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A Comprehensive Analysis of Gibbs Free Energy in Monte Carlo Simulations for Understanding 2D Protein Folding Dynamics

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Abstract. This paper presents a detailed analysis of Gibbs free energy changes in 2D protein folding using Monte Carlo simulations. It starts by introducing fundamental concepts about proteins and the critical role of Gibbs free energy in the free energy landscape of protein folding. The study utilizes a Monte Carlo-based protein prediction server to simulate the folding process of protein sequences and investigates how temperature variations impact this process. Inspired by the work outlined in "Evolutionary Monte Carlo for protein folding simulations," the analysis extends to explore the specific properties of amino acids and their influence on protein folding dynamics in two dimensions. The simulation results offer insightful observations on how different parameters affect the protein folding process, providing a better understanding of the underlying mechanisms. This comprehensive review of the application of Monte Carlo methods to study 2D protein folding dynamics highlights the significance of temperature and Gibbs free energy in shaping the protein folding landscape, demonstrating the utility of these simulations in biochemical research.

Keywords: Protein folding, Gibbs free energy, Monte Carlo, Temperature changes.

1. Introduction

Proteins, essential macromolecules in all living organisms, consist of amino acids linked by peptide bonds. The folding of proteins into their functional conformations is a crucial biochemical process where the aim is to achieve the lowest Gibbs free energy state. Misfolding of proteins can lead to loss of function and is associated with various diseases. The study of protein folding, particularly in a simplified two-dimensional (2D) environment, provides insights into the fundamental mechanisms that underlie protein structure and function. Each protein's unique structure dictates its specific biological role, and aggregates of proteins can form functional domains, critical in understanding cellular processes.

Current Research Status: The concept of a free energy landscape is central to the study of 2D protein folding, offering a visual representation of all potential folding states along with the energy barriers that must be overcome to reach the stable conformation. In this landscape, proteins must navigate through high-energy states and intermediate folds to reach a state of minimum energy, representative of their functional form. Understanding these transitions and the role of each amino acid in the folding process is crucial for decoding how proteins work and why certain misfoldings lead to disease. Current methods

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employ computational models such as Monte Carlo simulations and lattice-based models to simulate these processes, providing a clearer picture of how proteins fold and the energy dynamics involved.

This paper focuses on the application of 2D protein folding studies using advanced computational tools to analyze the Gibbs free energy changes throughout the folding process. By employing Monte Carlo simulations within a lattice-based model framework, this study examines the specific properties of amino acids and how they contribute to the formation of structural features like alpha helices and beta strands. Two prominent protein prediction servers, PSIpred and Jpred 4, will be utilized to simulate the folding process, allowing for an examination of the sequence-to-structure transition in silico. This approach not only aids in tracking the protein folding energy (ΔG) and focusing on intermediate states but also enhances the prediction accuracy of the protein's final conformation. The insights gained from these simulations are expected to contribute significantly to the broader understanding of protein folding dynamics and its implications in health and disease.

2. Theoretical Foundations

In this section, there will be some basic information about how the protein was made from amino acid, what the process is, protein structure, relationship of Gibbs free energy and free energy landscape.

2.1. Amino acids and protein structures

In the process of protein synthesis, there are two major steps, transcription and translation. They collaborate by using enzymes to interpret the genetic code to produce the proteins.

The first step is called transcription [1]. In the initiation step, the enzyme polymerase will bind to a specific region of DNA called promoter, then the DNA will be unwound so it is available for the enzyme to read. The polymerase will come and bind to the promoter and start to move downstream from 5' to 3' until it detect a terminator sequence. RNA polymerase will bind to this new RNA strand and doing proofread, although it is not very powerful. This is called elongation. The mRNA strand will release and the DNA rewinds back to its original double helix structure. This mRNA will have uracil instead of thymine. transcription finished with the step called termination.

The mRNA will go to the ribosome for the next step, translation. Translation is the process of encoded mRNA into protein [2]. It happens in a large ribosome, which has an A site and a P site. A site is the place for the incoming tRNA, which has specific amino acid, and P site holds the growing polypeptide chain. The first step is initiation, a small subunit of the ribosome binding to the mRNA at the start codon, which is AUG, an amino acid for methionine. An initiator tRNA will bind to AUG in the translation initiation region, when the tRNA reads the codon and translates to its amino acid, the complementary tRNA will enter the region. Two amino acids will join by covalent bonds. In elongation, the first tRNA will then leave the ribosome and the new tRNA will join the ribosome and enter the A site of the ribosome, waiting to be translated. This step will go on until it reads a stop codon UAA, UGA, or UAG (corresponding to glutamine for UAA and UAG, tryptophan for UGA) on the mRNA, the translation ends, which comes to the termination step. The newly synthesized polypeptide will be released, and the ribosomal subunits will dissociate. After all these steps, it will come to protein folding.

