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Recent Advances in Protein Structure Prediction (2019–2024): A Literature Review from Traditional Methods to AI Models

Haris Ali Paracha
School of Computer Science and IT, Institute of Management Sciences, Peshawar
Fatima Orakzai
School of Computer Science and IT, Institute of Management Sciences, Peshawar
Afsheen Khalid
School of Computer Science and IT, Institute of Management Sciences, Peshawar
Dilawar Khan*
Department of Computer Science, University of Engineering and Technology, Peshawar
Sumaira Johar
School of Computer Science and IT, Institute of Management Sciences, Peshawar
Omar bin Samin
School of Computer Science and IT, Institute of Management Sciences, Peshawar

Email: dilawarkhan@uetpeshawar.edu.pk
Corresponding Author: Dilawar Khan (Email: dilawarkhan@uetpeshawar.edu.pk)

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Abstract:

Protein structure prediction (PSP) remains one of the most challenging and impactful problems in computa- tional biology. This review systematically examines the evolution of PSP methodologies, from traditional computational approaches to cutting-edge deep learning techniques. We begin with classical methods such as homology modeling and molecular dynamics, then explore machine learning-based approaches including neural networks and protein language models. Special emphasis is placed on revolutionary deep learning architectures like AlphaFold2 and RoseTTA Fold, which have achieved remarkable accuracy in recent CASP competitions. We also discuss emerging directions in reinforcement learning for protein folding simulation and design. Throughout the review, we highlight key biological insights, computational innovations, and remaining challenges in the field.

Keywords: *Protein Structure Prediction, Deep Learning, AlphaFold, Reinforcement Learning, Protein Language Models, Computational Biology*

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Introduction

ROTEINS serve as fundamental macromolecules within living organisms, playing pivotal roles in virtually every biological process. From catalyzing metabolic reactions to maintaining structural integrity, their functionality is both vast and vital. The cor- nerstone of this diverse functionality lies in the three- dimensional (3D) structure of proteins, determined by their amino acid sequences.[1] This sequence-structure relationship forms the essence of the ”protein fold- ing problem,” a longstanding challenge in molecular biology.[2]

The importance of solving this problem cannot be overstated, with implications spanning drug discovery, enzyme engineering, and disease mechanism studies [3]. Misfolded proteins are implicated in numerous diseases including Alzheimer’s and Parkinson’s [4], making accurate structure determination essential for biomedical research.

Historically, three experimental techniques have dominated protein structure determination:

- X-ray crystallography (high resolution but re-quires crystallization) [5]
- NMR spectroscopy (solution studies but limited to small proteins) [6]
- Cryo-EM (powerful for large complexes but resource-intensive) [7]

Due to these limitations, computational PSP meth- ods have become increasingly crucial. The widening gap between known sequences (UniProt) and solved structures (PDB) underscores the need for reliable computational prediction methods [8].

Prior to the deep learning revolution in protein structure prediction, foundational methods such as the Chou–Fasman algorithm [1], GOR method [2], and early homology modeling efforts laid the ground- work for structural bioinformatics. A comprehensive pre-deep learning review can be found in [20], which summarizes approaches before 2019. These early mod- els, although limited in accuracy and scalability, intro- duced core concepts in residue prediction, statistical potentials, and comparative modeling that remain relevant today

2. Traditional Computational Methods

The emergence of computational protein structure prediction stemmed from the recognition that experi- mental methods, although precise, are often slow and resource-intensive. Techniques such as X-ray crystal- lography and nuclear magnetic resonance (NMR) spec- troscopy, while capable of delivering high-resolution structures, cannot keep up with the rapidly expanding volume of protein sequence data generated by modern high-throughput sequencing. To address this dispar- ity, researchers developed traditional computational strategies based on established biological knowledge and fundamental physical principles. These meth- ods aimed to provide approximate yet useful struc- tural insights, relying on information such as sequence homology, evolutionary conservation, and energetics. Traditional approaches—such as homology modeling, protein threading, and ab initio prediction—laid the essential groundwork that enabled the emergence of more sophisticated machine

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learning and deep learn- ing models in recent years.

2.1 Homology Modeling

Homology modeling, also known as comparative mod- eling, is predicated on the evolutionary concept that proteins with similar sequences tend to adopt similar structures. This methodology involves aligning a tar- get protein sequence to one or more template proteins with known structures and then constructing a 3D model based on this alignment.

The process of homology modeling generally in- volves four major steps: template identification, se- quence alignment, model building, and model vali- dation. Template identification relies on searching databases of experimentally solved structures, such as the Protein Data Bank (PDB), to find suitable templates sharing significant sequence similarity. Se- quence alignment is a critical step where the target and template sequences are aligned, ensuring homol- ogous residues correspond spatially. Tools such as MODELLER automate the model-building process, which generates atomic coordinates for the target based on the aligned template structures [14, 16].

Homology modeling excels when the sequence iden- tity between the target and the template exceeds 30%, allowing accurate inference of the backbone confor- mation and side-chain packing. However, its accuracy diminishes significantly in the so-called ”twilight zone” (sequence identity below 30%), where alignment errors and structural divergence limit reliability [15]. More- over, homology models often inherit the imperfections and missing residues present in the template struc- tures, especially in flexible loop regions or disordered segments, which are challenging to model accurately. Despite these drawbacks, homology modeling remains a cornerstone technique due to its efficiency and rela- tive accuracy when suitable templates exist.

2.2 Threading (Fold Recognition)

Threading, also called fold recognition, was developed to tackle cases where sequence similarity is too low to identify homologous templates but where structural similarity might still exist. Unlike homology modeling, threading methods attempt to ”thread” the target sequence onto a library of known protein folds and evaluate how well the sequence fits each fold based

Table 1: Comparison of Traditional Protein Structure Prediction Approaches (2019–2024)

Method	Key Features	Best Use Case	Example Paper (Citation)	Year
Homology Modeling	Uses known structure templates with high sequence identity	Sequence identity > 30%	Zhang and Xie, ”Deep learning in protein structure pre-diction” [13]	2021
Threading (Fold Recognition)	Aligns sequence to known folds; works with low identity	Remote homology detection	Zhou and Liu, ”Understand-ing protein folding...” [15]	2020
Ab Initio Modeling	No template required; energy minimization	Small proteins (<100	Singh et al.,”Advances in deep neural	2020

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		residues)	networks...” [17]	
Fragment Assembly	Reconstructs protein from known frag-ments	Medium-length pro-teins with unknown fold	Nguyen et al., ”Review using deep learning” [18]	2020
Loop Modeling	Predicts flexible loop regions	Gaps in templates or active site mod-eling	Alniss et al., ”ML models for PSP” [14]	2020
Side-Chain Modeling	Optimizes side-chain conformations using rotamers	Enzyme active sites or interface model-ing	Alford et al., ”Deep learning models for PSP” [16]	2020
Molecular Dynamics (MD)	Refines structure using physical simulations	Local refinement, Conformation al flexibility	Li et al., ”From sequence to structure” [19]	2019
Hybrid Methods (e.g., I-TASSER)	Combines threading, ab initio, refinement	Targets with weak templates	Jumper et al., ”AlphaFold accuracy” [7]	2021

On physicochemical and structural compatibility [17]. This approach uses scoring functions that incorpo- rate residue environment, secondary structure propen-sity, solvent accessibility, and residue-residue contact potentials to assess the fitness of the sequence in a particular fold. Methods like Phyre2 and MUSTER apply sophisticated threading algorithms to detect remote homologs and recognize folds even without detectable sequence similarity.

