PROTEIN FOLDING: THE GIBBS FREE ENERGY

YI FANG

Abstract. The fundamental law for protein folding is the Thermodynamic Principle: the amino acid sequence of a protein determines its native structure and the native structure has the minimum Gibbs free energy. If all chemical problems can be answered by quantum mechanics, there should be a quantum mechanics derivation of Gibbs free energy formula \( G(X) \) for every possible conformation \( X \) of the protein. We apply quantum statistics to derive such a formula. For simplicity, only monomeric self folding globular proteins are covered.

We apply quantum statistics to derive such a formula. For simplicity, only monomeric self folding globular proteins are covered.

We point out some immediate applications of the formula. We show that the formula explains the observed phenomena very well. It gives a unified explanation to both folding and denaturation; it explains why hydrophobic effect is the driving force of protein folding and clarifies the role played by hydrogen bonding; it explains the successes and deficiencies of various surface area models. The formula also gives a clear kinetic force of the folding: \( F_i(X) = -\nabla_{x_i} G(X) \). This also gives a natural way to perform the ab initio prediction of protein structure, minimizing \( G(X) \) by Newton’s fastest descending method.

1. Introduction

The newly synthesized peptide chain of a protein automatically folds to its native structure and only in this native structure the protein can perform its biological function. Wrong structure will cause disasters [1]. Why and how the protein folds to its native structure and how to predict the native structure from only the knowledge of the peptide chain are topics of protein folding [2].

The fundamental law for protein folding is the Thermodynamic Principle: the amino acid sequence of a protein determines its native structure and the native structure of the protein has the minimum Gibbs free energy among all possible conformations [3]. Let \( X \) be a conformation of a protein, is there a natural Gibbs free energy function \( G(X) \)? The answer must be positive, as G. N. Lewis said in 1933: “There are can be no doubt but that in quantum mechanics one has the complete solution to the problems of chemistry.” (quoted from [4, page 130].) Protein folding is a problem in biochemistry, why we have not found such a formula \( G(X) \)? The answer is also ready in hand. In 1929 Dirac wrote: “The underlying physical laws necessary for the mathematical theory of ... the whole of chemistry are thus completely known, and the difficulty is only that the exact application of these laws leads to equations much too complicated to be soluble.” (quoted from [3, page 132]). Yes, the complex of the Shrödinger equation for protein folding is beyond our ability to solve, no matter how fast and how powerful of our computers. But mathematical theory guarantees that

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there are a complete set of eigenvalues (energy levels) and eigenfunctions to the Shr"{o}dinger equation in the Born-Oppenheimer approximation. Then consider that in the statistical mechanics, ensembles classify all (energy) states of the system, although we cannot have exact solutions to the Shr"{o}dinger equation, we can apply the grand canonical ensemble to obtain the desired Gibbs free energy formula $G(X)$. This is the main idea of our derivation. The interested readers can read the details in the Appendix A.

Here we first state the formulae and the assumptions in deriving them. Then we will point out some immediate applications and will use $G(X)$ to explain well known facts such as hydrophobic effect and its relations with the hydrogen bonding, the denaturation of proteins, and the success in discriminating native and closely nearby compact non-native structures by empirical surface area models. Other inferences from $G(X)$, such as the kinetic force in protein folding, the common practice of measuring $\Delta G$, etc., are also discussed. The derivation itself will be put in Appendix A so that uninterested readers can skip it. In Appendix B we will give the kinetic formulae $F_i(X)$.

1.1. Assumptions. All assumptions here are based on well-known facts of consensus. Let $U$ be a protein with $M$ atoms $(a_1, \cdots, a_i, \cdots, a_M)$. A structure of $U$ is a point $X = (x_1, \cdots, x_i, \cdots, x_M) \in \mathbb{R}^{3M}$, $x_i \in \mathbb{R}^3$ is the atomic center (nuclear) position of $a_i$. Alternatively, the conformation $X$ corresponds to a subset in $\mathbb{R}^3$, $P_X = \bigcup_{i=1}^{M} B(x_i, r_i) \subset \mathbb{R}^3$, where $r_i$ is the van der Waals radius of the atom $a_i$ and $B(x, r) = \{ y \in \mathbb{R}^3; |y - x| \leq r \}$ is a closed ball with radius $r$ and center $x$.

(1) The proteins discussed here are monomeric, single domain, self folding globular proteins.

(2) Therefore, in the case of our selected proteins, the environment of the protein folding, the physiological environment, is pure water, there are no other elements in the environment, no chaperons, no co-factors, etc. This is a rational simplification, at least when one considers the environment as only the first hydration shell of a conformation, as in our derivation of the $G(X)$.

(3) During the folding, the environment does not change.

(4) Anfinsen [3] showed that before folding, the polypeptide chain already has its main chain and each residue’s covalent bonds correctly formed. Hence, our conformations should satisfy the following steric conditions set in [5] and [6]: there are $\epsilon_{ij} > 0, 1 \leq i < j \leq M$ such that for any two atoms $a_i$ and $a_j$ in $P_X = \bigcup_{k=1}^{M} B(x_k, r_k)$, 
\[
\begin{align*}
\epsilon_{ij} &\leq |x_i - x_j|, & \text{no covalent bond between } a_i \text{ and } a_j; \\
\delta_{ij} - \epsilon_{ij} &\leq |x_i - x_j| \leq \delta_{ij} + \epsilon_{ij}, & \delta_{ij} \text{ is the standard bond length between } a_i \text{ and } a_j.
\end{align*}
\]

We will denote all conformations satisfying (1) as $\mathcal{X}$ and only consider $X \in \mathcal{X}$ in this paper.

(5) A water molecule is taking as a single particle, centered at $w \in \mathbb{R}^3$, the oxygen nuclear position, and the covalent bonds in it are fixed. In the Born-Oppenheimer approximation, only the conformation $X$ is fixed, all particles, water molecules or electrons in the first hydration shell of $P_X$, are moving.

(6) We agree that simply classifying amino acids as hydrophobic or hydrophilic is an oversimplification [7]. All atoms should be classified according to the hydrophobicity of moieties or atom
groups it belongs. Suppose there are $H$ hydrophobic levels $H_i$, $i = 1, \cdots, H$, such that $\cup_{i=1}^{H} H_i = (a_1, \cdots, a_i, \cdots, a_M)$. For example, we may assume that the classification is as in [7], there are $H = 5$ classes, C, O/N, O$^-$, N$^+$, S. Unlike in [7], we also classify every hydrogen atom into one of the $H$ classes according to whom it is bounded with. There are many different hydrophobicity classifications. Our derivation is valid for any of them.