The structure can be described in four levels: primary, secondary, tertiary, and quaternary structure. Primary structure contains all the information of the later folding [3]. It is simply all the amino acids linked together using peptide bonds, each amino acid has a central carbon atom attached to a hydrogen, an amino group (NH2), an acidic carboxyl group (COOH), and a R group. R groups make each amino acid unique. Some R groups are polar or nonpolar, or even aromatic properties. Each polypeptide has two ends known as N terminal and C terminal, N terminal has a free amino group and C terminal has a free carboxyl group.

Secondary structures have two main conformations, alpha helix and beta sheet. Alpha helix usually has 10-15 amino acid residues, and it is the most stable structure. Hydrogen on amino acids will form a hydrogen bond, connecting the backbone amide and the carbonyl group. This kind of hydrogen bond will make the polypeptide chain a right-hand spiral. Beta pleated sheets usually have 8-10 amino acid residues. It is the second most stable structure, and the hydrogen bond will form between two different

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beta strands, making it into a beta sheet. Beta sheet can have parallel or antiparallel configuration. Antiparallel configuration usually has the sheet structured as crisscross, and parallel refers to the beta strand pointing the same way. A beta sheet is most likely going to have more antiparallel configuration, and then mixture of both, lastly parallel configuration. Some of the protein will have a mixture of alpha helix and beta pleated sheet, but some will only have one of those, it depends on the amino acid that protien has.

Tertiary structures have a three-dimensional structure, and it is the folding stage. Amino acids that contain aliphatic or aromatic hydrophobic side chains will have hydrophobic interaction, which usually happens between non-polar groups. Hence protein will fold into a hydrophobic amino acid core and hydrophilic amino acid left on the outside. This kind of conformation and the hydrophobic interaction will also stabilize the structure.

Quaternary structure involves the connection between two or more similar polypeptide subunits. When each subunit combines, they can perform a specific function. There are three kinds of structure: homodimer, homotrimer, and heterooligomer. Homodimer and homotrimers are formed by identical molecules, however, heterotrimer would be formed by three non-identical subunits. The level of protein folding into tertiary or quaternary structures depends on amino acid sequence, subunit interaction, etc.

2.2. Gibbs free energy and free energy landscape

Gibbs free energy widely used in chemistry, usually measures the energy of a reaction. From the Gibbs free energy, experimenters can understand if this reaction is spontaneous or not. In the context of protein folding, it is a concept that describes the amount of energy to do work. It usually uses the equation:

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

Equation 1: general Gibbs free energy in protein folding during the protein folding process, specifically to the change in energy. Where ΔG is the change of Gibbs free energy, ΔH is the change in enthalpy, T is the temperature in kelvin, and ΔS is the change in entropy.

During the folding, there are three states: unfolded state, folding state, and folded state. Protein in their unfolded or denatured state usually has a high Gibbs free energy, and the folded state will have lower energy [4]. Negative ΔG means the process is spontaneous, means the protein is likely to fold into its structure without any given condition. However, if the outcome is Positive ΔG, it means the process is non-spontaneous, and the protein must in certain condition to do the folding.

Free energy landscape is a model that includes all possible conformation of a protein and their corresponding Gibbs free energy [5]. It usually has an inverted cone shape or funnel graph. On the top of the funnel, it is wide and has a high conformational entropy. This is the unfolded state. As it moves down the funnel, the entropy will decrease, and the enthalpy will increase. As ΔS decreases, the system because more ordered, which correspond to a decrease in temperature based on the equation TΔS. To maintain a stable energy, the ΔH usually increases, and overall stability increases as it goes down the funnel. As show in the figure 1.

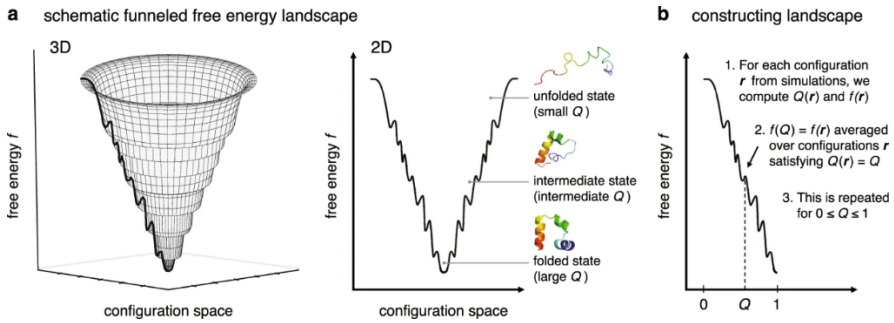

Figure 1. The relationship between free energy and protein structure in different states. From the schematic funneled free energy landscape (left), the unfolded state will have the small Q, and it will have a large ΔG; the folded state or native state on the bottom with a large Q, and small Δ [2].