Threading is especially valuable for proteins lack- ing close homologs with known structures, expanding the coverage of structural prediction. However, its accuracy heavily depends on the quality of the scoring functions and the completeness of the fold library. In- correct scoring or absence of the correct fold template in the database can lead to erroneous fold assignment [14].

2.3Ab Initio (De Novo) Modeling

Ab initio modeling aims to predict protein structures without relying on homologous templates, based solely on physicochemical principles and thermodynamics. It attempts to identify the lowest free-energy confor-mation of a protein sequence by sampling the vast conformational space accessible to the polypeptide chain [19].

The central challenge of ab initio modeling lies in the astronomical size of this

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conformational space — a dilemma known as Levinthal's paradox. To overcome this, methods like Rosetta utilize fragment assembly approaches, sampling small peptide fragments ex- tracted from known structures and assembling them into full-length models guided by energy functions [16].

While ab initio methods have shown success in accu- rately predicting small protein folds, their applicabil- ity to larger proteins is limited due to computational complexity and the difficulty of accurately modeling long-range interactions. Nonetheless, ab initio prin- ciples have driven improvements in energy functions and sampling strategies.

2.4 Fragment Assembly

Fragment assembly is a hybrid technique that simpli- fies the folding problem by dividing it into manageable subproblems. It involves selecting short structural fragments from a library of known protein structures that match segments of the target sequence. These fragments are then assembled into complete structures using stochastic sampling algorithms [12, 16].

This method reduces the complexity of conforma- tional sampling by constraining possible backbone conformations to those observed in solved structures. Tools such as Rosetta have demonstrated remarkable success using fragment assembly, especially for small to medium-sized proteins.

2.5 Knowledge-Based Potentials

Knowledge-based potentials are derived by analyzing the frequencies of residue-residue interactions, dis- tances, and angular relationships in protein struc- tures. These statistical observations are translated into scoring functions used to evaluate model quality [19, 18].

Common examples include DFIRE, DOPE, and sta- tistical pairwise contact potentials. These are widely used for model validation and structure refinement.

2.6 Loop Modeling

Loops are flexible protein regions connecting sec- ondary structures. Accurate modeling of loops is crucial for functional relevance, especially in homology models. Loop modeling uses both database searches and ab initio sampling to find conformations compat- ible with flanking regions [15, 19].

2.7 Side-Chain Modeling

Side-chain modeling aims to place amino acid side chains onto a fixed backbone using rotamer libraries.

Tools use energy functions and optimization algo- rithms such as Monte Carlo or dead-end elimination for this task [19].

This modeling is especially important for detailed studies of enzyme active sites or docking simulations [17].

2.8 Molecular Dynamics Simulations

Molecular dynamics (MD) simulates atomic motions over time using Newtonian mechanics. MD is pri- marily used for refining protein models by allowing relaxation to energetically favorable states [15, 10].

Due to computational intensity, MD is often limited to local refinement but is valuable for studying protein dynamics and validating predicted models.

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2.9 Hybrid Approaches

Hybrid methods combine various modeling techniques to enhance prediction accuracy. For example, I- TASSER uses threading, fragment assembly, and structural refinement. These systems exploit the strengths of multiple methodologies while minimizing individual weaknesses [8, 7, 3].

Hybrid approaches are now standard in structural prediction pipelines and have been instrumental in community challenges like CASP [10, 14].

3 Machine Learning Approaches

The advent of machine learning (ML) revolutionized protein structure prediction by introducing models capable of learning complex, non-linear relationships between protein sequences and structural features. Unlike traditional methods that relied heavily on hand- crafted rules or physical simulations, ML methods can learn directly from data, making them particularly suited for capturing subtle evolutionary and biophys- ical patterns [14, 13]. This section explores various ML-based approaches used in the different stages of protein structure prediction.

Table 2: Comparison of Traditional Protein Structure Prediction Approaches

Method	Key Features	Best Use Case	Typical Accuracy (RMSD or TM-score)
Homology Modeling	Uses known structure tem-plates with high sequence identity	Sequence identity ¿ 30%	RMSD: 1–3 ˚A or TM-score ¿ 0.7
Threading(Fold Recognition)	Aligns sequence to known folds; works with low iden-tity	Remote homology detection	RMSD: 3–5 ˚A or TM-score ≈ 0.5–0.7
Ab Initio Modeling	No template required; en-ergy minimization	Small proteins (¡100 residues)	RMSD: 4–8 ˚A or TM-score ¡ 0.5
Fragment Assembly	Reconstructs protein from known fragments	Medium-length pro-teins with unknown fold	RMSD: 2–6 ˚A
Loop Modeling	Predicts flexible loop re-gions	Gaps in templates or active site mod-eling	RMSD: 1.5–5 A˚ (for loops)
Side-Chain Model- ing	Optimizes side-chain con-formations using rotamers	Enzyme active sites or interface model-ing	Side-chain RMSD: 1–2 ˚A

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Molecular Dynam- ics (MD	Refines structure using physical simulations	Local refinement, conformational flexibility	Improves by 0.5–2 ˚A
Hybrid Methods (e.g., I-TASSER)	Combines threading, abinitio, refinement	Targets with weak templates	RMSD: 2–4 ˚A or TM-score ≈ 0.6–0.8

3.1 Secondary Structure Prediction

One of the first successful ML applications in struc- tural bioinformatics was secondary structure predic- tion. Traditional statistical methods struggled with capturing long-range dependencies and required care- ful curation of feature sets [2]. ML-based techniques, particularly Support Vector Machines (SVMs), Ran- dom Forests, and ensemble learning models, brought significant performance improvements by integrating a wide variety of sequence-based and evolutionary features [19].

Methods such as PSIPRED and SPIDER3 utilized position-specific scoring matrices (PSSMs), amino acid composition, hydrophobicity scales, and pre- dicted solvent accessibility to classify residues into helix, strand, or coil states [14, 17]. Ensemble methods combining several weak classifiers improved accuracy by reducing individual model biases. However, these models depended heavily on the quality of the input features and struggled with low-homology sequences. Later methods began to incorporate windowed fea- tures, where a local segment of the protein (typically 15–21 residues) was used as the input for ML classifiers. Although effective, these window-based approaches limited the model's ability to capture global dependen- cies, a problem eventually addressed by deep learning models such as convolutional and recurrent neural networks [13, 18].

