponding to each normal mode and they have the following relation with the frequencies of the unperturbed Hamiltonian,

i.e;

$$\Omega_i = 2\pi\nu_i$$

where ν_i are the oscillations frequencies. Because our assumption is based on the periodicity of perturbed Hamiltonian, $H(w_i, J_i)$, one can find a new set of action-angle variables $(\underline{w}_i, \underline{J}_i)$ so that perturbed Hamiltonian can be expressed as a function of \underline{J}_i only. Let us denote the functional dependence of the perturbed Hamiltonian on \underline{J}_i and ϵ by \underline{H} . Similar to the perturbed Hamiltonian which is a function of the old action-angle variables, the transformed Hamiltonian \underline{H} can be expanded as follows

$$\underline{H}(J_i, \epsilon) = \sum_i \underline{H}_0(J_i) + \epsilon \underline{H}_1(J_i) + \epsilon^2 \underline{H}_2(J_i) \quad (3.101)$$

Observe that the perturbed Hamiltonian \underline{H} is a constant of the motion due to fact that it is cyclic in all new angle variables, \underline{w}_i .

Similar to the Equation (3.53), the generating function of canonical transformation from (w_i, J_i) to $(\underline{w}_i, \underline{J}_i)$ is given by

$$Y(w_i, \underline{J}_i) = \sum_i w_i \underline{J}_i + \epsilon Y_1(w_i, \underline{J}_i) + \epsilon^2 Y_2(w_i, \underline{J}_i) + \dots \quad (3.102)$$

where w_i and \underline{J}_i are old angle variables and new action variables, respectively.

Now Hamilton-Jacobi equation is

$$H\left(w_i, \frac{\partial Y}{\partial W_i}\right) = \underline{H}(\underline{J}_i) \quad (3.103)$$

Since the right side of Equation (3.103) is independent of the action variable \underline{w}_i , and H is analytic, we can expand both sides of the equality as follows

$$H_0\left(\frac{\partial Y}{\partial w_i}\right) + \epsilon H_1\left(w_i, \frac{\partial Y}{\partial w_i}\right) + \dots = \underline{H}_0(\underline{J}_i) +$$

$$+ \epsilon \underline{H}_1(\underline{J}_i) + \epsilon^2 \underline{H}_2(\underline{J}_i) + \dots \quad (3.104)$$

Similar to Hamilton-Jacobi equation in which we replaced all momenta by the derivatives of the generating functions with respect to the coordinates, the conjugate momenta J_i are replaced by the derivatives of the generating function Y with respect to the coordinates, w_i . Thanks to this relation, we obtain the following transformation equation

$$J_i = \frac{\partial Y}{\partial w_i} = \underline{J}_i + \epsilon \frac{\partial Y_1}{\partial w_i} + \epsilon^2 \frac{\partial Y_2}{\partial w_i} \quad (3.105)$$

where we take advantage of generating function given in the Equation (3.102).

Using the Equation (3.102), one can get the following forms for the left side of the Equation (3.104)

$$\begin{aligned} H_0 \left(\underline{J}_i + \epsilon \frac{\partial Y_1}{\partial w_i} + \epsilon^2 \frac{\partial Y_2}{\partial w_i} + \dots \right) &= H_0(\underline{J}_i) + \epsilon \sum_i \frac{\partial Y_1}{\partial w_i} \frac{\partial H_0}{\partial J_i} \Big|_{J_i=\underline{J}_i} + \epsilon^2 \sum_i \frac{\partial Y_2}{\partial w_i} \frac{\partial H_0}{\partial J_i} \Big|_{J_i=\underline{J}_i} \\ &+ \epsilon^2 \sum_i \frac{1}{2} \frac{\partial^2 Y_1}{\partial w_i^2} \frac{\partial^2 H_0}{\partial J_i^2} \Big|_{J_i=\underline{J}_i} \end{aligned} \quad (3.106)$$

$$H_1 \left(w_i, \underline{J}_i + \epsilon \frac{\partial Y_1}{\partial w_i} \right) = H_1(w_i, \underline{J}_i) + \epsilon \sum_i \frac{\partial Y_1}{\partial w_i} \frac{\partial H_1}{\partial J_i} \Big|_{J_i=\underline{J}_i} \quad (3.107)$$

where we have expand the terms H_0 and H_1 in a Taylor series around $J_0 = \underline{J}_i$. Since H_0 is linear in J_i the last term in the Equation (3.106) drops out. Furthermore, using the following relation, one can immediately rewrite the Equations (3.107) and (3.106) as follows

$$H_0 = H_0(\underline{J}_i) + \epsilon \sum_i \nu_i \frac{\partial Y_1}{\partial w_i} + \epsilon^2 \sum_i \nu_i \frac{\partial Y_2}{\partial w_i} \quad (3.108)$$

$$H_1 = H_1(w_i, \underline{J}_i) + \epsilon \sum_i \frac{\partial Y_1}{\partial w_i} \frac{\partial H_1}{\partial J_i} \Big|_{J_i=\underline{J}_i} \quad (3.109)$$

From the Equation (3.104) immediately one can see the following relations

$$\underline{H}_0(\underline{J}_i) = H_0(\underline{J}_i) \quad (3.110)$$

$$\underline{H}_1(\underline{J}_i) = \sum_i \nu_i \frac{\partial Y_1}{\partial w_i} + H_1(w_i, \underline{J}_i) \quad (3.111)$$

$$\underline{H}_2(\underline{J}_i) = \sum_i \nu_i \frac{\partial Y_2}{\partial w_i} + \sum_i \frac{\partial Y_1}{\partial w_i} \frac{\partial H_1}{\partial J_i} \Big|_{J_i=\underline{J}_i} \quad (3.112)$$