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2.3. Monte carlo method and parallel tempering

The Monte Carlo method is a statistical technique that uses random samples to solve problems, and it usually involves high-dimensional spaces. The monte Carlo fits well with the randomness of matter, so the technique is also used in protein folding.

Parallel tempering is a method that usually swaps the temperature of two different conformations i and j and compares them with the original two conformations. The probability of swap acceptance use equation [6, 7]:

$$p = \min\left(1,\ \exp\left(-(\beta_j - \beta_i)(E_i - E_j)\right)\right) \quad (2)$$

Equation 2: probability of swap acceptance equation. Where K_B is Bolzman constant, $\beta_i = \frac{1}{K_B T_i}$ is the inverse temperature, and E_i is the energy of the conformation for i, or j for E_j.

According to Alexander Schug, Thomas Herges, Abhinav Verma and Wolfgang Wenzel, "we have used an adaptive temperature control for the simulations: starting with an initial, ordered set of geometrically distributed temperatures we monitored the rate of exchange between adjacent temperatures. If the rate of exchange between temperature i and i +1 was below 0.5%, then all temperatures above t_i were lowered by 10% of t_i+1 − t_i . If the exchange rate was above 2%, then all temperatures above t_i were increased by the same difference." (p. 3, Investigation of the parallel tempering method for protein folding). Based on this, arranging the geometrically distributed temperatures in a nonlinear order can provide better coverage of the different range of energy states. So, lowering the higher temperatures can reduce the energy gap, making it easier for replicas to exchange between neighboring temperatures. For replicas at lower temperatures, increasing the energy gap is needed, it is for preventing too frequent exchange. Thus, this adaptive property makes the simulation more flexible and better suited to the energy profile of the system as it evolves.

Overall, the Monte Carlo method can help with determine the structure by exploring a wide range of possible conformations through random sampling. It evaluates their stability based on the energy, to get the lowest energy conformation.

3. System Analysis and Application Research

This part will mainly focus on protein compactness from the perspective of temperature changes, introduce two monte Carlo based simulation servers to predict a sample protein, and lastly to analyze the Gibbs free energy changes in 2D protein folding.

3.1. Impact of temperature changes on protein compactness

When temperature increases, the kinetic energy of protein molecules also increases, which leads to enhanced molecular motion. This can interrupt the delicate balance of non-covalent interactions of protein compactness. As the temperature continues to rise, these non-covalent interactions have broken and cause the protein to unfold, which loses its compact structure and become unstable [8]. This is also known as denaturation.

Stability is also closely related to the protein compactness when the temperature is changing. It is a measure of the protein's ability to maintain its structure under various conditions. On the free energy landscape, each protein has a unique temperature range, and in these conditions, they will be stable and have the best ability to show their function. When the temperature exceeds this range, it will lead to unfolding and denaturation. This unfolding usually appears in secondary and tertiary structures.

When this applies to living creatures, proteins constantly influenced by temperature can cause serious problems. For example, when humans get fever, rising temperature of one's body can lead to denaturation of heat-sensitive proteins. Continuing fever can cause brain, cardiovascular, gastrointestinal, liver kidney, and hemostasis problems. One of the gastrointestinal effects is closely linked to protein. According to, "Above 40 °C (104 F), there is a reduction in blood flow to the GI tract." [9]. This ultimately increases the likelihood of inflammation and edema in the gastrointestinal tract.

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3.2. Monte carlo simulation framework

When predicting 2D protein structure, two of the tools that experimenters can use are PSIpred and Jpred 4, both allow user to get the protein secondary structure prediction. It simply inputs a single protein sequence or multiple protein sequence and gets the prediction. It is a great tool for getting alpha helix, beta sheet and coil. Based on the paper The PSIPRED protein structure prediction server written by Liam J. McGuffin, Kevin Bryson, and David T. Jones, "A new highly accurate secondary structure prediction method, PSIPRED". This server was used because it has a high accuracy of 76.3%. This can provide the user with an accurate graphical analysis output and also support e-mail results.