3.2 Contact Map Prediction

Contact map prediction is essential for inferring the 3D topology of a protein from its sequence. It in-

Table 3: Comparison of Machine Learning Approaches in Protein Structure Prediction (2019–2024)

ML Technique	Key Features	Best Use Case	Example Paper (Cita- tion)	Year
Secondary Structure Prediction	Uses evolutionary profiles, PSSMs, SVMs, ensemble models	Predicting helix, strand, coil labels	Singh et al., "Advances in DNNs for protein struc- ture" [17]	2020
Contact Map Predic-tion	Co-evolutionary fea-tures + SVMs or Na¨ıve Bayes; later CNNs	Inferring residue-residue contacts	Nguyen et al., "Comprehen- sive review DL PSP" [18]	2020

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Hidden Markov Models (HMMs)	Models inser- tions/deletions in MSAs using pro- file HMMs	Homology detec- tion, fold recogni- tion	Zhang and Xie, ”DL in protein structure predic- tion” [13]	2021
Disorder Region Predic-tion	Uses SVMs, logis- tic regression with disorder- related fea- tures	Predicting flexible or unstructured pro- tein regions	Alford et al., ”DL models for PSP” [16]	2020
Solvent Accessibility & Torsion Angle Predic-tion	Regression with sliding windows and kNN/SVM models	Predicting local residue exposure or phi/psi angles	Zhou and Liu, ”Protein fold-ing and drug discovery” [15]	2020
Template-Free Embed-ding / Representation Learning	Unsupervised learn-ing (e.g.autoen- coders, early trans- formers)	Feature generation From sequences without MSAs	Li et al., ”Protein structure via DL” [19]	2019

volves predicting whether pairs of residues are in contact (typically within 8˚A). ML models initially used evolutionary couplings from MSAs and applied classifiers such as SVMs or Na¨ıve Bayes models to predict contact probabilities [16].

Later methods like MetaPSICOV and DeepCon- tact used richer feature sets, combining evolutionary profiles, predicted secondary structures, and coevolu- tion metrics [13]. The incorporation of convolutional neural networks allowed these methods to capture spatial dependencies across the sequence and improve long-range contact prediction [19].

These methods were further enhanced with the use of ensemble strategies, dropout-based regularization, and multi-task learning frameworks, allowing for im- proved generalization [14]. The output of these models often feeds into downstream structure reconstruction algorithms that use contact maps to fold proteins using optimization-based methods [16].

3.3 Hidden Markov Models (HMMs)

Hidden Markov Models (HMMs) revolutionized se- quence alignment and homology detection. In protein structure prediction, HMMs were primarily used for profile construction and fold recognition [13]. Tools like HMMER and HHpred built probabilistic models of MSAs, capturing insertions, deletions, and muta- tions [14].

HMMs enabled sensitive detection of remote ho- mologs, which are essential for accurate template se- lection in homology modeling [16]. These models also played a role in domain annotation, transmembrane region identification, and functional site prediction [17].

Despite their widespread use, HMMs were even- tually limited by their Markovian assumptions and inability to capture long-range or hierarchical depen- dencies. As a

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result, they have been increasingly augmented or replaced by deep learning-based profile models [18].

3.4 Disorder Region Prediction

Intrinsically disordered regions (IDRs) are segments of proteins that lack a fixed 3D structure under phys- iological conditions. Predicting IDRs is important for understanding protein flexibility, interaction sites, and regulatory functions. ML-based models, particularly logistic regression and SVMs, were among the first to predict disorder regions from primary sequence data [14].

Feature sets for IDR prediction typically include amino acid propensities, physicochemical properties, hydrophobicity, and evolutionary conservation. Tools like IUPred and DISOPRED utilized such features within machine learning frameworks to identify flexi- ble and unstructured regions [17].

Recent advances incorporate deep recurrent net- works and bidirectional LSTMs that model sequential dependencies across the full length of the protein, sig- nificantly improving prediction of both short and long disordered regions [13, 16]. These predictions also feed into structure refinement pipelines to prevent misfolding of flexible loops or termini [19].

3.5 Solvent Accessibility and Torsion Angle Prediction

ML models have also been applied to predict residue- level properties such as solvent accessibility and tor- sion angles (ϕ, ψ), which provide fine-grained struc- tural information useful for tertiary structure predic- tion. Early models used regression techniques like SVM regression and k-nearest neighbors (kNN) to predict continuous values for these properties [16].

Tools such as SPINE-X and Real-SPINE used neu- ral networks trained on sliding window features to predict residue exposure and backbone angles [18]. These predictions helped constrain conformational search spaces for tertiary structure prediction algo- rithms.

Modern deep learning models for protein structure prediction employ fully connected and convolutional architectures trained end-to-end to capture complex relationships between amino acid sequences and their three-dimensional structures. These models are often implemented within multi-task learning frameworks, predicting multiple residue-level attributes simultane- ously, such as secondary structure, solvent accessibil- ity, torsion angles, disorder regions, and contact maps [17, 13]. By sharing learned representations across tasks, multi-task models enhance generalization, par- ticularly for proteins with limited evolutionary infor- mation, and allow richer internal feature extraction from sequence data.

Incorporating physicochemical constraints into modeling pipelines—such as solvent exposure, tor- sion angles, and residue-residue distances—improves both model convergence and the physical plausibility of predicted structures [19]. Advanced architectures, including residual networks, graph neural networks, and transformer-based attention models, capture long- range dependencies and structural context, which is crucial for predicting complex folds. Furthermore, many models leverage evolutionary information from multiple

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sequence alignments (MSAs) or embeddings from pretrained protein language models, providing additional sequence-based features that improve ac- curacy even for proteins without close homologs. To- gether, these strategies have significantly enhanced the precision of computational protein structure predic- tion, bringing it closer to experimentally determined structures.

3.6 Template-Free Structural Embedding and Representation Learning

In recent years, unsupervised and self-supervised learn- ing methods have been employed to learn structural embeddings directly from large protein databases. These models, inspired by natural language process- ing (NLP), use architectures like Transformers and masked language modeling (MLM) to learn contextual representations of protein sequences [6, 5]. Models such as ESM (Evolutionary Scale Modeling), ProtBert, and TAPE have shown that pretraining on massive unlabeled datasets allows these models to capture structural and functional information implic- itly [6, 5, 19]. Fine-tuning these representations on downstream tasks like secondary structure prediction, contact prediction, or binding site prediction has led to state-of-the-art results in many cases [11].

These learned embeddings serve as generalized fea- ture extractors that outperform hand-crafted features across diverse prediction tasks. Moreover, such embed- dings have been used to cluster protein folds, discover new functional domains, and even predict effects of point mutations on structure and function [9, 10].