We should take average the equations above over the period of angle variable w_i

$$\langle f \rangle = \int \prod_i dw_i f(w_i, \dots) \quad (3.113)$$

$$\underline{H}_0(\underline{J}_i) = H_0(\underline{J}_i) \quad (3.114)$$

$$\underline{H}_1(\underline{J}_i) = \left\langle \nu_i \frac{\partial Y_1}{\partial w_i} \right\rangle + \langle H_1(w_i, \underline{J}_i) \rangle, \quad (3.115)$$

$$\underline{H}_2(\underline{J}_i) = \left\langle \nu_i \frac{\partial Y_2}{\partial w_i} \right\rangle + \left\langle \frac{\partial Y_1}{\partial w_i} \frac{\partial H_1}{\partial J_i} \Big|_{J_i=\underline{J}_i} \right\rangle \quad (3.116)$$

where the averages are taken over the periods of the angle variables w_i . Now, the first set of terms in the Equations (3.115) and (3.116) can be regarded as non-effective terms on the contribution of \underline{J}_i to the new transformed Hamiltonian, $\underline{H}(\underline{J}_i)$. In other words, Y_1 and Y_2 have zero mean, i.e :

$$\left\langle \frac{\partial Y_1}{\partial w_i} \right\rangle = 0, \quad \left\langle \frac{\partial Y_2}{\partial w_i} \right\rangle = 0$$

The fact that Y_1 and Y_2 have zero mean is a consequence of a general property of the angle variables w_i and \underline{w}_i . Suppose that the old action variable J_i is held fixed and w_i increases by 1, then \underline{w}_i also increases by 1, or if the new action variable \underline{J}_i is

held fixed and \underline{w}_i increases by 1, w_i increases by 1.

At constant J_i , let $\Delta\underline{w}_i$ denote the change in \underline{w}_i when w_i increases by 1. So,

$$\Delta\underline{w}_i = \int_0^1 \frac{\partial w_i}{\partial \underline{w}_i} dw_i \quad (3.117)$$

Since \underline{w}_i is equal to $\frac{\partial Y}{\partial J_i}$, the Equation (3.117) can be modified to the following form

$$\Delta\underline{w}_i = \int_0^1 \frac{\partial^2 Y}{\partial J_i \partial w_i} dw_i \quad (3.118)$$

Meanwhile, because \underline{J}_i is constant, it can be taken out of the integral, hence the Equation (3.118) becomes

$$\Delta\underline{w}_i = \frac{\partial}{\partial J_i} \int_0^1 J_i dw_i \quad (3.119)$$

The fact that the range $[0,1]$ describe exactly one period of motion, integration over one complete cycle gives exactly \underline{J}_i , i.e :

$$\int_0^1 J_i dw_i = \underline{J}_i. \quad (3.120)$$

In other words, the average of J_i over one complete period of w_i gives the the new action variable \underline{J}_i , namely

$$\langle J_i \rangle = \underline{J}_i \quad (3.121)$$

So after taking the average of the Equation (3.105), one can get

$$\langle J_i \rangle = \langle \underline{J}_i \rangle + \epsilon \left\langle \frac{\partial Y_1}{\partial w_i} \right\rangle + \epsilon^2 \left\langle \frac{\partial Y_2}{\partial w_i} \right\rangle \quad (3.122)$$

Taking the Equation (3.121) into consideration, the Equation (3.122) becomes

$$\underline{J}_i = \langle \underline{J}_i \rangle + \epsilon \left\langle \frac{\partial Y_1}{\partial w_i} \right\rangle + \epsilon^2 \left\langle \frac{\partial Y_2}{\partial w_i} \right\rangle \quad (3.123)$$

Because \underline{J}_i is independent of w_i , one can conclude that $\langle \underline{J}_i \rangle = \underline{J}_i$. Thus,

$$\underline{J}_i = \underline{J}_i + \epsilon \left\langle \frac{\partial Y_1}{\partial w_i} \right\rangle + \epsilon^2 \left\langle \frac{\partial Y_2}{\partial w_i} \right\rangle \quad (3.124)$$

and finally one can get the following results after some cancelations of \underline{J}_i in the both sides of the equality

$$\left\langle \frac{\partial Y_1}{\partial w_i} \right\rangle = 0, \quad \left\langle \frac{\partial Y_2}{\partial w_i} \right\rangle = 0$$

So, the Equations (3.115) and (3.116) become

$$\underline{H}_1(\underline{J}_i) = \left\langle H_1(w_i, \underline{J}_i) \right\rangle \quad (3.125)$$

$$\underline{H}_2(\underline{J}_i) = \left\langle \sum_i \frac{\partial Y_1}{\partial w_i} \frac{\partial H_1}{\partial J_i} \Big|_{J_i = \underline{J}_i} \right\rangle \quad (3.126)$$