In this example, sample Homo Sapiens was used to go through the server. First, finding the translation in NCBI GenBank, following is the sequence. As show in the figure 2.

```
/translation="MPHKGHSGVNQLGGVFVNGRPLPDSTRQKIVELAHSGARPCDIS
RILQTHADAKVQVLDNQNVSNGCVSKILGRYYETGSIRPRAIGGSKPRVATPEVVSKI
AQYKRECPSIFAWEIRDRLLSEGVCTNDNIPSVSSINRVLRNLASEKQQMGADGMYDK
LRMLNGQTGSWGTRPGWYPGTSVPGQPTQDGCQQQEGGGENTNSISSNGEDSDEAQMR
LQLKRKLQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNR
RAKWRREEKLRNQRRQASNTPSHIPISSSFSTSVYQPIPQPTTPVSSFTSGSMLGRTD
TALTNTYSALPPMPSFTMANNLPMQDSFPLVCQFQFKFPEVNLICLNTGQDYSKKKKK
KKKERKYCVNSVSDYGDTTVELSGKKEKWLLEPLQFYNCVLYCTTGEGMDLKQGPLYT
EGTISVGTNLHFGIQTFIHFGVLFVNGHLYVIMKKRTM"
```

Figure 2. Translation sequence from NCBI, Homo Sapiens chromosome 11 (Photo credit: Original).

In the home page of PSIPRED, input the above sequence into the textbox, and the result will be displayed [10]. As show in the figure 3.

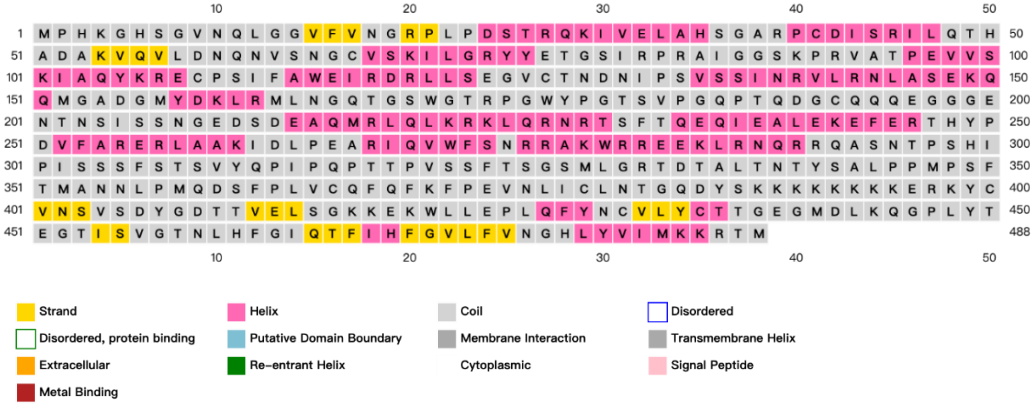

Figure 3. Output of the sequence, yellow indicates beta strands, pink indicates the alpha helix, and the grey area indicates the coils (Photo credit: Original).

When clicking "show aatypes" on the output page, it will show each amino acid's description, as shown below. As show in the figure 5 and figure 4.

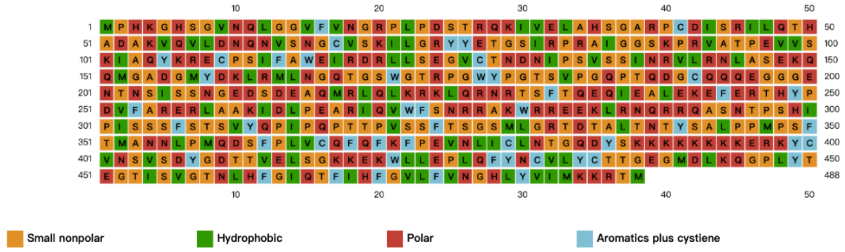

Figure 4. Output of sequence that shows properties of each amino acid, yellow indicates small nonpolar, green shows hydrophobic, red shows polar or hydrophilic, and light blue shows aromatics plus cysteine (Photo credit: Original).

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Figure 5. Graph of PSIPRED Cartoon. On this graph, the bar in dark or light blue shows the confidence of prediction, higher the bar is, the more confident it is. The cart shows the structure, yellow indicates the beta strands, pink indicates the alpha helices, and grey means coils. The pred is same with cart but shows in letter. Lastly, AA shows amino acids shows in origin protein sequence (Photo credit: Original).

When click the next portion, PSIPRED cartoon, it shows bar graph with labels of yellow and pink, as shown below.

Another function bottom on the page was “show memsat”, which indicates the transmembrane protein structure prediction. It simply PSIPRED which part has extracellular protein and transmembrane helix. In this sequence, show memsat does not apply. After introducing PSIPRED, now I will move on to Jpred 4 [11]. In the home page of Jpred 4, enter the above sequence into the textbox and click make prediction. The result will be displayed on the screen in a while. As show in the figure 6.