1.2. The Formula. The formula has two versions, the chemical balance version is:

$$G(X) = \mu_e N_e(X) + \sum_{i=1}^{H} \mu_i N_i(X),$$

where $N_e(X)$ is the mean number of electrons in the space included by the first hydration shell of $X$, $\mu_e$ is its chemical potential. $N_i(X)$ is the mean number of water molecules in the first hydration layer that directly contact to the atoms in $H_i$, $\mu_i$ is the chemical potential.

Let $M_X$ (see Figure 1) be the molecular surface for the conformation $X$, defining $M_{X_i} \subset M_X$ as the set of points in $M_X$ that are closer to atoms in $H_i$ than any atoms in $H_j$, $j \neq i$. Then the geometric version of $G(X)$ is:

$$G(X) = a\mu_e V(\Omega_X) + a d_w \mu_e A(M_X) + \sum_{i=1}^{H} \nu_i \mu_i A(M_{X_i}), \quad a, \nu_i > 0,$$

where $V(\Omega_X)$ is the volume of the domain $\Omega_X$ enclosed by $M_X$, $d_w$ the diameter of a water molecule, and $A(M_X)$ and $A(M_{X_i})$ the areas of $M_X$ and $M_{X_i}$, $a[V(\Omega_X) + d_w A(M_X)] = N_e, \nu_i A(M_{X_i}) = N_i(X), 1 \leq i \leq H$. The $a$ and $\nu_i$ are independent of $X$, they are the average numbers of particles per unit volume and area.

2. Applications

2.1. Structure Prediction. Prediction of protein structures is the most important method to reveal proteins’ functions and working mechanics, it becomes a bottle neck in the rapidly developing life science. With more and more powerful computers, this problem is attacked in full front. Various models are used to achieve the goal, homologous or $ab$ initio, full atom model or coarse grained, with numerous parameters of which many are quite arbitrary. But although our computer power growths exponentially, prediction power does not follow that way. At this moment, we should take a deep breath and remind what the great physicist Fermi said: “There are two ways of doing calculations in theoretical physics. One way, and this is the way I prefer, is to have a clear physical picture of the process that you are calculating. The other way is to have a precise and selfconsistent mathematical formalism.” And “I remember my friend Johnny von Neumann used to say, with four parameters I can fit an elephant, and with five I can make him wiggle his trunk.” Quoted from [8].

These should also apply to any scientific calculation, not just theoretical physics. Look at the current situation, all $ab$ initio prediction models are actually just empirical with many parameters to ensure some
success. Fermi’s comments remind us that a theory should be based on fundamental physical laws, and contain no arbitrary parameters. Look at formulae (2) and (3), we see immediately that they are neat, precise and self-consistent mathematical formulae. Furthermore, they including no arbitrary parameter, all terms in them have clear physical meanings. Chemical potentials $\mu_e$ and $\mu_i$’s, geometric constants $a$ and $\nu_i$’s, can be valued by theory or experiments, they are not arbitrary at all.

But a theory has to be developed, tested, until justified or falsified. For interested researchers, the tasks are to determine the correct values of the chemical potentials in (2) and the geometric ratios $a$ and $\nu_i$ in (3). There are many estimates to them, but they are either for the solvent accessible surface area such as in [7] hence not suit to the experiment data as pointed out in [9], or do not distinguish different hydrophobicity levels as in [9]. To get the correct values of the chemical potentials and geometric constants, commonly used method of training with data can be employed, in which we can also test the formulae’s ability of discriminating native and nearby compact non-native structures. After that, a direct test is to predict the native structure from the amino acid sequence of a protein by minimizing the following:

$$G(X_N) = \inf_{X \in X} G(X).$$

(4)

This is the first time that we have a theoretically derived formula of the Gibbs free energy. Before this, all ab initio predictions are not really ab initio. A combined (theoretical and experimental) search for the values of chemical potentials will be the key for the success of the ab initio prediction of protein structure.

2.2. Energy Surface or Landscape. An obvious application is the construction of Gibbs free energy surface or landscape. We do not need any empirical estimate anymore, the Gibbs free energy formula $G : X \to \mathbb{R}$ gives a graph $(X, G(X))$ over the space $X$ (all eligible conformations for a given protein), and this is nothing but the Gibbs free energy surface. Mathematically it is a $3M$ dimensional hyper-surface. Its characteristics concerned by students of energy surface theory, such as how rugged it is, how many local minima are there, is there a funnel, etc., can be answered by simple calculations of the formula.

Since the function $G$ is actually defined on the whole $\mathbb{R}^{3M}$ (on an domain of $\mathbb{R}^{3M}$ containing all $X$ is enough), we can explore mathematical tools to study its graph, and compare the results with the restricted conformations. One important question is: Does the absolute minimum structure belongs to $X$?

2.3. Kinetics. It is observed that while we apply the thermodynamic principle, a difficulty is that we do not have kinetics and have use other method to present it [10]. The advantage of a theoretical formula for Gibbs free energy in the form $G(X)$ is that it connects the thermodynamics with the kinetics. In fact, for any atomic position $x_i$, the kinetic force is $F_i(X) = -\nabla x_i G(X)$, [11]. With formula (3) these quantities are really calculable, mathematical formulae and implementations on molecular surface $M_X$ are given in [12].

We will give the mathematical formulae in Appendix B. The resulting Newton’s fastest descending method was used in the simulation in [6].
3. Discussions

We are theoretically treating the protein folding by introducing quantum statistics. A theory is useful only if it can make explanations to the observed facts and if it can simplify and improve research methods as well as clarify concepts. We will show that $G(X)$ can do exactly these.

![Figure 1](image_url)

**Figure 1.** Two dimensional presenting of molecular surface [13] and solvent accessible surface [14]. This figure was originally in [6].
Figure 2. Note that $R_{Xi}$ generally are not connected, i.e., having more than one block.

If the same theoretical result can be derived from two different disciplines, it is often not just by chance. We will show an early phenomenological mathematical model [5], starting from purely geometric reasoning, has achieved formula (3), with just two hydrophobic levels, hydrophobic and hydrophilic.

A theory also has to be falsifiable, that is making a prediction to be checked. The fundamental prediction is that minimizing formulae (2) or (3) we will get the native structures from the amino acid sequences of proteins covered in the assumptions of the formulae. That can only be done after we have the actual values in the physiological environment of the chemical potentials appear in the formulae.