Even though Y_1 has zero mean, the average the product of it with $\frac{\partial H_1}{\partial J_i}$ does not have to be zero. To carry the average of the multiplication in the Equation (3.126) out, we can find the following relation. Furthermore, with the help of the Equations (3.98) and (3.101), one can quickly recognize that

$$\underline{H}(\underline{J}_i) = \langle H_0(\underline{J}_i) \rangle + \epsilon \langle H_0(\underline{J}_i) \rangle = H_0(\underline{J}_i) + \epsilon H_0(w_i, \underline{J}_i) \quad (3.127)$$

or clearly

$$\nu \underline{J}_i + \epsilon \langle H_1 \rangle = \nu J_i + \epsilon H_1 \quad (3.128)$$

Taking the Equation (3.105) into consideration up to order ϵ , one can get the following result

$$\frac{\partial Y_1}{\partial w_i} = \frac{\langle H_1 \rangle - H_1}{\nu_i} \quad (3.129)$$

Using the result we have obtained in the equation above, so the Equation (3.126) becomes

$$\underline{H_2}(J_i) = \left\langle \frac{\langle H_1 \rangle - H_1}{\nu_i} \frac{\partial H_1}{\partial J_i} \Big|_{J_i = \underline{J_i}} \right\rangle \quad (3.130)$$

From the Equation (3.100), in terms of action angle variables, H_1 was

$$H_1(w_i, J_i) = \sum_{i,j,k} \Gamma_{ijk} \left(\frac{J_i J_j J_k}{\pi^3 \Omega_i \Omega_j \Omega_k} \right)^{\frac{1}{2}} \sin 2\pi w_i \cdot \sin 2\pi w_j \cdot \sin 2\pi w_k \quad (3.131)$$

Whatever the indices might be, the average of H_1 over one complete period of w_i, w_j, w_k gives zero, i.e:

$$\langle H_1 \rangle = 0 \quad (3.132)$$

Hence, the Equation (3.130) reduces to the following equation:

$$\underline{H_2}(J_i) = \left\langle \sum_i \frac{-H_1}{\nu_i} \frac{\partial H_1}{\partial J_i} \Big|_{J_i = \underline{J_i}} \right\rangle \quad (3.133)$$

or

$$\underline{H_2}(J_i) = \left\langle \sum_i -\frac{1}{2\nu_i} \frac{\partial H_1^2}{\partial J_i} \Big|_{J_i = \underline{J_i}} \right\rangle \quad (3.134)$$

Collecting all the results we have obtained above, one can write $\underline{H}(J_i)$ as follows

$$\underline{H}(J_i) = H_0(J_i) - \epsilon^2 \sum_i \frac{1}{2\nu_i} \left\langle \frac{\partial}{\partial J_i} H_1^2 \Big|_{J_i = \underline{J_i}} \right\rangle \quad (3.135)$$

or simply, the equation above can be rewritten as follows

$$\underline{H}(\underline{J}_i) = H_0(\underline{J}_i) - \epsilon^2 \sum_i \frac{1}{2\nu_i} \left\langle \frac{\partial}{\partial \underline{J}_i} H_1^2 \right\rangle \quad (3.136)$$

Because \underline{J}_i is independent of w , the derivative operation commutes with the average process and hence the Equation (3.136) can be rewritten as follows

$$\underline{H}(\underline{J}_i) = H_0(\underline{J}_i) - \epsilon^2 \sum_i \frac{1}{2\nu_i} \frac{\partial}{\partial \underline{J}_i} \langle H_1^2 \rangle \quad (3.137)$$

Now, it is convenient to replace index i by l , i.e:

$$H_1(w_l, \underline{J}_l) = \sum_l \Gamma_{ljk} \left(\frac{J_l J_j J_k}{\pi^3 \Omega_l \Omega_j \Omega_k} \right)^{\frac{1}{2}} \cdot \sin 2\pi w_l \cdot \sin 2\pi w_j \cdot \sin 2\pi w_k \quad (3.138)$$

and hence H_1^2 becomes

$$\begin{aligned} H_1^2 &= \sum_{l'j'k'k'} \Gamma_{ljk} \Gamma_{l'j'k'} \left(\frac{J_l \cdot J_j \cdot J_k \cdot J_{l'} \cdot J_{j'} \cdot J_{k'}}{\pi^6 \Omega_l \Omega_j \Omega_k \Omega_{l'} \Omega_{j'} \Omega_{k'}} \right)^{\frac{1}{2}} \\ &\times \sin 2\pi w_l \cdot \sin 2\pi w_j \cdot \sin 2\pi w_k \cdot \sin 2\pi w_{l'} \cdot \sin 2\pi w_{j'} \cdot \sin 2\pi w_{k'} \end{aligned} \quad (3.139)$$

For simplicity, we can denote the part that contains the product of sinus functions by θ_ρ , and the other part by λ_ρ where ρ represents any particular combination of w_i 's in which the average of H_1^2 is different from zero. In the forthcoming calculation, since we will face with different combination of w 's, one can assign a number to each different combination. So, depending on the combination, the index ρ takes the numbers starting from 1. The different possible combination of the following set of indices will be used to compute the averaging process:

$$\rho \equiv \{l, l', j, j', k, k'\} \quad (3.140)$$

In λ and θ notation, H_1^2 can be expressed as follows :

$$H_1^2 = \sum_{\rho} \lambda_{\rho} \theta_{\rho} \quad (3.141)$$

where λ and θ are

$$\lambda_{\rho} = \sum_l \Gamma_{ljk} \Gamma_{l'j'k'} \left(\frac{J_l \cdot J_j \cdot J_k \cdot J_{l'} \cdot J_{j'} \cdot J_{k'}}{\pi^6 \Omega_l \Omega_j \Omega_k \Omega_{l'} \Omega_{j'} \Omega_{k'}} \right)^{\frac{1}{2}} \quad (3.142)$$

and

$$\theta_{\rho} = \sin 2\pi w_l \cdot \sin 2\pi w_j \cdot \sin 2\pi w_k \cdot \sin 2\pi w_{l'} \cdot \sin 2\pi w_{j'} \cdot \sin 2\pi w_{k'} \quad (3.143)$$

Now, we are going to take average of θ_{ρ} over the periods of w_i . However, before carrying the averaging process out, we should better determine the number of the particular combination of w_i 's in which θ_{ρ} becomes an even function. Thus, as an even function, θ_{ρ} brings non-zero results after the averaging of it is performed.