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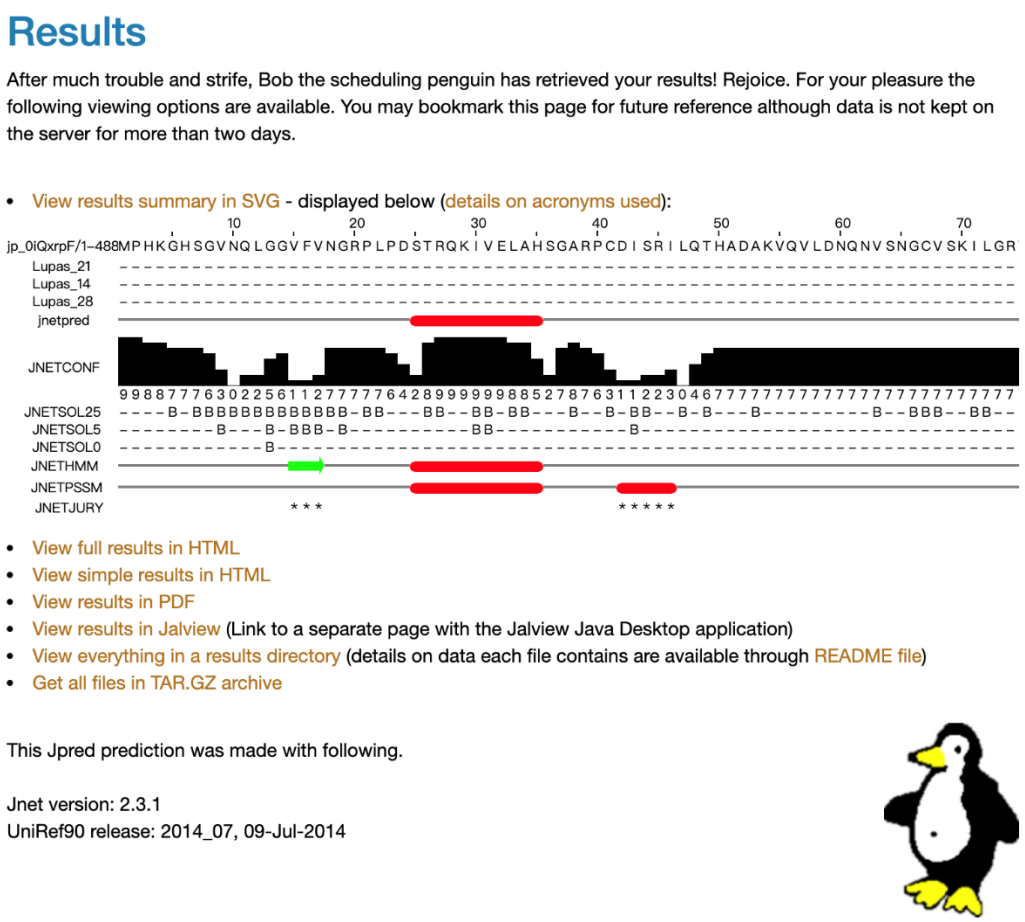

Figure 6. This is a result for chromosome 11 after entering the Jpred 4 (Photo credit: Original).

When the result Displays, there are many terms on the left. Lupas_21, Lupas_14, and Lupas_ 28 represent the coil prediction, there are dashes, small c and uppercase C. Dashes represent the coil in this region that are less than 50%, the letter c represents the region between 50% to 90%, and the uppercase C represents the regions that are greater than 90%. Jnetpred indicates the helices or shown in the letter H and the green arrow indicates the beta strand or shown in the letter E. JNETCONF represents the confident estimate for the prediction, the number indicate the value of confidence, high number means high confidence. JNETHMM annotation means the HMM prediction, it denotes Hidden Markov Models, which are used to convert multiple sequence pairs into position-specific scoring systems. JNETPSSM means the cyblast PSSM prediction. JNETSOL 25 means the relative solvent accessibility of an amino acid residue in the protein structure is less than 25% solvent accessibility, followed by JNETSOL5 and JNETSOL0, B represent the buried region.

When click into the full results in HTML, JNET on the left indicates the final secondary structure prediction, which for this one, is shown as the picture below. As show in the figure 7.