3.1. Unified Explanation of Folding and Denaturation. Protein denaturation is easy to happen, even if the environment is slightly changed, as described in [15] by Hsien Wu in 1931. (The reference [15] is the 13th article that theorizes the results of a series experiments, and a preliminary report was read before the
Xllith International Congress of Physiology at Boston, August 19-24, 1929, and published in the *Am. J. Physiol* for October 1929. In which Hsien Wu first suggested that the denatured protein is still the same molecule, only structure has been changed.) Anfinsen in various experiments showed that after denaturation by changed environment, if removing the denature agent, certain globular proteins can spontaneously refold to its native structure.\(^3\) The spontaneous renaturation suggests that protein folding does not need outside help, at least to the class of proteins in study. Therefore, the fundamental law of thermodynamics asserts that in the environment such that a protein can fold, the native structure must have the minimum Gibbs free energy. The same is true for denaturation, under the denatured environment, the native structure no longer has the minimum Gibbs free energy, some other structure(s), will have the minimum Gibbs free energy. Thus let \(E_n\) present environment, any formula of Gibbs free energy should be stated as \(G(X,E_n)\) instead of just \(G(X)\), unless specified the environment like in this paper. Let \(E_{nN}\) be the physiological environment and \(E_{nU}\) be some denatured environment, \(X_N\) be the native structure and \(X_U\) be one of the denatured stable structure in \(E_{nU}\), then the thermodynamic principle for both of protein folding and unfolding should be that

\[
G(X_N,E_{nN}) < G(X_U,E_{nN}), \quad G(X_N,E_{nU}) > G(X_U,E_{nU}). \tag{5}
\]

To check this, an experiment should be designed that can suddenly put proteins in a different environment. Formulae (2) and (3) should be written as \(G(X,E_n)\). Indeed, the chemical potentials \(\mu_e\) and \(\mu_i\)'s are Gibbs free energies per corresponding particles, \(\mu = u + Pv - Ts\). Two environment parameters, temperature \(T\) and pressure \(P\), explicitly appear in \(\mu\), the inner energy \(u\) and entropy \(s\) may also implicitly depend on the environment. According to formulae (2) and (3), if \(\mu_i < 0\), then make more \(H_i\) atoms to expose to water (make larger \(A(M_{X_i})\)) will reduce the Gibbs free energy. If \(\mu_i > 0\), then the reverse will happen. Increase or reduce the \(H_i\) atoms’ exposure to water (\(A(M_{X_i})\)), the conformation has to change. The conformation changes to adjust until we get a conformation \(X_N\), such that the net effect of any change of it will either increase some \(H_i\) atoms’ exposure to water while \(\mu_i > 0\) or reduce \(H_i\) atoms’ exposure to water while \(\mu_i < 0\). In other words, the \(G(X,E_{nN})\) achieves its minimum at \(G(X_N,E_{nN})\). Protein folding, at least for the proteins considered in the assumptions, is explained very well by formulae (2) and (3).

In changed environment, the chemical potentials \(\mu_e\) and \(\mu_i\)'s in formulae (2) and (3) changed their values. With the changed chemical potentials, \(G(X,E_{nU})\) has the same form as \(G(X,E_{nN})\) but different chemical potentials. Therefore, the structure \(X_U\) will be stable, according to the second inequality in (5), the process is exactly the same as described for the protein folding if the changing environment method does not include introducing new kinds (non-water) of particles, for example, if we only change temperature or pressure.

Even the new environment including new kinds of particles, formulae (2) and (3) can still partially explain the denaturation, only that more obstructs prevent the protein to denature to \(X_U\), but any way it will end in some structure other than the \(X_N\), the protein is denatured. Actually, this is a hint of how to modify the current formulae to extend to general proteins.
3.2. Why $G(X)$ Instead Of $\triangle G(X)$. Here is a chance to explain why we use $G(X)$ instead of $\triangle G(X)$. In various experiments of testing the Gibbs free energy difference ($\triangle G$) between $X_N$ and $X_U$, the common practice is essentially set
\[
\triangle G = G(X_U, En_U) - G(X_N, En_N).
\] (6)
see, for example, [16]. Though some interpolation was taken to adjust, but that is not the experiment observation. But formulae (2) and (3) suggest that what we need is
\[
\triangle G = G(X_U, En_N) - G(X_N, En_N).
\] (7)
Unfortunately, there is no method of denaturation without changing environment, at least currently no such method. Therefore, no way to experimentally measuring of $\triangle G$ in (7). We should reexamine the conclusion of $\triangle G$ is very small because it was essentially drawn from (6). Thus although we believe that it is true, the conclusion was achieved neither via theory nor via real experiment observation.

While experiment has no way to change the native structure without disturbing the environment, theory can play a role instead. Formulae (2) and (3) give us the chance to compare $\triangle G$, as long as we have accurate chemical potentials.

3.3. Explain Hydrophobic Effect and the Role Played by Hydrogen Bonding. In 1959, by reviewing the literature Kauzmann concluded that the hydrophobic effect is the main driving force in protein folding [17]. Empirical correlation between hydrophobic free energy and aqueous cavity surface area was noted as early as 1974 [18], giving justification of the hydrophobic effect. Various justifications of hydrophobic effect were published, based on empirical models of protein folding, for example, [19]. But the debate continues to present, some still insist that it is the hydrogen bond instead of hydrophobic effect plays the main role of driving force in protein folding, for example, [20]. The theoretically derived formulae (2) and (3) can explain why the hydrophobic effect is indeed the driving force. A simulation of reducing hydrophobic area alone ([6]) can explain the intra-molecular hydrogen bonds.

In fact, according to formulae (2) and (3), if $\mu_i < 0$, then make more $H_i$ atoms to appear in the boundary of $P_X$ will reduce the Gibbs free energy. If $\mu_i > 0$, then the reverse will happen, reducing the exposure of $H_i$ atoms to water will reduce the Gibbs free energy. This gives a theoretical explanation of the hydrophobic effect. The kinetic formulae $F_i = \nabla_X G(X)$ and those given in Appendix B are the force that push the conformation to change to the native structure.

The mechanics stated above works through the chemical potentials $\mu_i$ for various levels of hydrophobicity, in physiological environment, all hydrophobic $H_i$’s will have positive $\mu_i$, all hydrophilic $H_i$’s will have negative $\mu_i$. Thus changing conformation $P_X$ such that the most hydrophilic $H_i$ ($\mu_i = \min(\mu_1, \cdots, \mu_H)$) gets the first priority to appear on the boundary, and the most hydrophobic $H_i$ ($\mu_i = \max(\mu_1, \cdots, \mu_H)$) gets the first priority to hide in the hydrophobic core to avoid contacting with water molecules, etc. We should keep in mind that all the time, the steric conditions (1) have to be obeyed.
But the hydrophobic effect is actually partially working through hydrogen bond formation. This is well presented in the chemical potentials in (2) and (3). In fact, the values of the chemical potentials reflect the ability of the atoms or atom groups to form hydrogen bond, either with another atom group in the protein or with water molecules. This gives a way to theoretically or experimentally determine the values of hydrophilic chemical potentials: checking the actually energy of the hydrogen bond.

For hydrophobic ones, it will be more complicated, common sense is that it reduces the entropy that certainly comes from the inability of forming hydrogen bonds with water molecules. Hence although hydrophobic effect is the driving force of protein folding, it works through the atom’s ability or inability to form hydrogen bonds with water molecules.