The number of the combinations of w_i 's to obtain non-zero numbers after the averaging process can be determined from the Equation (3.140). From the Equation (3.140), one can easily recognize that there should be 3 different forms of the combinations of w_i 's.

Obviously, one of the forms of combination in which θ_{ρ} becomes an even function is the combination of identical w_i 's in 3 different pairs. In other words, 3 different subgroups of identical w_i 's in pairs result in a non-zero value. Thus, the number of possible combinations for this case is

$$\frac{1}{3!} \cdot \frac{6!}{2!2!2!} = 15 \quad (3.144)$$

Hence the number of possible combinations for this case is 15. The factor $\frac{1}{3!}$ is considered because each subgroup contains the same number of w_i .

In addition to possible combination of the identical w_i 's in pairs, the indices can combine in two subgroups in which one of them contains 2 identical w_i 's and the other contains 4 identical w_i 's. Thus, one can conclude that the number of the possible combination for this kind of gathering can be obtained as follows

$$\frac{6!}{4!2!} = 15 \quad (3.145)$$

As for the last form for non-zero results, it obviously happens if all the indices are the same. Thus, the number of the possible combination for non-zero numbers for this case is 1.

Finally, considering the number of all possible different combinations of w_i 's , there are $15 + 15 + 1$ forms of combination in which the average of θ_ρ is different from zero.

The following sets represent all the possible combination for the first case, i.e:

$$\{(l = l'), (j = j'), (k = k')\} \quad (3.146)$$

$$\{(l = j), (k = l'), (j' = k')\} \quad (3.147)$$

As we stated before, there are 15 different possible combination for this case. Clearly, 6 of them is of the form the Equation (3.146) and the remaining is of the form the Equation (3.147). Denoted this possible combinations by $\rho = 1$, one can write

$$\lambda_1 = \sum_{l,j,k(l \neq j \neq k)} \frac{1}{\pi^3 \Omega_l \Omega_j \Omega_k} (6\Gamma_{ljk} \Gamma_{ljk} + 9\Gamma_{llj} \Gamma_{jkk}) \underline{J}_l \underline{J}_j \underline{J}_k \quad (3.148)$$

and

$$\theta_1 = \sin^2 2\pi w_l \cdot \sin^2 2\pi w_j \cdot \sin^2 2\pi w_k \quad (3.149)$$

The average of θ_1 over the periods of w_i 's

$$\langle \theta_1 \rangle = \int_0^1 \int_0^1 \int_0^1 dw_l dw_j dw_k \sin^2 2\pi w_l \sin^2 2\pi w_j \sin^2 2\pi w_k = \frac{1}{8} \quad (3.150)$$

and therefore

$$\lambda_1 \langle \theta_1 \rangle = \sum_{l,j,k(l \neq j \neq k)} \frac{1}{\pi^3 \Omega_l \Omega_j \Omega_k} \left(\frac{6}{8} \Gamma_{ljk} \Gamma_{ljk} + \frac{9}{8} \Gamma_{llj} \Gamma_{jjk} \right) \underline{J}_l \underline{J}_j \underline{J}_k \quad (3.151)$$

Similar to the first case, the following possible combinations represent all the possible combinations for the second case, i.e:

$$\{(l = j = k = l'), (j' = k')\} \quad (3.152)$$

$$\{(l = j = j' = k'), (k = l')\} \quad (3.153)$$

Similarly, there are 15 different combinations for this case as well. Clearly, 6 of them is of the form the Equation (3.152) and the remaining is of the form the Equation (3.153). Let us denoted this situation by $\rho = 2$, so one can write

$$\lambda_2 = \sum_{l,j(l \neq j)} \frac{1}{\pi^3 \Omega_l^2 \Omega_j} (6 \Gamma_{lll} \Gamma_{ljj} + 9 \Gamma_{llj} \Gamma_{jll}) \underline{J}_l^2 \underline{J}_j \quad (3.154)$$

and θ_2 is

$$\theta_2 = \sin^4 2\pi w_l \cdot \sin^2 2\pi w_j \quad (3.155)$$

The average of θ_2 over the periods of w_i is

$$\langle \theta_2 \rangle = \int_0^1 \int_0^1 dw_l dw_{j'} \sin^4 2\pi w_l \sin^2 2\pi w_j = \frac{3}{16}. \quad (3.156)$$

and so the product of λ_2 and θ_2 becomes

$$\lambda_2 \langle \theta_2 \rangle = \sum_{l,j(l \neq j)} \frac{1}{\pi^3 \Omega_l^2 \Omega_j} \left(\frac{18}{16} \Gamma_{ll} \Gamma_{ljj} + \frac{27}{16} \Gamma_{llj} \Gamma_{jll} \right) \underline{J}_l^2 \underline{J}_j \quad (3.157)$$