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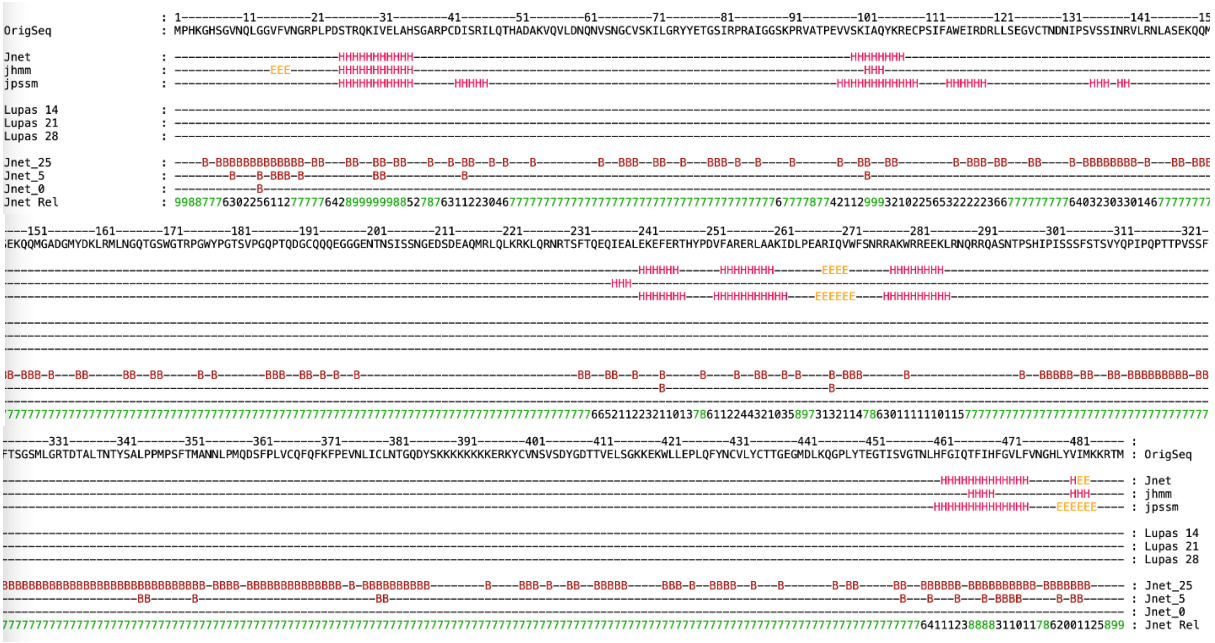

Figure 7. This is the sequence in HTML form. JNET indicates what does the secondary structure looks like (Photo credit: Original).

Based on the graph, H indicates the alpha helices, and E represents the beta sheet. When checking the LUPAS, as mentioned above, dashed means coil structure appears in the conformation, which it shows a coil less than 50%. When checking the JNET, B indicates the region that has less solubility, which shows the hydrophobic region. Jnet_25 shows the part that has hydrophobic region that is less than 25%, and Jnet_5 shows the part that has less than 5% exposure rate, lastly, Jnet_0 shows the part that has 0% exposure rate to any solvent. The number 7 shown on the bottom indicates the high accuracy, which in this protein, the confidence of predicting whether is alpha helices or beta sheet are high. The simulation graph would be shown below. As show in the figure 8.

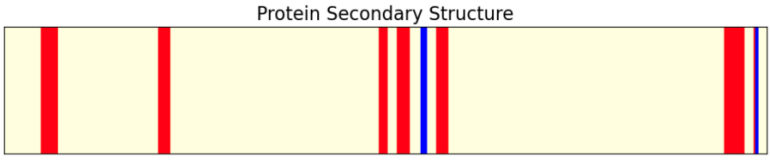

Figure 8. This image was run through python, it comes up with a simulation of the above strings with light yellow representing the coil (dash), red representing the alpha helices (H), and blue representing the beta sheets (E) (Photo credit: Original).

3.3. Gibbs free energy changes in 2D protein folding

To determine the Gibbs free energy changes in 2D protein folding, one of the essential concepts that needs to be used is Gibbs free energy landscape. As mentioned before, the landscape is a multidimensional representation of all possible conformations a protein can be, with free energy associated with each conformation. As show in the figure 9.