Table 4: Traditional vs ML-Based Approaches in Protein Structure Prediction

Approach	Limitations (Traditional)	Advantages (ML-Based)
Secondary Struc-ture	Rule-based methods lack global context	ML (SVMs, RNNs) capture sequence and evolutionary dependencies
Contact Maps	Co-evolution data is sparse	CNNs combine features for bet-ter long-range prediction
Homology/Threading	Depend on high-identity templates	ML infers structure via sequence embed- ings
Disorder Re-gions	Poor generaliza-tion from rules	DL improves prediction for short and long disorder
Solvent Accessi-bility	Limited multi-output regres-sion	Joint learning improves struc-tural accuracy
HMMs / Profiles	Markov assump-tion restricts context	Transformers model full se-quence context
Representation Learning	Need MSAs or handcrafted fea-tures	Self-supervised models general-ize broadly

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4 Deep Learning-Based Approaches

The advent of deep learning (DL) has revolutionized the field of protein structure prediction (PSP) by enabling models to autonomously learn complex, hi- erarchical mappings from protein sequences to their three-dimensional structures without the need for ex- tensive manual feature engineering. DL architectures are particularly well-suited to PSP because they can capture spatial, sequential, and long-range depen- dencies within protein sequences, which are essential for understanding the intricacies of protein folding [14, 19]. This section discusses key DL approaches historically and currently applied to PSP, highlight- ing their architectures, strengths, limitations, and biological impact.

4.1 Convolutional Neural Networks (CNNs)

Convolutional Neural Networks (CNNs), originally developed for computer vision tasks, represent one of the earliest DL architectures adapted for protein structure prediction. CNNs excel at extracting hier-archical spatial features through convolutional filters, making them ideal for analyzing spatially organized data such as images. In PSP, protein features such as contact maps, residue pairwise distance matrices, or secondary structure elements can be naturally repre- sented as 2D matrices, allowing CNNs to learn spatial patterns effectively [18, 20].

Initial applications of CNNs to PSP often focused on secondary structure prediction, where 1D CNNs were employed to capture local sequential motifs within protein sequences. By sliding convolutional filters over one-dimensional amino acid sequences or their associated profiles (such as position-specific scoring matrices), CNNs learned to identify characteristic se- quence patterns that correlate with helices, sheets, or coils. These approaches outperformed traditional machine learning methods reliant on handcrafted fea- tures due to the CNNs' ability to learn discriminative representations directly from raw input data [15, 19]. Extending beyond one-dimensional data, 2D CNNs were leveraged to predict residue-residue contact maps, which represent the proximity between amino acid pairs in the folded protein. By treating the contact

Table 5: Comparison of Deep Learning (DL) Approaches in Protein Structure Prediction (2019–2024)

DL Model	Key Features	Best Use Case	Representative Study	Year
Convolutional Neural Networks (CNNs)	Detect local sequence motifs and spatial features	Contact map and secondary structure prediction	Singh et al., "DNNs for Pro- tein Structure Prediction" [17]	2020
Recurrent Neur al Networks (RNNs)	Capture sequential dependencies via LSTM/GRU	Residue embedding, secondary structure	Zhang and Xie, "DL in Pro- tein Structure Prediction" [13]	2021

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End-to-End Differen- tiable Models	Predict 3D struc- ture directly from sequence or MSA using optimization	Full protein folding without intermedi- ate steps	AlQuraishi, ”End-to-End Dif- ferentiable Learning” [4]	2019
AlphaFold2	Evoformer block with pair and structure module	High-accuracy full structure prediction from MSAs	Jumper et al., ”AlphaFold” [7]	2021
RoseTTAFold	Three-track architec- ture (1D, 2D, 3D in- tegration)	Structure pre- diction and protein–protein interaction model- ing	Baek et al., ”RoseTTAFold” [8]	2021
Graph Neural Net- works (GNNs)	Residue/atom-level graph with message passing	Side-chain mod- eling, structure refinement	Alford et al., ”DL Models for PSP” [16]	2020
Transformers	Long-range attention over sequences; pre- cursor to PLMs	Structure modeling and embeddings	Rives et al., ”Scaling Unsuper- vised Learning” [6]	2021

map as a grayscale image, CNNs applied spatial fil- ters to capture interaction patterns between distant residues. Notably, networks such as DeepCov and DNCON2 employed multi-layer 2D CNNs to predict contacts with improved accuracy, enabling better con- straints for downstream folding algorithms. These CNNs exploited local spatial correlations, translation invariance, and hierarchical feature learning to detect subtle residue interactions critical for folding [14, 18].

Subsequent advancements introduced 3D CNNs that operate on volumetric representations of protein structures or fragment assemblies. These 3D CNNs can directly model spatial coordinates and atomic environments, allowing end-to-end learning of atomic interactions and facilitating tasks like structure refine- ment or loop modeling. Despite their promise, 3D

CNNs are computationally intensive and require large amounts of structural data for effective training [17]. However, CNNs face challenges in modeling very long-range dependencies intrinsic to protein folding,

where residues far apart in sequence form close con- tacts in three-dimensional space. While deeper CNNs and multi-scale aggregation strategies partially miti- gate this by increasing receptive fields, they inherently rely on local neighborhood operations, limiting their ability to capture global context fully. This limitation motivated the exploration of alternative architectures such as recurrent neural

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networks and, more recently, transformers [19].

Overall, CNNs laid the groundwork for DL in PSP by effectively modeling local and medium-range struc- tural patterns, improving prediction accuracy, and enabling end-to-end learning from sequence-derived features [14, 18].

4.2 Recurrent Neural Networks (RNNs)

Recurrent Neural Networks (RNNs) are designed to process sequential data by maintaining internal mem- ory states that capture information from previous inputs. Variants such as Long Short-Term Memory (LSTM) networks and Gated Recurrent Units (GRUs) were particularly popular for biological sequences, in- cluding proteins, because they can model dependen- cies over varying lengths and are capable of learning temporal or sequential relationships [19, 20].

In protein structure prediction, RNNs were ini- tially employed to model the sequential nature of amino acid chains, capturing long-range dependencies where distant residues influence folding and structural motifs. Applications included predicting secondary structures, disorder regions, and generating sequence profiles or embeddings for downstream tasks. For ex- ample, LSTM-based networks were used to generate context-aware residue representations by integrating information from both N-terminal and C-terminal di- rections (bidirectional RNNs), improving predictions of local structural features [14, 18].

Moreover, RNNs were explored for early folding simulations and contact prediction by treating residue pairs as sequence elements and attempting to infer spa- tial relationships through sequential processing. Their ability to remember contextual sequence information proved advantageous over traditional feed-forward net- works, particularly for capturing patterns extending beyond local neighborhoods [15].

Despite these strengths, RNNs faced fundamental limitations in PSP. The vanishing and exploding gradi- ent problems, while partially alleviated by LSTM and GRU architectures, still hindered learning over very long protein sequences. Furthermore, their inherently sequential nature makes parallelization challenging, limiting scalability for large-scale datasets and long sequences [19].

Most critically, RNNs are better suited to linear sequential relationships and have difficulty learning complex, global 3D spatial relationships that are cen- tral to protein folding. While they excel at model- ing sequential dependencies, they do not inherently encode the pairwise or higher-order interactions be- tween residues needed for accurate folding predictions [19, 20].

Consequently, the role of RNNs in state-of-the-art PSP has diminished with the advent of transformer architectures, which can simultaneously attend to all sequence positions and capture both local and global dependencies more efficiently. Nevertheless, RNNs contributed important foundational insights into se- quence modeling in PSP and remain useful in specific contexts, such as sequence embedding generation or disorder prediction [19].