In the last case, non-zero results are possible if all the indices are the same, i.e:

$$\{(l = j = k = l' = j' = k')\} \quad (3.158)$$

Denoted by $\rho = 3$, this case brings the following expressions

$$\lambda_3 = \sum_l \frac{1}{\pi^3 \Omega_l^3} \Gamma_{ll} \Gamma_{ll} \underline{J}_l^3 \quad (3.159)$$

and θ_3 is

$$\theta_3 = \sin^6 2\pi w_l \quad (3.160)$$

The average of θ_3 over the periods of w_i is

$$\langle \theta_3 \rangle = \int_0^1 dw_l \sin^6 2\pi w_l = \frac{15}{48} \quad (3.161)$$

and similarly, one can get the product of λ_3 and $\langle \theta_3 \rangle$ as follows

$$\lambda_3 \langle \theta_3 \rangle = \sum_l \frac{15}{48 \pi^3 \Omega_l^3} \Gamma_{ll} \Gamma_{ll} \underline{J}_l^3 \quad (3.162)$$

After collecting the Equations (3.151), (3.157) and (3.162), one can obtain

$$\langle H_1^2 \rangle = \sum_l \frac{15}{48 \pi^3 \Omega_l^3} \Gamma_{ll} \Gamma_{ll} \underline{J}_l^3 + \sum_{l,j(l \neq j)} \frac{1}{\pi^3 \Omega_l^2 \Omega_j} \left(\frac{18}{16} \Gamma_{ll} \Gamma_{ljj} + \frac{27}{16} \Gamma_{llj} \Gamma_{jll} \right) \underline{J}_l^2 \underline{J}_j +$$

$$+ \sum_{l,j,k(l \neq j \neq k)} \frac{1}{\pi^3 \Omega_l \Omega_j \Omega_k} \left(\frac{6}{8} \Gamma_{ljk} \Gamma_{ljk} + \frac{9}{8} \Gamma_{llj} \Gamma_{jkk} \right) \underline{J}_l \underline{J}_j \underline{J}_k \quad (3.163)$$

Taking the derivative of the Equation (3.163) with respect to \underline{J}_i gives

$$\begin{aligned} \sum_i \frac{\partial}{\partial \underline{J}_i} \langle H_1^2 \rangle &= \sum_i \frac{45}{48 \pi^3 \Omega_i^3} \Gamma_{iii} \Gamma_{iii} \underline{J}_i^2 + \sum_{i,j(j \neq i)} \frac{1}{\pi^3 \Omega_i^2 \Omega_j} \left(\frac{36}{16} \Gamma_{iii} \Gamma_{ijj} + \frac{54}{16} \Gamma_{iij} \Gamma_{jii} \right) \underline{J}_i \underline{J}_j + \\ &+ \sum_{i,j(j \neq i)} \frac{1}{\pi^3 \Omega_i \Omega_j^2} \left(\frac{18}{16} \Gamma_{jjj} \Gamma_{jii} + \frac{27}{16} \Gamma_{jji} \Gamma_{ijj} \right) \underline{J}_j^2 + \\ &+ \sum_{i,j,k(i \neq j \neq k)} \frac{1}{\pi^3 \Omega_i \Omega_j \Omega_k} \left(\frac{18}{8} \Gamma_{ijk} \Gamma_{ijk} + \frac{27}{8} \Gamma_{iij} \Gamma_{jkk} \right) \underline{J}_j \underline{J}_k \end{aligned} \quad (3.164)$$

where we have replaced l by j in the equation above.

Replacing the equation the Equation (3.165) into the Equation (3.136) results in

$$\begin{aligned} \underline{H}(\underline{J}_i) &= \sum_i \nu_i \underline{J}_i - \epsilon^2 \sum_i \frac{45}{24 \pi^2 \Omega_i^4} \Gamma_{iii} \Gamma_{iii} \underline{J}_i^2 - \\ &- \epsilon^2 \sum_{i,j(j \neq i)} \frac{1}{\pi^2 \Omega_i^3 \Omega_j} \left(\frac{36}{16} \Gamma_{iii} \Gamma_{ijj} + \frac{54}{16} \Gamma_{iij} \Gamma_{jii} \right) \underline{J}_i \underline{J}_j - \\ &- \epsilon^2 \sum_{i,j(j \neq i)} \frac{1}{\pi^2 \Omega_i^2 \Omega_j^2} \left(\frac{18}{16} \Gamma_{jjj} \Gamma_{jii} + \frac{27}{16} \Gamma_{jji} \Gamma_{ijj} \right) \underline{J}_j^2 - \\ &- \epsilon^2 \sum_{i,j,k(i \neq j \neq k)} \frac{1}{\pi^2 \Omega_i^2 \Omega_j \Omega_k} \left(\frac{18}{8} \Gamma_{ijk} \Gamma_{ijk} + \frac{27}{48} \Gamma_{iij} \Gamma_{jkk} \right) \underline{J}_j \underline{J}_k \end{aligned} \quad (3.165)$$

where we have used the following equations

$$H_0(\underline{J}_i) = \nu_i \underline{J}_i \quad (3.166)$$

and

$$\nu_i = \frac{\Omega_i}{2\pi} \quad (3.167)$$

To obtain the shifted frequency, we use the following expression

$$\underline{\nu}_i = \frac{\partial \underline{H}}{\partial \underline{J}_i} \quad (3.168)$$