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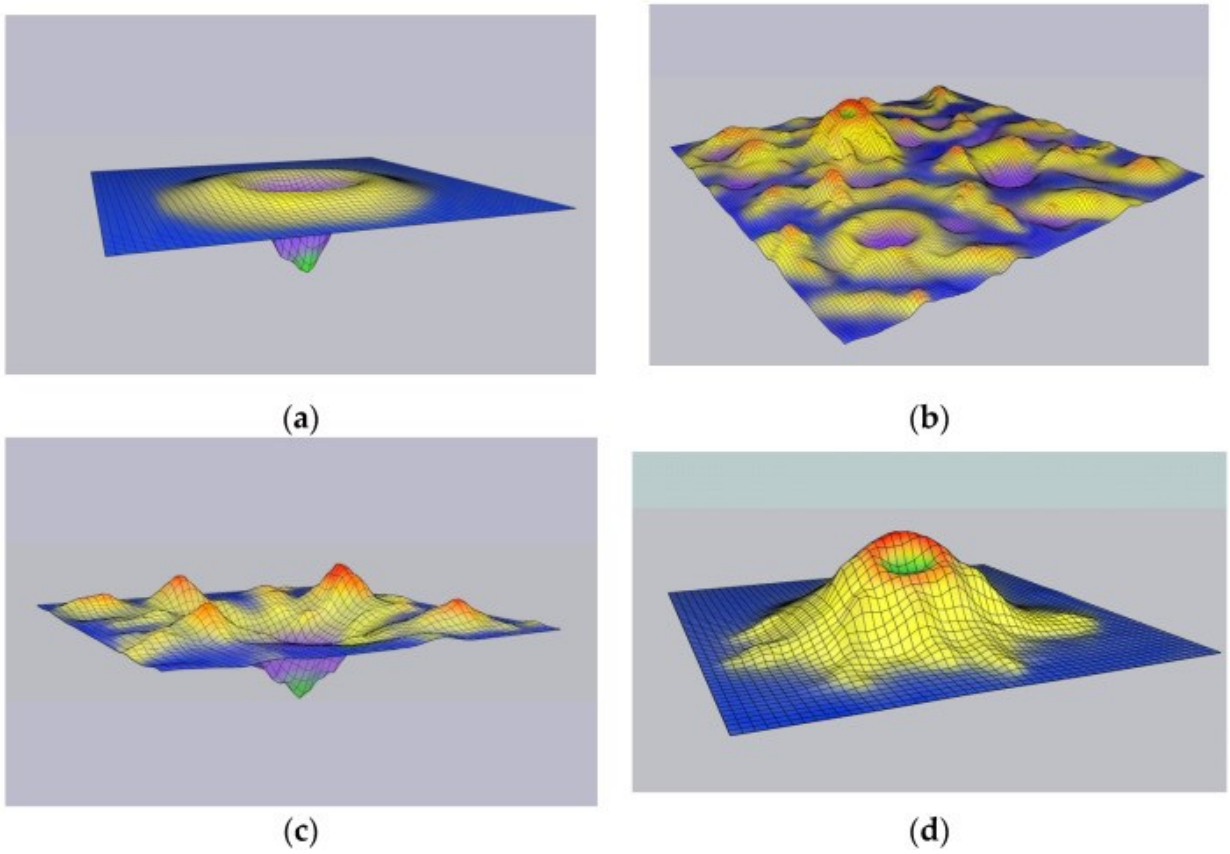

Figure 9. Picture of free energy landscape Blue regions indicate the unfolded state; yellow and purple region indicate the protein conformation during the folding process, but not in very compact shape yet; red region indicates the state almost reach the folded state, and the green region indicates the complete folded state. Looking at figure a, this can be applied to a simple protein folding model. Figure b above is very rugged and bumpy, and this also proves that the protein needs more time to find the best model. Figure c does not have the same rugged surface as figure b, but it also takes time to find the best model. The last figure d can be applied to most of the active proteins. Credit for https://www.ncbi.nlm.nih.gov/pmc/ articles/PMC8745595/ (Photo credit: Original).

Looking at the full state from unfolded to folded protein, in the unfolded state, protein would have high Gibbs free energy [12, 13]. Using the Gibbs free energy equation, protein polypeptide chain is more disordered, which corresponding to high entropy (ΔS) in the equation 1. However, another important role that makes the low Gibbs free energy is water molecules (Lotan et al.). It forms an ordered "cage" structure around the hydrophobic residues, making the residue have less contact with water, which results in a decrease in entropy of water molecules. Moreover, the enthalpy usually is higher in the unfolded state because of intramolecular hydrogen bonds and van der waals interaction, the breaking of interactions contributing to an increase in enthalpy. Thus, the overall Gibbs free energy has increased.

When the protein is in its folding state, which shows as a downhill slope on the graph. The delta G is getting smaller due to the conformation have more ordered and more thermodynamically stable compared to the unfolded state. Previous water molecules "cages" break, makes the entropy increase, leading to decrease in entropy and finally leads to lower Gibbs free energy.

In the process of folding, the protein may encounter barrier, they are the small uphill climb in the free energy landscape of protein must overcome. The entropy decreases, and this will cause the Gibbs free energy to increase temporarily, and it is simply the protein trying different structure, breaking and

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reforming bonds. In this step, if the protein does not overcome the barrier, it will form incorrect conformations, which leading to a disease like Alzheimer's disease or Parkinson's disease etc.

As the protein gets to its final formation, the Gibbs free energy will get smaller and smaller, until the protein finds its lowest Gibbs free energy, and it will stop there. It will correspond to the green region on the free energy landscape, and it is stable.

4. Challenges in Protein Folding Simulations

For the simulations part of the study, firstly, a random search was done through NCBI to find a scientific name and its protein sequence was found through GenBank. In this paper, the sample homo sapience chromosome 11 was used. Secondly, PSIPRED and jpred were used considering that the output might be more accurate if two different servers were used. PSIPRED uses less time to display the properties of each amino acid and uses rich colors to show whether each amino acid is constructed coils, alpha helices or beta strands. The server also can show the PSIPRED plot, memsat, and aatypes, which each corresponding to combination of sequence can be, prediction of transmembrane protein structure, and properties of each amino acid.

