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Deep Learning for Protein Structure Prediction: From AlphaFold to the Next Frontier

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Protein structure is closely linked with protein function. For many decades, scientists tried to predict the three-dimensional structure of a protein from its amino acid sequence. Experimental methods such as X-ray crystallography, nuclear magnetic resonance spectroscopy, and cryo-electron microscopy provide reliable structures. However, these methods can be expensive and time-consuming. Computational methods therefore became important alternatives. Early prediction methods were based mainly on sequence similarity, physical energy functions, and structural templates. Their accuracy was limited for many proteins.

Deep learning changed this field. AlphaFold2 showed that artificial intelligence can predict the structures of many proteins with near-experimental accuracy (Jumper et al., 2021). RoseTTAFold provided another powerful deep-learning approach (Baek et al., 2021). Protein language models such as ESMFold later showed that structural information can also be learned directly from very large collections of protein sequences (Lin et al., 2023). AlphaFold3 further expanded the field by predicting interactions among proteins, DNA, RNA, ions, small molecules, and modified residues (Abramson et al., 2024). Generative models such as RFdiffusion and ESM3 are now moving the field from structure prediction toward protein design (Watson et al., 2023; Hayes et al., 2025). Recent open models are also increasing access to advanced biomolecular modelling.

Despite this progress, major challenges remain. Proteins are dynamic molecules. They may adopt several conformations. Their structures are influenced by ligands, membranes, modifications, and the cellular environment. Future models therefore need to predict not only one structure, but also molecular dynamics, interactions, binding strength, and biological function. This review describes the development of deep learning for protein structure prediction and discusses the major directions that may define the next frontier.

Keywords: AlphaFold, deep learning, protein structure prediction, AlphaFold3, ESMFold, ESM3, RoseTTAFold, RFdiffusion, protein design

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1. Introduction

Proteins perform most of the important activities inside living cells. They act as enzymes, receptors, transporters, antibodies, structural components, and molecular signals. A protein is first produced as a chain of amino acids. This chain folds into a specific three-dimensional structure.

The structure of a protein is important because it influences its biological function. Even a small structural change may alter enzyme activity, ligand binding, stability, or interaction with another protein. Understanding protein structure is therefore important in molecular biology, medicine, agriculture, biotechnology, and drug discovery.

Determining protein structures experimentally is not always easy. X-ray crystallography requires suitable crystals. Nuclear magnetic resonance spectroscopy is more suitable for smaller proteins. Cryo-electron microscopy has become very powerful, especially for large complexes, but it requires expensive equipment and specialised expertise.

For this reason, scientists have tried for decades to predict protein structures by computer. This challenge became known as the **protein structure prediction problem**. The Critical Assessment of Structure Prediction, or CASP, was established to compare prediction methods using proteins whose structures were not yet publicly known.

Early computational methods gave useful results. However, they often failed when suitable structural templates were absent. The arrival of deep learning changed this situation dramatically.

2. Protein Structure Prediction Before AlphaFold2

Early protein modelling methods were mainly divided into template-based modelling and template-free modelling.

Template-based modelling uses the structure of a related protein. If two proteins have similar sequences, they often have similar structures. Homology modelling therefore works well when a close relative has already been experimentally solved.

The problem becomes more difficult when there is little sequence similarity.

Researchers then need to predict residue contacts, secondary structures, structural fragments, and possible energy-minimum conformations.

Another major advance came from evolutionary information. Amino acids that interact in a protein structure often change together during evolution. Multiple sequence alignments can therefore contain information about residues that are close in three-dimensional space.

Deep neural networks greatly improved the extraction of this information. The first AlphaFold system used deep learning to predict distances between amino-acid residues and then used these predictions to construct protein structures (Senior et al., 2020). It performed very well in CASP13.

However, an even greater advance appeared in the next generation.

3. AlphaFold2: A Major Breakthrough

AlphaFold2 was introduced by DeepMind and showed exceptional performance in CASP14. It could predict many protein structures at a level close to experimental structures (Jumper et al., 2021).

AlphaFold2 does not simply search for a known protein with a similar structure. It combines several types of information.

One important input is the **multiple sequence alignment**, or MSA. The MSA contains related protein sequences. Evolutionary patterns within these sequences provide information about which residues may interact.

AlphaFold2 processes sequence and pairwise residue information using a neural-network component called the **Evoformer**. Information is repeatedly exchanged between representations of individual residues and residue pairs. The model then produces three-dimensional atomic coordinates.

