Proceedings of ICBioMed 2025 Symposium: AI for Healthcare: Advanced Medical Data Analytics and Smart Rehabilitation
DOI: 10.54254/2753-8818/2025.AU28990

Advances and Challenges in AI-Based Protein Structure Prediction

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Abstract. Proteins are fundamental to life activities, and their three-dimensional structures are critical in determining their biological functions. Traditional methods of protein structure prediction, such as homology modeling and molecular dynamics simulations, have been constrained by template dependency and computational cost. Recent advances in artificial intelligence (AI), particularly deep learning models like AlphaFold2, have significantly improved prediction accuracy, even for proteins lacking homologous sequences. This review adopts a systematic literature review combined with theoretical analysis to examine major developments in AI-based structure prediction, evaluating their impact and current limitations. While AI models have revolutionized computational biology, this study identifies the lack of empirical validation as a critical constraint. Without integration with experimental techniques, such as cryo-electron microscopy or X-ray crystallography, AI-driven predictions risk limited applicability in complex biological contexts. Future research should prioritize interdisciplinary approaches that combine computational power with experimental validation, ensuring more robust, accurate, and practical outcomes for biological and biomedical applications.

Keywords: Protein structure prediction, artificial intelligence, deep learning, AlphaFold2, computational biology

1. Introduction

Proteins are fundamental molecules essential for life activities, and their three-dimensional structures directly determine their biological functions [1]. Accurate structure prediction is vital for understanding biological processes and has broad applications in drug design, disease research, and enzyme engineering [2]. However, due to the inherent complexity of protein folding and dynamic structural changes, prediction remains challenging. Traditional methods like homology modeling and molecular dynamics rely heavily on structural templates or require intensive computation, limiting scalability.

In recent years, the rapid development of artificial intelligence technology, especially deep learning models, has introduced transformative opportunities for structure prediction. The AlphaFold2 model, launched by the DeepMind team in 2020, achieved unprecedented results in the international protein structure prediction competition CASP14, with an average prediction accuracy approaching experimental resolution, marking a historic breakthrough in AI in the field of biological

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structure modeling [3]. The subsequent open-source release of AlphaFold2 has further accelerated its adoption and fueled advancements in AI-based structure prediction across the scientific community. Despite advances like AlphaFold2, challenges such as empirical validation and real-world application persist. This article aims to systematically review recent developments in AI-based structure prediction, beginning with the fundamentals of protein structures. It further assesses the real-world applicability and constraints of current technologies, offering recommendations to enhance their integration into biological research and life sciences.

2. Overview of the relationship between protein structure and function

2.1. Introduction to protein structures: from primary to quaternary levels

Proteins are biological macromolecules composed of 20 amino acids connected by peptide bonds, and their functions are highly dependent on their three-dimensional organization. Protein structures are commonly categorized into four levels based on their complexity (see Figure 1) [4]. AI can predict protein structures effectively because proteins have a hierarchical organization that follows underlying physicochemical and biological rules. This structure includes learnable patterns such as local folding motifs and residue interactions, which deep learning models can identify. Using methods like attention mechanisms and graph neural networks, AI systems can capture residue-level relationships and spatial constraints to reconstruct accurate 3D conformations from amino acid sequences. The layered organization of proteins matches well with AI's ability to learn multi-level patterns from complex data.

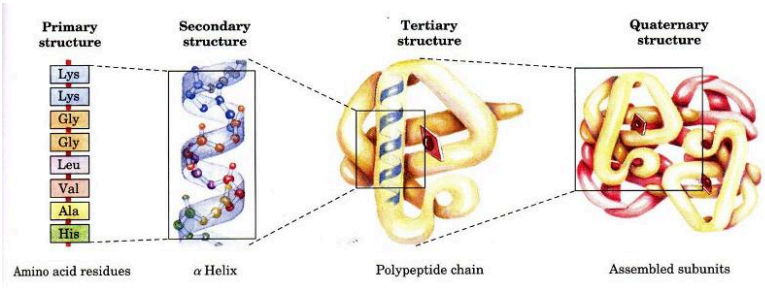

Figure 1. Four-level protein structure [5]

2.2. Advances in protein structure prediction

Protein structure prediction refers to the use of computational methods to infer the spatial conformation of an amino acid sequence without relying on direct experimental structural analysis. This research direction began with the "protein folding problem" in the 1970s [6]. With the improvement of computing power and the accumulation of databases, it has gradually developed into a frontier discipline at the intersection of molecular biology and artificial intelligence.

2.2.1. Traditional methods and their limitations

Early structural prediction relied on template-dependent methods, such as homology modeling. When the target protein shares high sequence similarity with a known structure, its predicted structure can be inferred through direct alignment and comparison. In contrast, threading aims to fit the target sequence into the framework of a known structure under conditions of low homology, seeking the most stable conformational match. Free modeling methods, which build structures de

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novo based solely on physical and chemical principles, are computationally intensive and often hindered by low predictive accuracy due to challenges in energy function modeling and the vastness of conformational space. Collectively, these traditional approaches made important contributions but were constrained by their dependence on existing structural templates and limited scalability.