In other words, in order to find the frequency of the perturbed Hamiltonian, one should immediately take the derivative of the Equation (3.165) with respect to \underline{J}_i . Thus, one can get the shifted frequency as follows

$$\underline{\Omega}_i = \Omega_i - k_B T \frac{\alpha_1}{\Omega_i^5} - \sum_{j,k(i \neq j \neq k)} k_B T \left\{ \frac{\alpha_2}{\Omega_i^3 \Omega_j^2} + \frac{\alpha_3}{\Omega_i \Omega_j^4} + \frac{\alpha_4}{\Omega_i \Omega_j^2 \Omega_k^2} \right\} \quad (3.169)$$

where we set ϵ equal to 1 because it was chosen arbitrarily at the beginning of the calculations to emphasize that the perturbation Hamiltonian, H_1 , is a slight deviation from the unperturbed Hamiltonian, H_0 . Meanwhile in the equation above we took advantage of the Equation (3.90) and considered that up to a first approximation the average energy per oscillator is

$$E_i \approx k_B T \quad (3.170)$$

The factor α 's in the equation above are as follows

$$\alpha_1 = \frac{15}{2\pi} \Gamma_{iii} \Gamma_{iii} \quad (3.171)$$

$$\alpha_2 = \frac{18}{\pi} \Gamma_{iii} \Gamma_{ijj} + \frac{27}{\pi} \Gamma_{ijj} \Gamma_{jii} \quad (3.172)$$

$$\alpha_3 = \frac{9}{\pi} \Gamma_{jjj} \Gamma_{jii} + \frac{27}{2\pi} \Gamma_{jji} \Gamma_{ijj} \quad (3.173)$$

$$\alpha_4 = \frac{18}{\pi} \Gamma_{ijk} \Gamma_{ijk} + \frac{27}{\pi} \Gamma_{ijj} \Gamma_{jkk} \quad (3.174)$$

To see how big the effects of nonlinear terms on the normal frequencies of the system, we consider a simplified force field describing protein dynamics[21]. The simplified force field between nodes is considered to be of the form

$$V(r_{ij}) = \sum_{i \neq j} c \exp\left(-\frac{|r_{ij}|^\lambda}{|r_0|^\lambda}\right) (|r_{ij}| - |R_{ij}^0|)^2 \quad (3.175)$$

where r_0 is a cutoff distance and it is 0.3 nm[22], The factor c is a constant and it has a dimension of $\frac{kJ}{molA^2}$. Usually the parameter λ is taken typically as $\lambda = 2$. R_{ij}^0 is the distance between residue pairs i and j at the equilibrium state, and r_{ij} is the difference between these nodes after they deviate from the equilibrium state. Let us express r_{ij} in new coordinates

$$r_{ij} = \eta_{ij} + R_{ij}^0 \quad (3.176)$$

In terms of new coordinates, the potential energy becomes

$$V(r_{ij}) = \sum_{i \neq j} c \exp\left(-\frac{|\eta_{ij} + R_{ij}^0|^2}{|r_0|^2}\right) (|\eta_{ij} + R_{ij}^0| - |R_{ij}^0|)^2 \quad (3.177)$$

Once we expand the potential we will obtain the following expansion equation for the potential

$$\begin{aligned} V_{ij} = & \sum_{i \neq j} ck(R_{ij}^0) \frac{(\eta_{ij} \cdot \widehat{R}_{ij}^0)^2}{|R_{ij}^0|} + \\ & + \sum_{i \neq j} ck(R_{ij}^0) \left\{ \frac{(\eta_{ij} \cdot \widehat{R}_{ij}^0)}{|R_{ij}^0|} \eta_{ij}^2 - \frac{(\eta_{ij} \cdot \widehat{R}_{ij}^0)^3}{|R_{ij}^0|} \left(1 + 2 \frac{|\widehat{R}_{ij}^0|^2}{|r_0|^2}\right) \right\} \end{aligned} \quad (3.178)$$

where

$$k(R_{ij}^0) = \exp\left(-\frac{|R_{ij}^0|^2}{|r_0|^2}\right) \quad (3.179)$$

From now on we are going to replace each index by double index, i.e:

$$i \rightarrow i\alpha \quad (3.180)$$

where i represents the residues and it varies between 1 and N , the index α , on the other hand, represents the coordinates and it varies between 1 and 3. Furthermore,

after we symmetrize the potential energy, it becomes

$$V = \sum_{i,j,\alpha,\beta} K_{(i\alpha)(j\beta)} \eta_{i\alpha} \eta_{j\beta} + \frac{1}{3} \sum_{i,j,k,\alpha,\beta,\gamma} \Gamma_{(i\alpha)(j\beta)(k\gamma)} \eta_{i\alpha} \eta_{j\beta} \eta_{k\gamma} \quad (3.181)$$

where K is constant force matrix

$$K_{(i\alpha)(j\beta)} = 2 \sum_{i,j,\alpha,\beta} ck(R_{ij}^0) \{ (\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta \delta_{ij} \delta_{\alpha\beta} - (\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta \} \quad (3.182)$$