How to explain the intra-molecular hydrogen bonds? It seems that formula (2) and (3) do not address this issue. The possible theory is that the amino acid sequence of a protein is highly selectable in evolution, in tact only a tiny number of amino acid sequences can really become a protein. With these specially selected sequences, while shrinking the various hydrophobic surfaces to form a hydrophobic core, residues are put in position to form secondary structures and their associated hydrogen bonds. This sounds a little bit too arbitrary. But a simulation of shrinking hydrophobic surface area alone indeed produced secondary structures and hydrogen bonds. The simulation was reported in [6]. Without calculating any dihedral angles or electronic charges, without any arbitrary parameter, paying no attention to any particular atom’s position, by just reducing hydrophobic surface area (there it was assumed that there are only two kinds of atoms, hydrophobic and hydrophilic), secondary structures and hydrogen bonds duly appeared. The proteins used in the simulation are 2i9c, 2hng, and 2ib0, with 123, 127, and 162 residues. No simulation of any kind of empirical or theoretical models had achieved such a success. More than anything, this simulation should prove that hydrophobic effect alone will give more chance of forming intra-molecular hydrogen bonds. Indeed, pushing hydrophilic atoms to make hydrogen bonds with water molecules will give other non-boundary hydrophilic groups more chance to form intra-molecular hydrogen bonds.

Again formula (3) can partly explain the success of this simulation, when there are only two hydrophobic classes in (3), the hydrophobic area presents the main positive part of the Gibbs free energy, reducing it is reducing the Gibbs free energy, no matter what is the chemical potential’s real value.

### 3.4. Explanation of the Successes of Surface Area Models

In 1995, Wang et al [21] compared 8 empirical energy models by testing their ability to distinguish native structures and their close neighboring compact non-native structures. Their models WZS are accessible surface area models with 14 classes of atoms, $\sum_{i=1}^{14} \sigma_i A_i$. Each two combination of three targeting proteins were used to train WZS to get $\sigma_i$, hence there are three models WZS1, WZS2, and WZS3. Among the 8 models, all WZS’s performed the best, distinguishing all 6 targeting proteins. The worst performer is the force field AMBER 4.0, it failed in distinguishing any of the 6 targets.
These testing and the successes of various surface area models such as [7], showed that instead of watching numerous pairwise atomic interactions, the surface area models, though looking too simple, have surprising powers. Now the formula (3) gives them a theoretic justification. On the other hand, the successes of these models also reinforce the theoretical results.

There is a gap between the accessible surface area model in [7] and the experiment results (surface tension), as pointed out in [9]. The gap disappeared when one uses the molecular surface area to replace the accessible surface area, in [9] it was shown that molecular surface area assigned of 72-73 cal/mol/Å² perfectly fits with the macroscopic experiment data. Later it was asserted that the molecular surface is the real boundary of protein in its native structure [22].

Figure 1 and Figure 2 show the water molecules contact to $\mathcal{P}_X$ and the accessible surface and molecular surface, we see that all water molecules must be outside the molecular surface $M_X$, but the assessable surface is in the middle of the first hydration shell. So it is better to use the molecular surface $M_X$ as the boundary of the conformation $\mathcal{P}_X$. Moreover, the conversion of the mean numbers $N_i(\mathcal{X})$ to surface area, $N_i(\mathcal{X}) = \nu_i A(M_X \cdot i)$, only works for the molecular surface, not for the accessible surface. This can explain the conclusions in [9] and [22].

In fact, the advantage of the solvent accessible surface is that by definition of it we know exactly each atom occupies which part of the surface, therefore, one can calculate its share in surface area. This fact may partly account why there are so many models based on the solvent accessible surface, even people knew the afore mentioned gap. For other surfaces, we have to define the part of surface that belongs to a specific hydrophobicity class. This was resolved in [5] via the distance function definition as we used here.

All surface area models neglected one element, the volume of the structure. As early as in the 1970’s, Richards and his colleagues already pointed out that the native structure of globular proteins is very dense, or compact, (density = 0.75, [13]). To make a conformation denser, obviously we should shrink the volume $V(\Omega_X)$. The model in [5] introduced volume term but kept the oversimplification of all atoms are either hydrophobic or hydrophilic. The derivation of (2) and (3) shows that volume term should be counted, but it may be that $a\mu_e$ is very small, in that case, volume maybe really is irrelevant.

3.5. Coincidence with Phenomenological Mathematical Model. If a theoretical result can be derived from two different disciplines, its possibility of correctness will be dramatically increased. Indeed, from a pure geometric consideration, a phenomenological mathematical model, $G(\mathcal{X}) = aV(\Omega_X) + bA(M_X) + cA(M_X^{1})$, $a, b, c > 0$ (it was assumed that there are only two hydrophobicity levels, hydrophobic and hydrophilic, the hydrophilic surface area $A(M_X^{2})$ is absorbed in $A(M_X)$ by $A(M_X^{2}) = A(M_X) - A(M_X^{1})$), was created in [5].

It was based on the well-known global geometric characteristics of the native structure of globular proteins: 1. high density; 2. smaller surface area; 3. hydrophobic core, as demonstrated and summarized in [13] and [23]. So that to obtain the native structure, we should shrink the volume (increasing the density) and surface
area, and form better hydrophobic core (reducing the hydrophobic surface area $A(M_{X_1})$) simultaneously and cohesively.

The coincidence of formula (3) and the phenomenological mathematical model of [5] cannot be just a coincidence. Most likely, it is the same natural law reflected in different disciplines. The advantage of (3) is that everything there has its physical meaning.

3.6. Potential Energy Plays No Role in Protein Folding. Formulae (2) and (3) theoretically show that hydrophobic effect is the driving force of protein folding, it is not just solvent free energy besides the pairwise interactions such as the Coulombs, etc., as all force fields assumed. Only in the physiological environment the hydrophobic effect works towards to native structure, otherwise it will push denaturation as discussed in explanation of folding and unfolding. Formulae (2) and (3) show that the Gibbs free energy is actually independent of the potential energy, against one’s intuition and a bit of surprising. The explanation is that during the folding process, all covalent bonds in the main chain and each side chain are kept invariant, the potential energy has already played its role in the synthesis process of forming the peptide chain, which of course can also be described by quantum mechanics. According to Anfinsen [3], protein folding is after the synthesis of the whole peptide chain, so we can skip the synthesis process and concentrate on the folding process.

The steric conditions (1) will just keep this early synthesis result, not any $X = (x_1, \cdots, x_i, \cdots, x_M)$ is eligible to be a conformation, it has to satisfy (1). The steric conditions not only pay respect to the bond length, it also reflect a lot of physi-chemco properties of a conformation: They are defined via the allowed minimal atomic distances, such that for non-bonding atoms, the allowed minimal distances are: shorter between differently charged or polarized atoms; a little longer between non-polar ones; and much longer (generally greater than the sum of their radii) between the same charged ones, etc. For example, we allow minimal distance between sulfur atoms in Cysteines to form disulfide bonds. And for any new found intra-covalent bond between side chains, we can easily modify the steric conditions to allow it to form during folding, though it may not necessarily form.