2.2.2. Breakthroughs via deep learning

Entering the 21st century, the rapid expansion of protein databases such as PDB and the enrichment of sequence homology information paved the way for the gradual introduction of artificial intelligence (AI) into protein structure prediction. Deep neural networks began to be used to predict and analyze sequence-derived information, including contact maps and residue distance matrices, which greatly improved prediction accuracy. The release of AlphaFold2 in 2020 marks the first time that the accuracy of structure prediction has reached the level of experimental analysis [5]. It introduces advanced algorithms such as multiple sequence alignment, attention mechanism, and graph neural network, making it possible to predict template-free proteins. The development of structure prediction has not only promoted basic research but also provided important tools for drug design and protein engineering. Its strategic value has been verified in scenarios such as vaccine design and enzyme catalysis design. Today, structure prediction is entering a new stage dominated by AI and driven by data. Despite these breakthroughs, empirical validation, generalization to dynamic or disordered proteins, and integration into broader biological workflows remain significant challenges. As a result, the field is transitioning to a new AI-driven, data-enabled phase.

3. Main applications of AI in protein structure prediction

3.1. Deep learning approaches

The core role of deep learning models in protein structure prediction is to establish a nonlinear mapping from linear amino acid sequences to three-dimensional spatial structures. Early convolutional neural networks (CNNs) were employed to predict protein contact maps or residue distance matrices, laying the foundation for subsequent three-dimensional structure reconstruction. With the development of natural language processing (NLP) technology, recurrent neural networks (RNNs) and long short-term memory networks (LSTMs) have gradually been used to process sequence features but are limited by their ability to capture long-distance dependencies between sequences [7].

In recent years, the Transformer architecture has become mainstream due to its global attention mechanism, which simultaneously captures the information relationship between any two residues in a sequence, greatly enhancing the ability to model long-range dependencies. AlphaFold2 builds upon an evolutionary structure (Evoformer) of Transformer, integrating multiple sequence alignment (MSA) with template information, capturing the co-evolutionary relationship between sequences through an information interaction module, and using graph neural networks to integrate this information into the geometric constraints required for structure construction [5]. Another representative model is RoseTTAFold, which uses a "three-track architecture" (sequence, distance map, coordinates) to synchronize sequence information with spatial geometric interactions more tightly [8]. Compared to AlphaFold, RoseTTAFold exhibits slightly lower accuracy but significantly faster inference speeds, making it more suitable for medium-precision, high-throughput screening tasks. Additionally, the advent of protein language models, such as ESM-2, along with unsupervised pre-training techniques, is gradually reducing the reliance on MSA. This shift enables AI-based

DOI: 10.54254/2753-8818/2025.AU28990

structure prediction to move towards single-sequence modeling, thereby enhancing its adaptability to proteins with low homology.

3.2. Structural quality assessment

AI not only plays a leading role in the structure prediction process but also plays an essential role in assessing the reliability and optimizing the quality of predicted structures. Traditionally, the evaluation of structure prediction results relies on the scoring criteria of template matching scores or energy functions, which often performed poorly for template-free models and lacked the ability to assess local prediction confidence. AI models represented by AlphaFold2 introduce the pLDDT score (Predicted Local Distance Difference Test), which is a method based on the internal confidence score of deep learning output results, which can evaluate the prediction accuracy at the level of each residue [5, 8]. The pLDDT score correlates highly with deviations from experimentally determined structure, allowing researchers to quickly identify high-confidence and low-confidence areas and guide subsequent structural modifications or experimental verification. AI models are also used to evaluate the conformational flexibility and dynamic change trends of proteins. For example, some studies use variational autoencoders (VAE) or generative adversarial networks (GAN) can map potential conformational states and reveal metastable structures, critical for understanding protein dynamics during ligand binding, activation, or mutational changes. This is particularly critical for studying the structural changes of proteins during functional activation, ligand binding, or mutation response. AI-assisted tools can also perform "post-processing" after structure generation, including repairing collisions between residues, correcting secondary structures, and minimizing structural energy. These tools are often combined with molecular dynamics simulations to improve the physical rationality and biological interpretability of the predicted structure.

3.3. Cross-modal AI models

With the continuous evolution of AI technologies, researchers are increasingly integrating multi-modal data into structure prediction models to enhance their biological relevance and generalization capabilities. A notable advancement is the development of Protein Language Models (PLMs), which capture structural and functional patterns in amino acid sequences through unsupervised pre-training on large datasets [9]. Models like ESM (Evolutionary Scale Modeling), ProtBERT, and ProtT5 can deliver high-quality structure predictions without relying on MSA, making them particularly effective for analyzing low-homology or de novo protein sequences [10]. Another promising direction is the fusion of AI models with experimental data, such as cryo-EM (cryo-electron microscopy) and NMR (nuclear magnetic resonance), which provide partial density maps or spatial constraints [11]. AI models can refine and optimize predicted structures using techniques like image recognition and volume-matching algorithms, achieving greater alignment with native biological structures. Through cross-modal learning, these AI models can more accurately reflect the true structures of proteins in living organisms and offer valuable tools for emerging fields like personalized drug design and synthetic biology. This trend suggests that future structure prediction models will not be confined to single-sequence inputs but will integrate diverse biological data, achieving a more comprehensive "panoramic structural modeling" approach.