and Γ is as follows

$$\begin{aligned} \Gamma_{(i\alpha)(j\beta)(k\gamma)} = & \sum_{i,j,k,\alpha,\beta,\gamma} \frac{ck(R_{ij}^0)}{|R_{ij}^0|} \{ (\hat{R}_{ij}^0)_\beta \delta_{ij} \delta_{\alpha\beta} \delta_{kj} \delta_{\gamma\beta} - (\hat{R}_{ij}^0)_\alpha \delta_{kj} \delta_{\gamma\beta} - \\ & - 2(\hat{R}_{ij}^0)_\beta \delta_{kj} \delta_{\gamma\beta} \delta_{\alpha\beta} + 2(\hat{R}_{ij}^0)_\alpha \delta_{ki} \delta_{\gamma\alpha} \delta_{\alpha\beta} + (\hat{R}_{ij}^0)_\beta \delta_{ki} \delta_{\gamma\alpha} - \\ & - (\hat{R}_{ij}^0)_\alpha \delta_{ji} \delta_{ki} \delta_{\beta\alpha} \delta_{\gamma\alpha} + ((\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta (\hat{R}_{ij}^0)_\gamma \delta_{ij} \delta_{kj} \delta_{\alpha\beta} \delta_{\gamma\beta} - \\ & - 3(\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta (\hat{R}_{ij}^0)_\gamma \delta_{kj} \delta_{\gamma\beta} + 3(\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta (\hat{R}_{ij}^0)_\gamma \delta_{ki} \delta_{\gamma\alpha} - \\ & - (\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta (\hat{R}_{ij}^0)_\gamma \delta_{ji} \delta_{ki} \delta_{\beta\alpha} \delta_{\gamma\alpha}) (1 + 2 \frac{|R_{ij}^0|^2}{|r_0|^2}) \} \quad (3.183) \end{aligned}$$

4. CONCLUSIONS

In this thesis, we study the nonlinear characters of collective vibrations of proteins. From normal mode analysis we know that slow modes are driven by small and amplitude sensitive frequencies.

Having motivated by this idea, we tried to find a general expression for the shifted frequencies of the system. To see how the big effects of the nonlinear on the normal frequencies, we find Γ and Ω from simplified force field and see how big shift took place in the normal frequencies.

The general expression of shifted frequency we have obtained is of the form

$$\underline{\Omega}_i = \Omega_i - \sum_{j(j \neq i)} k_B T \left(52 \frac{\Gamma_{iii} \Gamma_{iii}}{\Omega_i^5} + 15 \frac{\Gamma_{iii} \Gamma_{ijj}}{\Omega_i^3 \Omega_j^2} + 17 \frac{\Gamma_{ijj} \Gamma_{jii}}{\Omega_i^3 \Omega_j^2} \right) \quad (4.1)$$

From the expansion of the empirical potential which we have already worked out, it is clear that the factors Ω and Γ are as follows

$$\Omega^2 = 2 \sum_{i,j,\alpha,\beta} \frac{ck(R_{ij}^0)}{M} \{ (\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta \delta_{ij} \delta_{\alpha\beta} - (\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta \} \quad (4.2)$$

and

$$\begin{aligned} \Gamma_{(i\alpha)(j\beta)(k\gamma)} = & \sum_{i,j,k,\alpha,\beta,\gamma} \frac{ck(R_{ij}^0)}{M^{\frac{3}{2}} |R_{ij}^0|} \{ (\hat{R}_{ij}^0)_\beta \delta_{ij} \delta_{\alpha\beta} \delta_{kj} \delta_{\gamma\beta} - (\hat{R}_{ij}^0)_\alpha \delta_{kj} \delta_{\gamma\beta} - \\ & - 2(\hat{R}_{ij}^0)_\beta \delta_{kj} \delta_{\gamma\beta} \delta_{\alpha\beta} + 2(\hat{R}_{ij}^0)_\alpha \delta_{ki} \delta_{\gamma\alpha} \delta_{\alpha\beta} + (\hat{R}_{ij}^0)_\beta \delta_{ki} \delta_{\gamma\alpha} - \\ & - (\hat{R}_{ij}^0)_\alpha \delta_{ji} \delta_{ki} \delta_{\beta\alpha} \delta_{\gamma\alpha} + ((\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta (\hat{R}_{ij}^0)_\gamma \delta_{ij} \delta_{kj} \delta_{\alpha\beta} \delta_{\gamma\beta} - \\ & - 3(\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta (\hat{R}_{ij}^0)_\gamma \delta_{kj} \delta_{\gamma\beta} + 3(\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta (\hat{R}_{ij}^0)_\gamma \delta_{ki} \delta_{\gamma\alpha} - \\ & - (\hat{R}_{ij}^0)_\alpha (\hat{R}_{ij}^0)_\beta (\hat{R}_{ij}^0)_\gamma \delta_{ji} \delta_{ki} \delta_{\beta\alpha} \delta_{\gamma\alpha}) (1 + 2 \frac{|R_{ij}^0|^2}{|r_0|^2}) \} \quad (4.3) \end{aligned}$$

If we consider

$$\Omega^2 \sim \frac{ck(R_{ij}^0)}{M}, \quad \Gamma \sim \frac{ck(R_{ij}^0)}{M^{\frac{3}{2}}|R_{ij}^0|}$$

and additionally $k_B T$ as proportional to

$$k_B T \sim M \Omega^2 A^2$$

where A is a typical residue fluctuation from its equilibrium position and it is nearly 2 Angstrom. Therefore $\Gamma^2 k_B T / \Omega^5$ is proportional to

$$\frac{\Gamma^2 k_B T}{\Omega^5} \sim \Omega \left(\frac{A}{|R_{ij}^0|} \right)^2 \quad (4.4)$$