JPRED, after inputting the protein sequence, took one night to get the structure prediction. This server has a basic display of alpha helices, beta sheet, and coils, and it is also possible to observe the soluble behavior of different regions of the protein sequence, and the degree of confidence in the structure predicted for each part. Unlike the first one, Jpred4 does not put the amino acids on top of each result, so if you need to compare the results for each amino acid, you should choose PSIPRED, and you can invest less time in waiting. The original plan was to use GOR4 to continue double checking the results from Jpred4 because GOR4 has a better summarizing function. But it failed after loading the page after 3 hours. So, in the end, only PSIPRED and Jpred4 were used.

5. Conclusion

This study has detailed the utilization of Monte Carlo simulation-based prediction servers for protein structure analysis, illustrating how these tools enhance our understanding of protein conformations. By integrating analyses of the free energy landscape, this research has clearly demonstrated the ability to observe Gibbs free energy variations across different protein folds and assess whether proteins have achieved their native, functional, and stable conformations. The adoption of these computational methods extends beyond mere theoretical analysis and preliminary outcomes; it furnishes vital information that bridges the gap between theoretical frameworks and laboratory simulations of complex biological substances.

The application of computational models in protein research holds promising potential for broader scientific and practical applications. Future studies should focus on refining these computational techniques to enhance their accuracy and efficiency in simulating protein folding dynamics. There is also a significant opportunity to expand the application of these models in biotechnology, drug design, and disease understanding by exploring more complex protein interactions and the effects of genetic variations on protein behavior. Moreover, advancing our knowledge of intermediate states and kinetic behaviors through these models could revolutionize our approach to developing new therapies and understanding the underlying mechanisms of various diseases. By continuing to develop and apply these advanced computational tools, researchers can contribute more profoundly to the fields of biotechnology and personalized medicine.

References

[1] Vastag, B. (2002). Genome. gov launched. JAMA, 288(3), 307-307.

[2] Wang R., Zhu J., Wang S., Wang T., Huang J., Zhu X. Multi-modal emotion recognition using tensor decomposition fusion and self-supervised multi-tasking. International Journal of Multimedia Information Retrieval, 2024, 13(4): 39.

[3] Sanvictores, T., & Farci, F. (2022, October 31). Biochemistry, primary protein structure. StatPearls [Internet]. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK564343/#:~:

DOI: 10.54254/2753-8818/74/2024.LA18777

text=To% 20reiterate%2C%20the%20primary%20structure, created%20during%20the% 20protein%20biosynthesis

[4] Sorokina, I., Mushegian, A. R., & Koonin, E. V. (2022b, January 4). Is protein folding a thermodynamically unfavorable, active, energy-dependent process? International Journal of Molecular Sciences.

[5] Zhu, X., Guo, C., Feng, H., Huang, Y., Feng, Y., Wang, X., & Wang, R. (2024). A Review of Key Technologies for Emotion Analysis Using Multimodal Information. Cognitive Computation, 1-27.

[6] Sambridge, M. (2014). A parallel tempering algorithm for probabilistic sampling and multimodal optimization. Geophysical Journal International, 196(1), 357-374.

[7] Fang, Y. (2012). Gibbs free energy formula for protein folding. Thermodynamics—Fundamentals and Its Application in Science, Intech, Rijeka, 47-82.

[8] Harish, B., Gillilan, R. E., Zou, J., Wang, J., Raleigh, D. P., & Royer, C. A. (2021). Protein unfolded states populated at high and ambient pressure are similarly compact. Biophysical Journal, 120(12), 2592-2598.

[9] Balli, S., Shumway, K. R., & Sharan, S. (2022). Physiology, fever. In StatPearls [Internet]. StatPearls Publishing.

[10] Buchan, D. W., Ward, S. M., Lobley, A. E., Nugent, T. C. O., Bryson, K., & Jones, D. T. (2010). Protein annotation and modelling servers at University College London. Nucleic acids research, 38(suppl_2), W563-W568.

[11] Zhu, X., Huang, Y., Wang, X., & Wang, R. (2023). Emotion recognition based on brain-like multimodal hierarchical perception.Multimedia Tools and Applications, 1-19.

[12] Sorokina, I., Mushegian, A. R., & Koonin, E. V. (2022a, January 4). Is protein folding a thermodynamically unfavorable, active, energy-dependent process? International Journal of Molecular Sciences.

[13] Lotan, I., Schwarzer, F., & Latombe, J.-C. (n.d.). Efficient energy computation for Monte Carlo simulation of proteins.