4.3 End-to-End Differentiable Models

The most transformative advancement in DL-based protein structure prediction (PSP) has been the de- velopment of fully end-to-end differentiable mod- els. These models

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learn to map raw amino acid sequences—and often multiple sequence alignments (MSAs)—directly to three-dimensional atomic coor- dinates. This is achieved by jointly optimizing all components of the prediction pipeline via backprop- agation. Unlike traditional multi-stage systems that rely on handcrafted intermediate features, these mod- els integrate the entire process, enabling them to learn hierarchical representations and structural constraints in a unified framework [4, 7].

4.4 AlphaFold and AlphaFold2

The introduction of AlphaFold by DeepMind in 2018 marked a watershed moment for PSP. AlphaFold em- ployed deep convolutional neural networks to predict distance distributions between residue pairs, gener- ating a probabilistic representation of inter-residue distances. These distance maps were then converted into 3D coordinates using gradient-based optimiza- tion, enabling accurate structure reconstruction. Al- phaFold outperformed prior methods in the CASP13 competition, demonstrating the efficacy of DL-based distance predictions for structure modeling [3, 7].

Building on this success, AlphaFold2, released in 2020, introduced a radically novel architecture that integrated transformers with innovative modules to jointly model evolutionary, pairwise, and spatial in- formation. Key components of AlphaFold2 include:

- **MSA Attention:** This mechanism extracts evo- lutionary relationships from multiple sequence alignments, allowing the model to identify con- served and co-evolving residues that inform fold- ing [7].
- **Evoformer Blocks:** Deep neural network mod- ules that iteratively refine sequence and pairwise representations through attention and communi- cation between sequence and residue pair features [7].
- **End-to-End Structure Module:** A differen- tiable module that directly predicts 3D atomic co- ordinates from refined features, enabling gradient-based training of the entire network [7].

AlphaFold2 abandoned the traditional two-stage approach (predicting contacts or distances followed by folding) in favor of a unified end-to-end framework, enabling the model to learn effective folding strategies implicitly. This architecture achieved a median Global Distance Test (GDTTS) score of 92.4 at CASP14, rivaling the accuracy of experimental methods and revolutionizing the field [7, 10].

Importantly, AlphaFold2's open-source release and associated databases have democratized access to high- quality structure predictions, catalyzing advances in biology, drug discovery, and protein engineering [7, 13].

4.5 RoseTTAFold

RoseTTAFold, developed concurrently by the Baker Lab, introduced an alternative deep learning frame- work that employs a distinctive three-track network architecture. In contrast to AlphaFold2's primarily sequential processing, RoseTTAFold processes infor- mation across three interconnected tracks in parallel:

- **1D Sequence Track:** Processing raw amino acid sequences.

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- **2D Residue Pair Track:** Capturing pairwise relationships and spatial constraints.
- **3D Coordinate Track:** Responsible for reason- ing about atomic spatial arrangements within the protein structure.

Information flows bidirectionally between these tracks, allowing the model to integrate sequence, in- teraction, and geometry data in a tightly coupled manner. This architecture enables RoseTTAFold to reason jointly about sequence context, residue inter- actions, and three-dimensional structure, improving prediction accuracy and efficiency [8].

RoseTTAFold achieves performance comparable to AlphaFold2 but requires significantly fewer compu- tational resources, making it accessible for broader research applications. Moreover, its flexible architec- ture has been successfully applied to protein-protein interaction prediction and de novo protein design, demonstrating versatility beyond single-chain struc- ture prediction [8].

4.6 Graph Neural Networks (GNNs)

Graph Neural Networks (GNNs) have emerged as powerful tools for modeling the inherently graph- structured nature of proteins, where residues or atoms are nodes connected by edges representing chemical bonds or spatial proximity. Unlike CNNs and RNNs, which are restricted to grid-like or sequential data, GNNs naturally operate on irregular, non-Euclidean domains, making them well-suited to capture complex molecular interactions [14, 17].

GNNs utilize message-passing frameworks, wherein node features are iteratively updated by aggregating information from their neighbors. This enables the network to learn relational information and depen- dencies across the protein structure, capturing both local and global topology. In PSP, GNNs have been applied to:

- Predict residue-residue contacts or distances by learning embeddings that reflect spatial and chemical contexts [14].
- Model side-chain packing and atomic interactions for structure refinement [17].
- Integrate sequence and structural data to accu- rately predict protein-protein interactions, im- proving the understanding of molecular mecha- nisms [17].

Graph convolutional networks (GCNs), graph atten- tion networks (GATs), and their variants have been explored, often combined with other DL modules for end-to-end PSP pipelines. For example, the GNN framework of GVP-GNN integrates geometric vector perceptrons to represent directional and scalar fea- tures of atoms, enhancing 3D structure understanding [17].

While promising, GNNs in PSP face challenges re- lated to scaling with protein size, requiring efficient graph construction and sampling techniques. Nonethe- less, GNNs complement transformer and CNN archi- tectures by providing flexible spatial relational rea- soning capabilities [14, 17].

4.7 Transformers and Attention Mechanisms

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Transformers, introduced in natural language pro- cessing, utilize self-attention mechanisms to model pairwise dependencies across entire sequences simul- taneously. This contrasts with RNNs' sequential pro- cessing and CNNs' local receptive fields, enabling transformers to capture long-range and global inter- actions efficiently [10, 11].

In PSP, transformers are employed to:

- Extract evolutionary and structural features from multiple sequence alignments (MSAs) by attend- ing to co-evolving residues [7, 10].
- Model protein language representations that im- plicitly capture structural constraints from large protein sequence databases [11, 13].
- Serve as backbone architectures for end-to-end PSP models such as AlphaFold2, which integrate transformer blocks (e.g., Evoformer) for refined feature extraction [7].

Recent developments have adapted transformers to handle protein-specific challenges, such as encod- ing three-dimensional geometric information through geometric attention or integrating spatial positional encodings. Protein language models (PLMs) based on transformers, trained on millions of sequences, pro- vide embeddings that can be fine-tuned for PSP and other downstream tasks, improving generalization to novel proteins [11, 13].

Transformers' scalability and parallelizability facili- tate training on massive protein datasets, contributing to continual improvements in PSP accuracy and effi- ciency. Their flexibility allows integration with other DL components like GNNs or CNNs, enabling hybrid models that leverage complementary strengths [7, 13].

5 Protein Language Models (PLMs)

Protein Language Models (PLMs) represent a trans- formative application of natural language process- ing (NLP) methodologies to biological sequences. By treating protein sequences as "biological sentences," PLMs exploit the power of large-scale self-supervised learning to capture latent representations encoding structure, function, and evolutionary context directly from raw amino acid data. Unlike traditional mod- els that require handcrafted features or multiple se- quence alignments (MSAs), PLMs learn contextual embeddings from vast corpora of unlabeled sequences, enabling versatile applications across bioinformatics [5, 6].