Especially in the minimization of $G(X)$, steric conditions must be kept, thus the minimization in (4) is a constrained minimization. This, unfortunately, is a draw back, it increased the mathematical difficulty.

4. Conclusion

A quantum statistical theory of protein folding for monomeric, single domain, self folding globular proteins is suggested. The assumptions of the theory fit all observed realities of protein folding. The resulting formulae (2) and (3) do not have any arbitrary parameters and all terms in them have clear physical meaning. Potential energies involving pairwise interactions between atoms do not appear in them.

Formulae (2) and (3) have explanation powers. They give unified explanation to folding and denaturation, to the hydrophobic effect in protein folding and its relation with the hydrogen bonding. The formulae
also explain the relative successes of surface area protein folding models. Relation between kinetic and thermodynamic of protein folding is discussed, driving force formula comes from the Gibbs free energy formula (3) are also given. Energy surface theory will be much easier to handle. The concept of \( \Delta G \) is clarified.

**APPENDIX A. THE DERIVATION**

Let \( d_w \) be the diameter of a water molecule and \( M_X \) be the molecular surface of \( P_X \) as defined in [13] with the probe radius \( d_w/2 \), see Figure 1. Define

\[
\mathcal{R}_X = \{ x \in \mathbb{R}^3 : \text{dist}(x, M_X) \leq d_w \} \setminus P_X
\]

(8)
as the first hydration shell surrounding \( P_X \), where \( \text{dist}(x, S) = \inf_{y \in S} |x - y| \). Then \( T_X = P_X \cup \mathcal{R}_X \) will be our thermodynamic system of protein folding at the conformation \( X \).

We classify the atoms in \( \mathcal{U} \) into \( H \) hydrophobicity classes \( \mathcal{H}_i \), such that \( \bigcup_{i=1}^{H} \mathcal{H}_i = \{ a_1, a_2, \cdots, a_M \} \). Let \( I_i \subset \{ 1, 2, \cdots, M \} \) be the subset such that \( a_j \in \mathcal{H}_i \) if and only if \( j \in I_i \). Define \( P_{X,i} = \bigcup_{j \in I_i} B(x_j, r_j) \subset P_X \) and as shown in Figure 2,

\[
\mathcal{R}_{X,i} = \{ x \in \mathcal{R}_X : \text{dist}(x, P_{X,i}) \leq \text{dist}(x, P_X \setminus P_{X,i}) \}, \quad 1 \leq i \leq H,
\]

(9)

Let \( V(\Omega) \) be the volume of \( \Omega \subset \mathbb{R}^3 \), then

\[
\mathcal{R}_X = \bigcup_{i=1}^{H} \mathcal{R}_{X,i}, \quad V(\mathcal{R}_X) = \sum_{i=1}^{H} V(\mathcal{R}_{X,i}), \quad \text{and for } i \neq j, \quad V(\mathcal{R}_{X,i} \cap \mathcal{R}_{X,j}) = 0.
\]

(10)

Since \( M_X \) is a closed surface, it divides \( \mathbb{R}^3 \) into two regions \( \Omega_X \) and \( \Omega'_X \) such that \( \partial \Omega_X = \partial \Omega'_X = M_X \) and \( \mathbb{R}^3 = \Omega_X \cup M_X \cup \Omega'_X \). We have \( P_X \subset \Omega_X \) and all nuclear centers of atoms in the water molecules in \( \mathcal{R}_X \) are contained in \( \Omega'_X \). Moreover, \( \Omega_X \) is bounded, therefore, has a volume \( V(\Omega_X) \). Define the hydrophobicity subsurface \( M_{X,i} \), \( 1 \leq i \leq H \), as

\[
M_{X,i} = M_X \cap \mathcal{R}_{X,i}.
\]

(11)

Let \( A(S) \) be the area of a surface \( S \subset \mathbb{R}^3 \), then

\[
M_X = \bigcup_{i=1}^{H} M_{X,i}, \quad A(M_X) = \sum_{i=1}^{H} A(M_{X,i}), \quad \text{and if } i \neq j, \quad A(M_{X,i} \cap M_{X,j}) = 0.
\]

(12)

Although the shape of each atom in \( \mathcal{U} \) is well defined by the theory of atoms in molecules ([4] and [24]), what concerning us here is the overall shape of the structure \( P_X \). The cutoff of electron density \( \rho \geq 0.001 \text{au} \) ([4] and [24]), gives the overall shape of a molecular structure that is just like \( P_X \), a bunch of overlapping balls. Moreover, the boundary of the \( \rho \geq 0.001 \text{au} \) cutoff is much similar to the **molecular surface** \( M_X \) which was defined by Richards in 1977 [13] and was shown has more physical meaning as the boundary surface of the conformation \( P_X \) ([9] and [22]).
A.1. The Shrödinger Equation. For any conformation \( X \in \mathcal{X} \), let \( W = (w_1, \ldots, w_i, \ldots, w_N) \in \mathbb{R}^{3N} \) be the nuclear centers of water molecules in \( \mathcal{R}_X \) and \( E = (e_1, \ldots, e_i, \ldots, e_L) \in \mathbb{R}^{3L} \) be electronic positions of all electrons in \( \mathcal{T}_X \). Then the Hamiltonian for the system \( \mathcal{T}_X \) is
\[
\hat{H} = \hat{T} + \hat{V} = -\sum_{i=1}^{M} \frac{\hbar^2}{2m_i} \nabla_i^2 - \frac{\hbar^2}{2m_w} \sum_{i=1}^{N} \nabla_i^2 - \frac{\hbar^2}{2m_e} \sum_{i=1}^{L} \nabla_i^2 + \hat{V}(X, W, E),
\]
where \( m_i \) is the nuclear mass of atom \( a_i \) in \( \Omega \), \( m_w \) and \( m_e \) are the masses of water molecule and electron; \( \nabla_i^2 \) is Laplacian in corresponding \( \mathbb{R}^3 \); and \( V \) the potential.