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4. Limitations in AI prediction

4.1. The dual challenges of accuracy and interpretability

Although AI models have made significant progress in protein structure prediction, their predictive capabilities still face the dual challenges of accuracy and interpretability. First, most AI models predict a single, static conformation of a protein, the so-called "lowest free energy state," while proteins in real biological environments often present a dynamic balance between multiple conformations. For example, ligand binding, changes in ion concentration, pH fluctuations, or protein-protein interactions often induce structural transitions that current AI models do not explicitly capture. These physiological conditions are usually not explicitly modeled in current AI models, limiting their applicability in dynamic biological scenarios. Moreover, AI models function as "black boxes", with highly complex prediction mechanisms that are difficult to interpret. For instance, while the attention weights in Transformer models can be partially visualized, they do not directly elucidate the underlying physical mechanisms or the specific energy contributions involved in protein folding. Consequently, although models like AlphaFold2 achieve predictive accuracy that closely approximates experimental results, they fall short of answering deeper mechanistic questions —such as the origins of structural stability, the pathways of folding, and the factors determining the formation of active sites.

Besides, the "blind spots" in structure prediction, such as flexible regions, disordered fragments, or conformational heterogeneity, tend to exhibit the lowest confidence in AI models, even though these regions often govern key biological functions. Therefore, improving the interpretability and environmental adaptability of AI structure prediction is one of the key topics in future development, and it also determines whether AI can truly transform from an "auxiliary tool" to an "engine of scientific discovery."

4.2. Limited prediction accuracy for novel or low-homology proteins

Currently, most high-precision AI prediction models rely heavily on MSA pairs as input, which makes them perform well in predicting traditional proteins with a lot of evolutionary information. However, their performance deteriorates when facing novel proteins, especially de novo-designed proteins, low-homology sequences, membrane proteins, and large protein complexes. Novel proteins (such as artificial proteins designed by synthetic biology) are difficult to construct an MSA for due to the lack of natural evolutionary history. AI models cannot extract effective residue connections without sufficient co-evolutionary information, resulting in distorted prediction results. In some cases, even language model systems such as ESMFold can predict based on single sequences, but they still face challenges in identifying complex topological structures and functional domains. For proteins with complex structures and stability in hydrophobic environments, such as membrane proteins and protein complexes, current models often cannot fully simulate their conformational properties in biological membranes. For example, although AlphaFold-Multimer has preliminary complex prediction capabilities, it still performs poorly in identifying transmembrane helices and lipid environment effects [5]. AI models' high computing resource requirements also limit their application in large-scale structural screening tasks. Therefore, developing lightweight, MSA-independent models capable of handling novel structures with reduced computational costs is an urgent direction for future research.

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5. Discussion

While artificial intelligence has significantly enhanced the efficiency and accuracy of protein structure prediction, challenges remain in areas such as model interpretability, adaptability to biological environments, and generalization. To expand the application of AI in structural biology, it is crucial to strengthen the integration of AI models with experimental data. Future advancements should focus on combining AI predictions with experimental techniques like cryo-EM density maps, NMR residual constraints, and cross-linking mass spectrometry (XL-MS), which can effectively calibrate and optimize predictions, balancing both high throughput and accuracy. Furthermore, improving the interpretability and controllability of these models will be essential for their theoretical application in basic research. Introducing causal reasoning frameworks or attention visualization mechanisms within graph neural networks can enable models not only to "predict structures" but also to "explain structures." Additionally, incorporating AI prediction results with traditional methods such as energy functions and biophysical rules will enhance structural rationality and reproducibility. In addressing new proteins and low-homology sequences, the development of lightweight, non-MSA-dependent models is critical for future progress.

6. Conclusion

This paper highlights the significant advancements made in the field of protein structure prediction, particularly with the integration of artificial intelligence (AI) and deep learning technologies. AI models, such as AlphaFold2 and RoseTTAFold, have revolutionized the accuracy of protein structure prediction, surpassing traditional methods by leveraging vast datasets and advanced algorithms. These models have notably excelled in predicting protein structures with unprecedented precision, even in the absence of homologous proteins, marking a major leap forward in computational biology. Additionally, AI's role extends beyond structure prediction, assisting in structural quality assessment, optimization, and the study of protein dynamics. The integration of AI with experimental data, such as cryo-EM and NMR, further enhances the biological relevance and precision of predictions, enabling a more comprehensive understanding of protein function.

A notable limitation of the present study is its reliance on secondary data sources and theoretical analysis, without the incorporation of empirical validation. While the discussion advances understanding of the application of AI in protein structure prediction, the lack of experimental corroboration constrains the generalizability and practical applicability of the findings. Future investigations should prioritize the integration of computational approaches with empirical validation techniques, such as cryo-electron microscopy or X-ray crystallography. Such interdisciplinary efforts would enhance the robustness of AI-driven predictions and facilitate their translation into practical outcomes within biological and biomedical research.

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