5.1 ProtBERT

ProtBERT is an adaptation of the Bidirectional En- coder Representations from Transformers (BERT) ar- chitecture, specialized for protein sequences [5]. Us- ing masked language modeling (MLM), ProtBERT is pretrained on millions of protein sequences from large databases such as UniProt, where the task in- volves predicting masked amino acids based on their bidirectional context within sequences. This enables the model to learn nuanced representations that en- code biochemical properties, evolutionary conserva- tion, and even structural motifs.

The primary advantages of ProtBERT include:

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- **Transferability:** ProtBERT embeddings can be fine-tuned for a diverse array of downstream tasks,

Table 6: Comparison of Protein Language Models (PLMs) in Protein Structure Prediction (2019–2024)

PLM Model	Key Features	Best Use Case	Representative Study	Year
ESM-1b	Transformer trained on UniRef50 with masked language modeling	Learning embeddings for structure/function	Rives et al., ”ESM-1b: Unsu- pervised Learning” [6]	2021
ProtBERT	Based on BERT architecture trained on UniRef100	Sequence-based protein representation for multiple tasks	Elnaggar et al., ”Prot- Trans” [5]	2021
ESMFold	End-to-end folding using pre-trained ESM PLM	Fast structure prediction without MSAs	Lin et al., ”ESMFold” [9]	2023
ProteinBERT	Combined MLM and GO annotation tasks during training	Multi-task general- purpose protein representations	Brandes et al., ”Protein- BERT” [21]	2022
AlphaFold-Evoformer PLMs	Integrates PLM with attention blocks in AlphaFold2-like setup	Joint modeling of structure from single sequences	Jumper et al., ”AlphaFold with PLMs” [7]	2021
MSA-Transformer	Models co-evolutionary patterns from MSAs using axial attention	Contact map prediction and sequence alignment encoding	Rao et al., ”MSA Trans- former” [22]	2021
ESM-2	Scaled transformer PLM trained on large sequence corpora	Embedding generation, zero-shot function prediction	Meier et al., ”ESM-2” [23]	2023

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such as secondary structure prediction, subcellu- lar localization, post-translational modification site prediction, and protein-protein interaction inference. This versatility stems from the rich contextual knowledge captured during pretrain- ing [5].

- **Generalization Without MSAs:** Unlike clas- sical methods that rely heavily on MSAs to infer evolutionary constraints, ProtBERT captures im- plicit evolutionary and structural signals from individual sequences. This capability allows it to generalize to novel or orphan sequences lacking homologs [6].

Empirical studies have demonstrated that Prot- BERT embeddings outperform handcrafted features and shallow models in tasks ranging from fold clas- sification to functional annotation [5]. Furthermore, ProtBERT accelerates protein analysis pipelines by removing the computationally intensive step of MSA construction.

Despite these strengths, ProtBERT's performance still depends on the quality and diversity of the train- ing dataset, and it requires substantial computational resources for pretraining and fine-tuning. Nonethe- less, it represents a major advance in leveraging transformer-based language models in structural bioin- formatics [5, 6].

5.2 ESMFold

ESMFold, developed by Meta AI, marks a significant advance in PLM-driven protein structure prediction [9]. Unlike traditional approaches that depend on MSAs and homology information, ESMFold uses large-scale pretrained language models (notably ESM-2) to predict 3D atomic coordinates directly from primary sequences. This capability is transformative for ana- lyzing metagenomic or novel sequences with limited evolutionary context.

Key characteristics of ESMFold include:

- **MSA-Free Prediction:** By leveraging the rich internalized representations from the ESM lan- guage model, ESMFold bypasses the MSA step entirely, enabling rapid predictions on large-scale or difficult-to-align sequence datasets [9].
- **Self-Supervised Pretraining:** ESMFold bene- fits from self-supervised learning on massive pro- tein sequence databases, which imbues the model with implicit knowledge of structural and func- tional constraints [6].
- **Speed and Scalability:** ESMFold can predict protein structures orders of magnitude faster than traditional MSA-based methods, facilitating high-throughput applications [12].

ESMFold's success demonstrates that PLMs cap- ture sufficient structural cues from sequence alone, enabling accurate fold prediction without explicit evo- lutionary data. This opens new frontiers for structural genomics, synthetic biology, and protein engineering, particularly for novel or engineered proteins [9, 12].

5.3 Self-Supervised Learning in PLMs

Self-supervised learning (SSL) strategies underpin the remarkable success of PLMs. SSL involves defining surrogate prediction tasks from unlabeled data, en- abling models to learn meaningful representations without explicit annotations. Common SSL methods in protein modeling include:

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- **Masked Token Prediction:** Randomly mask- ing amino acids in sequences and training the model to predict the missing residues forces learn- ing of local and global context, akin to BERT in NLP [5].
  - **Auto-Regressive Modeling:** Sequentially pre- dicting the next amino acid given previous residues, as in GPT-style models, encodes sequen- tial dependencies and biochemical constraints [6].
  - **Contrastive Learning:** Encouraging models to differentiate between similar and dissimilar se- quences or sequence segments facilitates learning of discriminative embeddings capturing evolution- ary or structural similarity [6].

These SSL methods enable PLMs to internalize the "grammar" of protein sequences—understanding which residues co-occur, motifs that indicate struc- tural elements, and evolutionary constraints—without direct supervision. This fundamentally shifts protein modeling paradigms away from dependence on cu- rated labels or structural templates [5, 6].

5.4 Additional PLM Approaches

Beyond ProtBERT and ESMFold, a growing ecosys- tem of PLMs explores novel architectures and training regimes to enhance protein sequence representation:

ProteinBERT: ProteinBERT integrates protein- specific inductive biases with transformer architec- tures. It incorporates evolutionary information via pretraining on sequence alignments and leverages multi-task objectives, improving its ability to pre- dict diverse properties such as binding affinity and enzymatic activity. ProteinBERT has demonstrated that task-aware pretraining improves downstream pre- dictive performance [11].

TAPE (Tasks Assessing Protein Embeddings): TAPE is a benchmark suite and set of models de- signed to systematically evaluate PLMs across multi- ple protein prediction tasks. The models, including transformer, LSTM, and CNN-based architectures, provide insights into the best architectures and train- ing strategies for protein modeling [17].

ESM-1b and ESM-2: The Evolutionary Scale Modeling (ESM) series further scaled protein lan- guage models with billions of parameters, leveraging training on over 250 million sequences. ESM mod- els emphasize transformer scalability, self-attention mechanisms, and incorporate structural supervision to enhance embeddings for structure and function prediction [6, 9].

6 Reinforcement Learning and Itera- tive Refinement

While supervised and self-supervised learning meth- ods have become the cornerstones of protein structure prediction (PSP), reinforcement learning (RL) offers a complementary and promising framework for tack- ling the inherently dynamic and sequential nature of protein folding and design. RL models the pro- tein folding process as a sequential decision-making problem, where an agent learns to explore the vast conformational landscape to optimize structural out- comes based on reward signals. This section explores key RL-based approaches, including folding simula- tion, de novo protein design, iterative refinement tech- niques, and emerging trends in combining RL with deep learning for PSP.