A.2. The First Step of The Born-Oppenheimer Approximation. Depending on the shape of \( \mathcal{P}_X \), for each \( i, 1 \leq i \leq H \), the maximum numbers \( N_X,i \) of water molecules contained in \( \mathcal{R}_X,i \) vary. Theoretically we consider all cases, i.e., there are \( 0 \leq N_i \leq N_X,i \) water molecules in \( \mathcal{R}_X,i, 1 \leq i \leq H \). Let \( M_0 = 0 \) and \( M_i = \sum_{j=1}^{N_i} N_j \) and \( W_i = (w_{M_i-1+1}, \ldots, w_{M_i-1+j}, \ldots, w_{M_i}) \in \mathbb{R}^{3N_i}, 1 \leq i \leq H \), and \( W = (W_1, W_2, \ldots, W_M) \in \mathbb{R}^{3MH} \) denote the nuclear positions of water molecules in \( \mathcal{R}_X \). As well, there will be all possible numbers \( 0 \leq N_c < \infty \) of electrons in \( \mathcal{T}_X \). Let \( E = (e_1, e_2, \ldots, e_{N_e}) \in \mathbb{R}^{3N_e} \) denote their nuclear positions. For each fixed \( X \in \mathcal{X} \) and \( N = (N_1, \ldots, N_H, N_e) \), the Born-Oppenheimer approximation has the Hamiltonian
\[
\hat{H}_X = -\frac{\hbar^2}{2} \left\{ \frac{1}{m_w} \sum_{j=1}^{M_u} \nabla_j^2 + \frac{1}{m_e} \sum_{\nu=1}^{N_e} \nabla_\nu^2 \right\} + \hat{V}(X, W, E).
\]
The eigenfunctions \( \psi_i^{X,N}(W, E) \in L_0^2(\prod_{i=1}^{H} \mathcal{R}_X^{N_i} \times \mathcal{T}_X^{N_e}) = \mathcal{H}_{X,N}, 1 \leq i < \infty \), comprise an orthonormal basis of \( \mathcal{H}_{X,N} \). Denote their eigenvalues (energy levels) as \( E_{X,N}^{ij} \), then \( \hat{H}_X \psi_i^{X,N} = E_{X,N}^{ij} \psi_i^{X,N} \).

A.3. Grand Partition Function and Grand Canonic Density Operator. In the following we will use the notations and definitions in [25, Chapter 10]. Let \( k_B \) be the Boltzman constant, set \( \beta = 1/k_BT \). Since the numbers \( N_i \) and \( N_e \) vary, we should adopt the grand canonical ensemble. Let \( \mu_i \) be the chemical potentials, that is, the Gibbs free energy per water molecule in \( \mathcal{R}_X,i \). Let \( \mu_e \) be electron chemical potential. The grand canonic density operator is ([25] and [11])
\[
\hat{\rho}_X = \exp \left\{ -\beta \left[ \hat{H}_X - \sum_{i=1}^{H} \mu_i \hat{N}_i - \mu_e \hat{N}_e - \Omega(X) \right] \right\}.
\]
where the grand partition function is
\[
\exp[-\beta \Omega(X)] = \text{Trace} \left\{ \exp \left[ -\beta \left( \hat{H}_X - \sum_{i=1}^{H} \mu_i \hat{N}_i - \mu_e \hat{N}_e \right) \right] \right\}
\]
\[
= \sum_{i,N} e^{-\beta [E_{X,N}^{ij} - \sum_{\mu=1}^{H} \mu_i N_i - \mu_e N_e]}.
\]
A.4. The Gibbs Free Energy $G(X)$. According to [25, page 273], under the grand canonic ensemble the entropy $S(X) = S(\mathcal{T}_X)$ of the system $\mathcal{T}_X$ is

$$S(X) = -k_B \text{Trace}(\hat{\rho}_X \ln \hat{\rho}_X) = -k_B \langle \ln \hat{\rho}_X \rangle = k_B \beta \left( \langle \hat{H}_X \rangle - \Omega(X) - \sum_{i=1}^{H} \mu_i \hat{N}_i - \mu_e \hat{N}_e \right)$$

$$= \left[ \langle \hat{H}_X \rangle - \langle \Omega(X) \rangle - \sum_{i=1}^{H} \mu_i \langle \hat{N}_i \rangle - \mu_e \langle \hat{N}_e \rangle \right] / T$$

$$= \left[ U(X) - \Omega(X) - \sum_{i=1}^{H} \mu_i N_i(X) - \mu_e N_e(X) \right] / T. \quad (14)$$

Here we denote $\langle \hat{N}_i \rangle = N_i(X)$ the mean numbers of water molecules in $\mathcal{R}_{X,i}$, $1 \leq i \leq H$, and $\langle \hat{N}_e \rangle = N_e(X)$ the mean number of electrons in $\mathcal{T}_X$. The inner energy $\langle \hat{H}_X \rangle$ of the system $\mathcal{T}_X$ is denoted as $U(X) = U(\mathcal{T}_X)$. The term $\Omega(X)$ is a state function with variables $T, V, \mu_1, \cdots, \mu_H$, and $\mu_e$, and is called the grand canonic potential ([25, page 27]) or the thermodynamic potential ([11, page 33]). By the general thermodynamic equations [11, pages 5 and 6]:

$$d\Omega(X) = -SdT - PdV - \sum_{i=1}^{H} N_i d\mu_i - N_e d\mu_e, \quad \lambda\Omega(X) = \Omega(X)(T, \lambda V, \mu_1, \cdots, \mu_H, \mu_e),$$

we see that $\Omega(X)(T, V, \mu_1, \cdots, \mu_H, \mu_e) = -PV(X)$, where $V(X) = V(\mathcal{T}_X)$ is the volume of the thermodynamic system $\mathcal{T}_X$. Thus by (14) we obtain the Gibbs free energy $G(X) = G(\mathcal{T}_X)$ in (2):

$$G(X) = G(\mathcal{T}_X) = PV(X) + U(X) - TS(X) = \sum_{i=1}^{H} \mu_i N_i(X) + \mu_e N_e(X).$$

A.5. Converting Formula (2) to Geometric Form (3). Since every water molecule in $\mathcal{R}_{X,i}$ has contact with the surface $M_{X,i}$, $N_i(X)$ is proportional to the area $A(M_{X,i})$. Therefore, there are $\nu_i > 0$, such that

$$\nu_i A(M_{X,i}) = N_i(X), \quad 1 \leq i \leq H. \quad (15)$$

Similarly, there will be an $a > 0$ such that $aV(\mathcal{T}_X) = N_e(X)$.

By the definition of $\mathcal{T}_X$ and $\Omega_X$, we have roughly $V(\mathcal{T}_X \setminus \Omega_X) = d_w A(M_X)$. Thus

$$N_e(X) = aV(\mathcal{T}_X) = a[V(\Omega_X) + V(\mathcal{T}_X \setminus \Omega_X)] = aV(\Omega_X) + ad_w A(M_X). \quad (16)$$

Substitute (15) and (16) into (2), we get (3).

We are applying fundamental physical laws directly to protein folding. The question is, can we do so? We will try to check how rigorous is the derivation and ask that are there any fundamental errors? We will also discuss possible ways to modify the formula or the derivation.
A.6. How Rigorous Is The Derivation? We adopted two common tools in physics, the first step of the Born-Oppenheimer approximation in quantum mechanics and the grand canonic ensemble in statistical physics to obtain formula \[2\].