A typical distance between residue pairs, $|R_{ij}^0|$, is nearly 7 Angstrom. So, $\Gamma^2 k_B T / \Omega^5$ becomes proportional to

$$\frac{\Gamma^2 k_B T}{\Omega^5} \sim \Omega \frac{1}{10} \quad (4.5)$$

Hence the shifted frequency will be of the form

$$\underline{\Omega} \sim \Omega - \sum_m \Omega_m \frac{1}{10} \quad (4.6)$$

Together with the Equations (4.2) and (4.3), one can conclude that the nonlinear effect in the Equation (4.6) seem to as not negligible as it is thought by Normal Mode Analysis and Gaussian Network Model.

In order to show that the nonlinear effects are not really negligible, we should find a numerical solution for the result we have obtained for the shifted frequencies of the system.

APPENDIX A: DETERMINATION OF Γ_{ijk}

In general, once the potential energy of the system has been expanded around an equilibrium point, the perturbed Hamiltonian of the system can be given as follows

$$H = \sum_{i,j} \frac{1}{2} M_{ij} \dot{X}_i \dot{X}_j + \sum_{i,j} \frac{1}{2} K_{ij} X_i X_j + \sum_{i,j,k} \Gamma_{ijk} X_i X_j X_k \quad (\text{A.1})$$

Introduce mass-weighted coordinates as

$$\zeta_a = (M^{\frac{1}{2}})_{ai} X_i \quad (\text{A.2})$$

$$\zeta_b = (M^{\frac{1}{2}})_{bj} X_j \quad (\text{A.3})$$

$$\zeta_c = (M^{\frac{1}{2}})_{ck} X_k \quad (\text{A.4})$$

Thus, the Hamiltonian becomes

$$\begin{aligned} H = & \frac{1}{2} \dot{\zeta}_a (M^{-\frac{1}{2}})_{ai} M_{ij} (M^{-\frac{1}{2}})_{jb} \dot{\zeta}_b + \frac{1}{2} \zeta_a (M^{-\frac{1}{2}})_{ai} K_{ij} (M^{-\frac{1}{2}})_{jb} \zeta_b + \\ & + \Gamma_{ijk} (M^{-\frac{1}{2}})_{ia} (M^{-\frac{1}{2}})_{jb} (M^{-\frac{1}{2}})_{kc} \zeta_a \zeta_b \zeta_c \end{aligned} \quad (\text{A.5})$$

and

$$H = \sum_{a,b} \frac{1}{2} \dot{\zeta}_a \dot{\zeta}_b \delta_{ab} + \sum_{a,b} \frac{1}{2} K_{ab} \zeta_a \zeta_b + \sum_{a,b,c} \Gamma_{abc} \zeta_a \zeta_b \zeta_c \quad (\text{A.6})$$

so H becomes

$$H = \sum_a \frac{1}{2} \dot{\zeta}_a \dot{\zeta}_a + \sum_{a,b} \frac{1}{2} K_{ab} \zeta_a \zeta_b + \sum_{a,b,c} \Gamma_{abc} \zeta_a \zeta_b \zeta_c \quad (\text{A.7})$$

where

$$\underline{K}_{ab} = (M^{-\frac{1}{2}})_{ai} K_{ij} (M^{-\frac{1}{2}})_{jb} \quad (\text{A.8})$$

and

$$\underline{\Gamma}_{abc} = \Gamma_{ijk} (M^{-\frac{1}{2}})_{ia} (M^{-\frac{1}{2}})_{jb} (M^{-\frac{1}{2}})_{kc} \quad (\text{A.9})$$

Since constant force matrix \underline{K}_{ab} is a symmetric matrix, there should be an orthogonal matrix which diagonalizes it, i.e:

$$(O^{-1})_{la} \underline{K}_{ab} (O)_{bk} = \underline{\Omega}_l^2 \delta_{kl} \quad (\text{A.10})$$

At this point one can introduce normal coordinates as follows

$$q_l = (O^{-1})_{la} \zeta_a \quad (\text{A.11})$$

$$q_m = (O^{-1})_{mb} \zeta_b \quad (\text{A.12})$$

and

$$q_n = (O^{-1})_{nc} \zeta_c \quad (\text{A.13})$$

hence the Hamiltonian in terms of normal coordinates can be expressed as follows

$$H = \sum_l \frac{1}{2} \dot{q}_l^2 + \sum_l \frac{1}{2} \underline{\underline{\Omega}}_l^2 q_l^2 + \sum_{lmn} \underline{\underline{\Gamma}}_{lmn} q_l q_m q_n \quad (\text{A.14})$$

where

$$\delta_{kl} \underline{\underline{\Omega}}_l^2 = (O^{-1})_{la} (M^{-\frac{1}{2}})_{ai} K_{ij} (M^{-\frac{1}{2}})_{jb} (O)_{bk} \quad (\text{A.15})$$

and

$$\underline{\underline{\Gamma_{lmn}}} = \Gamma_{ijk}(M^{-\frac{1}{2}})_{ia}(M^{-\frac{1}{2}})_{jb}(M^{-\frac{1}{2}})_{kc}(O)_{al}(O)_{bm}(O)_{cn} \quad (\text{A.16})$$

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