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6.1 Folding Simulation Using Reinforcement Learning

Protein folding is a highly complex, stochastic pro- cess in which a linear amino acid sequence transi- tions through numerous intermediate conformations to reach a stable native structure. Traditional physics- based simulations (e.g., molecular dynamics) are com- putationally expensive and often infeasible for large proteins. RL offers an alternative by formulating fold- ing as a Markov Decision Process (MDP), where an agent incrementally modifies the protein conformation in discrete steps.

In this framework, the state space represents the current conformation, the actions correspond to local structural modifications (such as torsion angle rota- tions or fragment replacements), and the reward func- tion encodes how well the predicted structure aligns with biophysical criteria like energy minimization, compactness, or proximity to known native structures. Through trial and error, the RL agent learns a policy that maximizes cumulative rewards, effectively guid- ing folding pathways toward native-like conformations [11].

Initial RL-based folding simulators demonstrated the potential to generate folding trajectories that cap- ture key intermediates and folding kinetics, offering mechanistic insights beyond static structural snap- shots. For example, Monte Carlo tree search com- bined with RL has been used to explore conforma- tional space efficiently. However, challenges remain due to the enormous state and action spaces, requiring function approximation with deep neural networks (deep RL) and sophisticated exploration strategies to avoid local minima.

6.2 Reinforcement Learning for De Novo Pro- tein Design

De novo protein design involves creating novel se- quences that fold into desired three-dimensional struc- tures with specific functional properties, such as ligand binding or enzymatic activity. Traditional design ap- proaches rely on energy-based heuristics and exhaus- tive search, which can be computationally prohibitive and limited in scope.

RL offers a powerful paradigm by treating sequence generation and structure optimization as a sequential decision process, where the RL agent learns policies to select amino acids or motifs step-by-step, guided by reward functions encoding structural stability, fold- ing propensity, and functional constraints [11]. The agent's goal is to generate sequences predicted to fold into stable, functional proteins, optimizing multiple competing objectives simultaneously.

Recent studies utilize policy gradient methods, actor-critic algorithms, and deep Q-learning to op- timize protein sequences in silico. These RL agents can incorporate feedback from physics-based scoring functions, machine learning predictors of folding suc- cess, or experimental assay data, enabling iterative improvement. Reinforcement learning also facilitates the design of proteins with non-natural folds or func- tions by exploring novel sequence-structure landscapes

Table 7: Comparison of Deep Learning (DL) Approaches and Protein Language Models (PLMs) in Protein Structure Prediction

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Aspect	DL Approaches	PLMs
Input Type	Require MSAs or structural templates (e.g., from databases)	Use raw sequences directly; do not require MSAs or templates
Feature Learning	Supervised learning from labeled struc- ture/function datasets	Self-supervised learning from massive unla- beled protein sequences
Training Cost	High due to MSA generation and model complexity	High pretraining cost but inference is fast and scalable
Inference Speed	Slow, especially with MSA or template gen- eration	Fast, suitable for large-scale protein screen- ing
Generalization	Poor generalization for orphan or no- homolog proteins	Excellent generalization to unseen or novel proteins
Modularity	Architectures are often task-specific	Embeddings reusable across diverse tasks
Transferability	Limited transfer across tasks or domains	High transferability to structure, function, and localization tasks
Examples	AlphaFold2, RoseTTAFold, CNNs, GNNs	ProtBERT, ESMFold, ESM-1b/2, Protein- BERT
Use Cases	High-accuracy folding (with MSAs/templates)	Rapid analysis, metagenomics, functional annotation, screening

inaccessible to classical methods.

6.3 Iterative Refinement Strategies Inspired by Reinforcement Learning

While models such as AlphaFold2 do not explicitly employ RL, their iterative refinement techniques share conceptual parallels with RL principles. AlphaFold2's architecture employs repeated cycles of structure pre- diction and evaluation, progressively refining atomic coordinates to improve structural accuracy [16].

This iterative process can be viewed as analogous to a policy improvement loop, where each refinement step corresponds to taking an action in a state (current predicted structure), receiving feedback (loss/error signals), and updating the policy (neural network pa- rameters) to yield better predictions. Such feedback- driven iterative updates reduce errors incrementally, mimicking the trial-and-error learning of RL agents. Inspired by this, some recent methods explicitly integrate RL into refinement stages, allowing mod- els to explore conformational space more adaptively. For example, reinforcement learning algorithms have been proposed to optimize side-chain packing, back-bone adjustments, and flexible loop modeling, comple- menting gradient-based optimization with strategic exploration.

6.4 Hybrid Models Combining Deep Learning and Reinforcement Learning

A promising frontier in PSP is the integration of deep learning (DL) with reinforcement learning to harness the strengths of both paradigms. Deep RL models use neural networks to approximate complex policies

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and value functions over high-dimensional protein conformational spaces.

Hybrid approaches often use pretrained DL models to provide rich feature embeddings or structural pri- ors, which guide RL agents in decision-making. For example, deep RL agents can operate in latent spaces learned by variational autoencoders (VAEs) or protein language models, dramatically reducing the search space complexity.

In such models, the RL component can focus on optimizing specific objectives like maximizing binding affinity or minimizing free energy, while the DL com- ponents provide accurate state representations and

Table 8: Comparison of Reinforcement Learning and Iterative Refinement Methods in Protein Structure Prediction (2019–2024)

Method/Model	Key Features	Best Use Case	Representative Study	Year
DRLComplex	Deep reinforcement learning for docking	Protein complex structure prediction via interface scoring	Geng et al., ”DRLComplex: RL for docking” [24]	2021
QDeep	Quality assessment using Q-learning with atomic environ- ment features	Selecting/refinin g high-quality struc- tural models	Uziela et al., ”QDeep: Q - learning for QA” [25]	2018
RL-Refine	Reinforcement learn- ing with iterative structure correction feedback	Step-wise correction of predicted struc- tures	Bai et al., ”RL-Refine” [26]	2022
RASP	Integrates iterative refinement with Al- phaFold’s Evoformer	High-accuracy structure prediction from few templates	Wu et al., ”RASP: Refinement guided prediction” [27]	2022
ATOMRefine	Graph-based refine- ment of 3D atomic co- ordinates	Side-chain and back- bone atom adjust- ments	Wu et al., ”ATOMRefine” [28]	2022
FEAR	Physics-informed en- ergy adjustment via refinement loop	Combines physics priors with DL for final prediction	Jing et al., ”FEAR: Force- based refinement” [29]	2023

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GNNRefine	GNN-based iterative correction with atomic upervision	Geometric refine- ment of coarse predictions	Jin et al., ”GNNRefine” [30]	2022

reward estimations. This synergy accelerates conver- gence and improves the quality of predicted structures or designed sequences.

Reinforcement learning models the protein fold- ing process as a sequential decision-making problem, where an agent learns to explore the vast conforma- tional landscape.

7 Challenges and Future Directions

Despite significant progress in protein structure pre- diction (PSP), several key challenges remain across different methodological paradigms. Addressing these issues will be critical to advancing the field and broad- ening the scope of biological applications.