A.6.1. The Born-Oppenheimer Approximation. The Born-Oppenheimer approximation “treats the electrons as if they are moving in the field of fixed nuclei. This is a good approximation because, loosely speaking, electrons move much faster than nuclei and will almost instantly adjust themselves to a change in nuclear position.” \[24\]. Since the mass of a water molecule is much less than the mass of a protein, we can extend this approximation to the case of when \(X\) changes the other articles, electrons and water molecules, will quickly adjust themselves to the change as well.

A.6.2. The Statistic Physics in General and the Grand Canonic Ensemble in Particular. “Up to now there is no evidence to show that statistical physics itself is responsible for any mistakes,” \[11, Preface\]. Via the ensemble theory of statistical mechanics we consider only one protein molecule and particles in its immediate environment, it is justified since as pointed out in \[11\] page 10 “When the duration of measurement is short, or the number of particles is not large enough, the concept of ensemble theory is still valid.” And among different ensembles, “Generally speaking, the grand canonic ensemble, with the least restrictions, is the most convenient in the mathematical treatment.” \[11\] page 16]. In fact, we have tried the canonic ensemble and ended with a result that we have to really calculate the eigenvalues of the quantum mechanics system.

Our derivations only put together the two very common and sound practices: the Born-Oppenheimer approximation (only the first step) and the grand canonic ensemble, and apply them to the protein folding problem. As long as protein folding obeys the fundamental physical laws, there should not be any serious error with the derivation.

A.7. Equilibrium and Quasi-Equilibrium. A protein’s structure will never be in equilibrium, in fact, even the native structure is only a snapshot of the constant vibration state of the structure. The best description of conformation \(X\) is given in \[4\] Chapter 3], we can simply think that a conformation \(X\) acturally is any point \(Y\) contained in a union of tiny balls centered at \(x_i\), \(i = 1, \ldots, M\). In this sense, we can only anticipate a quasi-equilibrium description (such as the heat engine, \[26\] page 94]) of the thermodynamic states of the protein folding. This has been built-in in the Thermodynamical Principle of Protein Folding. So the quantities such as \(S(X)\), \(\Omega(X)\), and \(G(X)\) can only be understood in this sense. That is, observing a concrete folding process one will see a series conformations \(X_i\), \(i = 1, 2, 3, \ldots\). The Thermodynamic Principle then says that if we measure the Gibbes free energy \(G(X_i)\) then eventually \(G(X_i)\) will converge to a minimum value and the \(X_i\) will eventually approach to the native structure. While all the time, no conformation \(X_i\) and thermodynamic system \(T_{X_i}\) are really in equilibrium state.
Any such Lie vector field \( \vec{L} \) will induce a Lie vector field \( \vec{L} : \mathbb{R}^3 \to \mathbb{R}^3 \). For example, moving \( x_i \) from \( x_i \) to \( x_i + (\Delta x_i, 0, 0) \) while keep other nuclear center fixed will induce \( L_{x_i} : \mathbb{R}^3 \to \mathbb{R}^3 \), such that \( \vec{L}_{x_i}(x_i) = (1, 0, 0) \) and \( \vec{L}_{x_i}(x_j) = (0, 0, 0) \) for \( j \neq i \). Similarly we can describe \( \vec{L}_{y_i} \) and \( \vec{L}_{z_i} \). Then write \( G_{x_i} = \vec{L}_{x_i} \), etc. and

\[
\nabla_{\vec{L}} G(\mathbf{X}) = (G_{L_{x_i}}, G_{L_{y_i}}, G_{L_{z_i}})(\mathbf{X}),
\]

(17)

Rotating around a covalent bond \( b_{ij} \) also induce a Lie vector field \( \vec{L}_{b_{ij}} : \mathbb{R}^3 \to \mathbb{R}^3 \). In fact if \( a_i a_j \) form the covalent bond \( b_{ij} \), then the bond axis is

\[
b_{ij} = \frac{x_j - x_i}{|x_j - x_i|}.
\]

If \( b_{ij} \) is rotatable, i.e., 1. it is chemically allowed to rotate; 2. cutting off \( b_{ij} \) from the molecular graph of \( \mathbf{X} \) (see, for example, \([4, \text{page} \ 32]\)) with two components, denoted all nuclear centers in one component by \( R_{b_{ij}} \) and others in \( F_{b_{ij}} \). We can rotate all centers in \( R_{b_{ij}} \) around \( b_{ij} \) for certain angle while keep all centers in \( F_{b_{ij}} \) fixed. The induced Lie vector field \( \vec{L}_{b_{ij}} \) will be

\[
\vec{L}_{b_{ij}}(x_k) = (x_k - x_i) \wedge b_{ij}, \text{ if } x_k \in R_{b_{ij}};
\]

(18)

\[
\vec{L}_{b_{ij}}(x_k) = 0, \text{ if } x_k \in F_{b_{ij}}.
\]

(19)

Any such a Lie vector field \( \vec{L} \) will generate a family of conformations \( \mathbf{X}_t = (x_{1t}, \cdots, x_{1t}, \cdots, x_{Mt}) \), where \( x_{kt} = x_k + t \vec{L}(x_k), k = 1, \cdots, M \).

The derivative \( G_{\vec{L}}(\mathbf{X}) \) is given by

\[
G_{\vec{L}}(\mathbf{X}) = a_0 \mu_0 V_{\vec{L}}(\Omega_\mathbf{X}) + a_0 \mu_0 A_{\vec{L}}(M_{\mathbf{X}}) + \sum_{i=1}^{H} \nu_i \mu_i A_{\vec{L}}(M_{\mathbf{X}_i}),
\]

(20)

where

\[
V_{\vec{L}}(\Omega_\mathbf{X}) = -\int_{M_{\mathbf{X}}} \vec{L} \cdot \vec{N} \mathrm{d} H^2, \quad A_{\vec{L}}(M_{\mathbf{X}}) = -2 \int_{M_{\mathbf{X}}} H(\vec{L} \cdot \vec{N}) \mathrm{d} H^2,
\]

(21)

where \( \vec{N} \) is the outer unit normal of \( M_{\mathbf{X}} \), \( H \) the mean curvature of \( M_{\mathbf{X}} \), and \( H^2 \) the Hausdorff measure. Define \( f_{t,i} : \mathbb{R}^3 \to \mathbb{R} \) as \( f_{t,i}(\mathbf{x}) = \text{dist}(\mathbf{x}, M_{\mathbf{X}_{i-1}}) - \text{dist}(\mathbf{x}, M_{\mathbf{X}_i \setminus M_{\mathbf{X}_{i-1}}}) \), and denote

\[
\nabla_{M_{\mathbf{X}}} f_{0,i} = \nabla f_{0,i} - (\nabla f_{0,i} \cdot \vec{N}) \vec{N}, \quad f'_{0,i} = \frac{\partial f_{t,i}}{\partial t} \bigg|_{t=0}, \quad \frac{\partial f_{0,i}}{\partial t} = \vec{L} \cdot \nabla f_{0,i} + f'_{0,i},
\]