The model also uses a process called **recycling**. Its predicted structure is sent through parts of the network again. This allows the prediction to be progressively improved.

Another important feature is confidence estimation. The **predicted Local Distance Difference Test**, or pLDDT, gives a confidence value for different parts of the structure.

The **Predicted Aligned Error**, or PAE, helps assess the predicted positions of domains relative to one another.

These scores are very useful. A predicted structure should not be accepted simply because it looks realistic. Low-confidence regions require careful interpretation.

AlphaFold2 changed the scale of structural biology. The AlphaFold Protein Structure Database later expanded to contain more than 214 million predicted protein structures (Varadi et al., 2024). These predictions are now widely used as starting points for biological research.

4. RoseTTAFold and Wider Access to Deep-Learning Prediction

AlphaFold2 was not the only important development. RoseTTAFold was developed by researchers at the University of Washington (Baek et al., 2021).

RoseTTAFold introduced a three-track neural network. It processes information at three connected levels. These are the one-dimensional protein sequence, two-dimensional residue relationships, and three-dimensional coordinates.

Information moves between these tracks. This allows sequence information and structural information to influence each other throughout prediction.

RoseTTAFold also demonstrated that deep-learning methods could predict many protein-protein complexes.

Access to advanced prediction was further improved by **ColabFold**. ColabFold combines fast sequence-search methods with AlphaFold2 and related tools. It greatly reduces the computational burden of preparing MSAs and makes structure prediction easier for researchers with limited computational resources (Mirdita et al., 2022).

These developments helped move protein structure prediction from a specialist computational field into routine biological research.

5. Protein Language Models and ESMFold

Most AlphaFold2 predictions depend strongly on evolutionary information obtained from MSAs. However, building large MSAs can take time. Some proteins also have few known relatives.

Protein language models offer a different approach.

A protein language model learns patterns from very large numbers of amino-acid sequences. This is similar in concept to language models that learn relationships among words. In proteins, the model learns relationships among amino acids.

ESMFold showed that a large protein language model can infer three-dimensional structural information directly from sequence representations (Lin et al., 2023).

This approach can be much faster because it does not always require a large MSA search. Lin et al. (2023) used the approach to predict structures for hundreds of millions of metagenomic proteins.

Protein language models are important for another reason. They learn more than structural patterns. Their internal representations may also contain information related to protein evolution, function, and biochemical properties.

This makes them attractive as general foundation models for protein science.

6. AlphaFold3: From Individual Proteins to Molecular Interactions

AlphaFold2 mainly transformed prediction of protein structures. However, biological molecules rarely act alone.

Proteins interact with other proteins. They also interact with DNA, RNA, metabolites, metal ions, drugs, cofactors, and modified amino acids.

AlphaFold3 was developed to address this larger problem (Abramson et al., 2024).

The architecture of AlphaFold3 differs significantly from AlphaFold2. One important change is the use of a diffusion-based structure generation process. Diffusion models learn to produce structured outputs by progressively removing noise.

AlphaFold3 can model complexes containing proteins, nucleic acids, small molecules, ions, and modified residues. It showed improved performance for several classes of molecular interaction compared with many earlier specialised methods (Abramson et al., 2024).

This represents a significant conceptual shift in the field. The central question is no longer confined to determining the static structure of an individual protein; rather, it has expanded to encompass a broader and more dynamic inquiry: how do biological macromolecules assemble, interact, and organize within three-dimensional space? This reformulation more closely approximates the conditions that prevail within the cellular environment, where molecules do not exist in isolation but engage in continuous, context-dependent interactions.

By March 2026, this movement toward complex prediction was also visible in the AlphaFold Protein Structure Database. Large-scale predicted protein complexes began to be added to the resource. Approximately 1.75 million homodimer predictions were made available during the March 2026 expansion (Callaway, 2026).

7. From Structure Prediction to Protein Design

Deep learning is now moving beyond predicting natural protein structures.

Researchers increasingly want to **design new proteins**.

Protein design is an inverse problem. In normal structure prediction, the amino-acid sequence is known and the structure is predicted. In protein design, researchers may start with a desired structure or function and ask which sequence could produce it.

ProteinMPNN is an important deep-learning method for this task. It predicts amino-acid sequences that are compatible with a given protein backbone (Dauparas et al., 2022).

RFdiffusion represents another major development. It uses a diffusion model to generate new protein structures (Watson et al., 2023). The method can create protein backbones that satisfy specific structural requirements.