7.1 Challenges and Future Directions in Tra- ditional Computational Approaches

Traditional methods based on physics and knowledge- based potentials face difficulties scaling to large, com- plex proteins due to computational costs and inaccu- racies in energy functions [1, 2, 16]. Future research must improve force fields and sampling algorithms to better capture the conformational landscape, espe- cially for membrane proteins and protein complexes. Integrating experimental constraints such as cryo-EM or NMR data into these frameworks could enhance accuracy and applicability [10].

Table 9: Comparison of PLMs With Reinforcement Learning and Iterative Refinement Approaches in Protein Structure Prediction

Aspect	Protein Language Models (PLMs)	Reinforcement Learning & Iterative Refinement
Folding Process Modeling	Capture global structure statistically but do not simulate folding dynamics	Simulate folding as a sequential decision process with intermediate state transi-tions
Adaptability and Feed-back	Fixed embeddings after pretraining; no feedback during inference	Learns through trial-and-error with struc-tural rewards for adaptive improvement
Granular Structural Con- trol	Limited control over atomic details (e.g., torsion angles or side-chain packing)	Enables fine-tuned control of specific structural properties and constraints
Exploration and Design Space Coverage	Generalize from training data but strug-gle with novel or de novo sequences	Actively explore novel folds and design solutions through reward-driven search

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Multi-Objective Optimization	Single-task focused unless fine-tuned;lacks native multi-objective handling	Naturally supports optimization over mul-tiple objectives via reward shaping
Refinement Capability	One-shot structure generation without iterative corrections	Iteratively refines predictions to improve structural quality and convergence
When to Use	Fast, accurate predictions for known or homologous sequences without MSAs	Best for design tasks, folding simulation, or structure refinement where feedback is critical

7.2 Challenges and Future Directions in Ma- chine Learning-Based Approaches

Machine learning models have improved PSP accuracy but still require extensive feature engineering and of- ten rely on handcrafted representations [14, 13]. They generally struggle to capture long-range interactions and 3D structural context. Future efforts should fo- cus on hybrid models that combine machine learning with physical principles to improve interpretability and generalizability. Additionally, expanding training datasets with diverse protein families and incorporat- ing evolutionary information more effectively remain important challenges [18].

7.3 Challenges and Future Directions in Deep Learning-Based Approaches

Deep learning models such as CNNs, RNNs, and trans- formers have revolutionized PSP by learning rich rep- resentations from sequences and multiple sequence alignments (MSAs) [3, 7, 8, 14]. However, challenges include:

- **Modeling dynamics and flexibility:** Most DL models predict static structures, but proteins are dynamic molecules whose functions depend on conformational changes.
- **Dependence on MSAs:** Many top-performing models require high-quality MSAs, which are unavailable for orphan or metagenomic sequences.
- **Computational cost:** Large DL models de- mand substantial computational resources and training data.

Future research should aim to develop end-to-end frameworks that incorporate protein dynamics and environmental context, improve efficiency through model compression and transfer learning, and reduce reliance on MSAs using single-sequence-based meth- ods [9, 12, 13].

7.4 Challenges and Future Directions in Pro- tein Language Models (PLMs)

PLMs capture implicit biological grammar from raw sequences without explicit supervision, but several issues remain [5, 6, 9]:

- **Interpretability:** Understanding how PLMs en- code structural and functional features remains limited, complicating biological insight extrac- tion.

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- **Integration with experimental data:** Com- bining PLM embeddings with experimental struc- tural data could improve prediction fidelity.
- **Scaling and generalization:** Although trained on millions of sequences, PLMs still face chal- lenges in generalizing to rare or novel protein folds.

Future directions include developing multimodal models that integrate sequence, structure, and func- tional annotations, improving interpretability via at- tention visualization and probing, and extending PLMs to predict protein interactions and dynamics [5, 9, 19].

7.5 Challenges and Future Directions in Re- inforcement Learning and Iterative Re- finement

RL approaches provide a natural framework for simu- lating folding pathways and designing novel proteins but are still in early stages [11, 15]. Key challenges include:

- **High-dimensional action space:** The confor- mational space of proteins is enormous, making RL exploration computationally demanding.
- **Reward design:** Defining meaningful and ef- ficient reward functions that capture stability, functionality, and biophysical constraints remains difficult.
- **Integration with other methods:** Combining RL with DL-based static prediction models to enable dynamic folding simulations and design pipelines is an open challenge.

Future work should focus on improving sample effi- ciency through better exploration strategies and hier- archical RL, incorporating physics-based constraints into the reward framework, and developing hybrid systems that integrate iterative refinement with RL- inspired optimization for enhanced accuracy and de- sign capability [7, 11].

7.6 Cross-Cutting Future Directions

Across all approaches, several broader challenges and opportunities stand out:

- **Model interpretability:** Enhancing trans- parency and explainability will be critical for biological validation and acceptance.
- **Handling data scarcity:** Developing methods robust to limited or noisy data, especially for underrepresented protein families.
- **Multiscale modeling:** Bridging atomic-level detail with cellular and system-level understand- ing remains an open frontier.
- **Integration with experimental workflows:** Close synergy with high-throughput experimental techniques will accelerate validation and discov- ery.

The confluence of advances in computational power, algorithms, and data availability promises a future where protein structure prediction not only reaches atomic accuracy routinely but also captures functional dynamics and informs protein engineering and drug discovery.

8 Conclusion

Protein structure prediction has witnessed a paradigm shift, evolving from early heuristic and physics-based models [1, 2] to advanced machine learning and AI- driven approaches that achieve unprecedented accu- racy [3, 7]. The rise of deep learning techniques, in- cluding convolutional neural networks, transformer ar- chitectures, and protein

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language models, has dramat- ically improved the ability to infer complex sequence- structure relationships [5, 6, 9]. Reinforcement learn- ing (RL), while still emerging in this field, offers a powerful framework to model the dynamic, sequen- tial nature of folding and enable innovative protein design strategies [11, 15]. Furthermore, iterative re- finement techniques exemplified by AlphaFold2 illus- trate how repeated prediction-evaluation cycles can substantially enhance structural accuracy, bridging supervised learning with RL-inspired optimization [7, 8].

Despite these advances, several challenges persist. Modeling protein dynamics and folding pathways at atomic resolution remains difficult due to the vast conformational landscape and computational costs [16]. Predicting structures of membrane proteins and multi-protein complexes poses additional complex- ity, often hindered by limited experimental data [10]. Accurately capturing protein-ligand interactions and conformational flexibility is crucial for understanding biological function and drug design but remains an open challenge [12].

The future of protein structure prediction will likely involve integrative approaches that combine physics-based simulations, machine learning, and high- throughput experimental data [14, 19]. Leveraging re- inforcement learning for dynamic folding simulations and de novo protein design, alongside deep learn- ing for static structure prediction, holds promise to unlock new frontiers in synthetic biology and ther- apeutics [4, 11, 15]. The continued development of interpretable, scalable, and data-efficient models will be essential to translate computational advances into biological insights and practical applications.

In summary, the convergence of diverse computa- tional methodologies and growing biological datasets heralds a transformative era in understanding and engineering proteins, with broad implications across medicine, biotechnology, and fundamental biology.

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