(22)

then let \( \vec{\eta} \) be the unit outward conormal vector of \( \partial M_{\mathbf{X}_i} \) (normal to \( \partial M_{\mathbf{X}_i} \) but tangent to \( M_{\mathbf{X}_i} \)),

\[
A_{\vec{L}}(M_{\mathbf{X}_i}) = -2 \int_{M_{\mathbf{X}_i}} H(\vec{L} \cdot \vec{N}) \mathrm{d} H^2 + \int_{\partial M_{\mathbf{X}_i}} \left[ \vec{L} \cdot \vec{\eta} - \frac{\partial f_{0,i}}{\partial t} \right] \mathrm{d} H^1.
\]

(23)
The $X_t$ is all the information we need in calculating the molecular surface $M_X$. But the kinetic formula $G(X)$ can help us quickly achieve a new conformation $Y$ from $X$ without really calculating $X_t$. For example, we can list all rotatable covalent bonds of the protein as $(b_1, \cdots, b_i, \cdots, b_L)$ and then simultaneously rotate them to get new conformations very quickly by moving along the negative of the gradient

$$G_{\vec{L}}(X_{b_1}, \cdots, G_{\vec{L}}(X_{b_i}, \cdots, G_{\vec{L}}(X_{b_L}))(X).$$

To calculate the above formulae we actually have to translate them into formulae on the molecular surface $M_X$. These translations are given in [12], they are calculable (all integrals are integrable, i.e., can be expressed by analytic formulae with variables $X$) and were calculated piecewisely on $M_X$. If the rotation around $b_i$ with rotating angle $-sG_{\vec{L}}(X)$ be denoted as $R_i$, we can then get the new conformation $Y_s = R_L \circ R_{L-1} \circ \cdots \circ R_1(X)$, where $s > 0$ is a suitable step length. The order of rotations in fact is irrelevant, i.e., by any order we will always get the same conformation $Y_s$, as proved in [12].

This actually is the Newton’s fastest desciending method, it reduces the Gibbs free energy $G(X)$ most efficiently. Afore mentioned simulations in [6] used this method.

References

[1] C. Branden and J. Tooze, *Introduction to Protein Structure*, (Second Edition, Garland, 1999).
[2] L. A. Dill, S. B. Ozkan, M. S. Shell, and T. R Weikl, The Protein Folding Problem. *Annu. Rev. Biophys.* 37, 289-316 (2008).
[3] C. B. Anfinsen, Principles that govern the folding of protein chains. *Science* 181, 223-230 (1973).
[4] R. F. W. Bader, *Atoms in Molecules: A Quantum Theory*. (Clarendon Press · Oxford, 1990).
[5] Y. Fang, Mathematical protein folding problem. In: D. Hoffman, Ed, *Global Theory of Minimal Surfaces. (Proceedings of the Clay Mathematical Proceedings, 2005)* pp. 611-622.
[6] Y. Fang and J. Jing, Geometry, thermodynamics, and protein. *Journal of Theoretical Biology*. 262, 382-390 (2010).
[7] D. Eisenberg and A. D. McLachlan, Solvation energy in protein folding and binding. *Nature* 319, 199-203 (1986).
[8] F. Dyson, A meeting with Enrico Fermi: How an intuitive physicist rescued a team from fruitless research. *Nature* 427, 297 (2004).
[9] I. Tuñón, E. Silla, and J. L. Pascual-Ahuir, Molecular surface area and hydrophobic effect. Protein Engineering 5(8), 715-716 (1992).
[10] D. Thirumalai, E. P. O’Brien, G. Morrison, and C. Hyeon. Theoretical perspectives on protein folding. *Annu. Rev. Biophys.* 39, 159-183 (2010).
[11] X. Dai, *Advanced Statistical Physics*. (Fudan University Press, Shanghai, 2007).
[12] Y. Fang and J. Jing, Implementation of a mathematical protein folding model. *International Journal of Pure and Applied Mathematics*, 42(4), 481-488 (2008).
[13] F. M. Richards, Areas, volumes, packing, and protein structure. *Annu. Rev. Biophys. Bioeng.* 6, 151-176 (1977).
[14] B. Lee and F. M. Richards, The interpretation of protein structures: estimation of static accessibility. *J. Mol. Biol.* 55:379-400, (1971).
[15] H. Wu, Studies on denaturation of proteins XIII. A theory of denaturation. *Chinese Journal nof Physiology* 4, 321-344 (1931). A preliminary report was read before the XIIIth International Congress of Physiology at Boston, Aug. 19-24, 1929 and published in the *Am. J Physiol.* for Oct. 1929. Reprinted in *Advances in Protein Chemistry* 46, 6-26 (1995).
[16] A. Cooper, Thermodynamics of protein folding and stability. In: G. Allen, Ed, (Protein: A Comprehensive Treatise, *Volume 2* 1999) pp. 217-270.
[17] W. Kaushmann, Some factors in the interpretation of protein denaturation. *Adv. Protein Chem.* 14, 1-63 (1959).
[18] J. A. Reynolds, D. B. Gilbert, and C. Tanford, Empirical correlation between hydrophobic free energy and aqueous cavity surface area. *Proc. Natl. Acad. Sci. USA* 71(8), 2925-2927 (1974).
K. A. Dill, Dominant forces in protein folding. *Biochemistry*, **29**, 7133-7155, 1990.

G. D. Rose, P. J. Fleming, J. R. Banavar, and A. Maritan, A backbone based theory of protein folding. *PNAS* **103**(45), 16623-16633, 2006.

Y. Wang, H. Zhang, W. Li, and R. A. Scott. Discriminating compact nonnative structures from the native structure of globular proteins. *PNAS*, **92**, 709-713 (1995).

R. M. Jackson and M. J. E. Sternberg, Protein surface area defined. *Nature* **366**, 638, (1993).

J. Novotny, R. Bruccoleri, R., and M. Karplus, An analysis of incorrectly folded protein models. Implications for structure predictions. *J. Mol. Biol.* **177**, 787-818 (1984).

P. Popelier, *Atoms in Molecules: An Introduction*. (Prentice Hall, 2000).

W. Greiner, L. Neise, and H. Stöker, *Thermodynamics and Statistical Mechanics*. (Springer-Verlag, New York, Berlin, ... 1994).

M. Bailyn. *A Survey of Thermodynamics*. American Institute of Physics New York, 1994.

M. Connolly, M. L. Analytical molecular surface calculation. *J. Appl. Cryst.*, **16**:548-558, 1983.

Department of Mathematics, Nanchang University, 999 Xuefu Road, Honggutan New District, Nanchang, China, 330031, yifang3@gmail.com