It has been used for the design of binders, symmetric proteins, and other new structures.

These models can also be combined.

A possible workflow is:

Desired function → RFdiffusion backbone generation → ProteinMPNN sequence design → AlphaFold structure validation → laboratory testing

This creates a powerful design cycle.

Instead of studying only proteins produced by evolution, scientists can begin exploring proteins that have never existed in nature.

8. ESM3 and Multimodal Protein Models

The next generation of protein language models is becoming multimodal.

ESM3 is an important example. It was developed to work across protein sequence, structure, and function information (Hayes et al., 2025).

Instead of treating these properties as completely separate problems, ESM3 learns relationships among them.

A user may therefore provide information about part of a protein sequence, a structural requirement, or a functional property. The model can use these different forms of information together.

Hayes et al.(2025) demonstrated this capability by generating functional fluorescent proteins that were substantially different in sequence from known fluorescent proteins.

This suggests that future protein models may behave less like simple structure predictors and more like general biological design systems.

They may eventually connect:

sequence → structure → dynamics → interaction → function → design

This is one of the most important directions in modern computational biology.

9. Open Models After AlphaFold3

Another important development is the growth of open biomolecular prediction systems.

AlphaFold3 demonstrated very strong performance. However, full access to advanced models is also important for reproducibility, method development, and independent benchmarking.

Boltz-1 was developed as an open model for biomolecular interaction prediction. It was designed to approach the capabilities of AlphaFold3 while providing access to model components and training resources (Wohlwend et al., 2025).

Table 1: Major deep-learning approaches in modern protein structure prediction and design

Model	Main advance	Major strength	Important limitation
AlphaFold	Deep-learning distance prediction	Improved template-free prediction	Lower accuracy than later models
AlphaFold2	Evoformer and end-to-end structure prediction	Very high accuracy for many proteins	Mainly predicts a dominant static structure
RoseTTAFold	Three-track network	Structure and complex prediction	Accuracy can vary for difficult targets
ColabFold	Fast AlphaFold-based workflow	Easy and rapid access	Still depends on underlying prediction models
ESMFold	Protein language model	Fast sequence-to-structure prediction	Can be less accurate for difficult proteins
AlphaFold3	Diffusion-based biomolecular modelling	Proteins, DNA, RNA, ligands and ions	Molecular dynamics and affinity remain difficult
ProteinMPNN	Inverse protein folding	Designs sequences for structures	Does not by itself prove biological function
RFdiffusion	Generative diffusion model	Generates new protein structures	Experimental testing is essential
ESM3	Multimodal protein language model	Links sequence, structure and function	Very large models require substantial computation
Boltz-1 / Protenix	Open biomolecular modelling	Accessibility and reproducibility	Benchmarking and continued validation are needed

Protenix is another effort to reproduce and extend AlphaFold3-like biomolecular modelling.

Updated Protenix-v1 work released in February 2026 focused on high-accuracy open-source prediction and improved evaluation strategies (Zhang et al., 2026).

These models are important because structural biology benefits from independent implementations.

Open systems allow researchers to test algorithms, investigate failure cases, improve training procedures, and develop specialised models for particular biological problems.

10. Major Limitations of Present Models

The success of AlphaFold should not be interpreted as meaning that all problems of protein folding have been solved.

10.1 Proteins are Dynamic

Most prediction systems produce one dominant structure. Real proteins move continuously.

Domains rotate. Loops move. Binding sites open and close. Proteins can shift between active and inactive conformations.

A single static structure cannot fully describe these processes.

10.2 Intrinsically Disordered Proteins Remain Difficult

Some proteins or protein regions do not have one stable three-dimensional structure.

These intrinsically disordered regions are biologically important. They are common in signalling and regulation.

Low confidence in such regions may represent real structural disorder rather than simple prediction failure.

10.3 Mutations are Difficult to Interpret

A single amino-acid mutation may alter stability, dynamics, activity, or binding.

Structure predictors may sometimes produce very similar structures for wild-type and mutant sequences even when the mutation has a major biological effect.

Therefore, predicted structural similarity does not prove that a mutation has no functional effect.

10.4 Prediction is not the Same as Molecular Dynamics

AlphaFold predicts likely structures. It does not reproduce the complete physical folding pathway of a protein.

It also does not replace molecular dynamics simulations when researchers need information about motion, thermodynamics, energy barriers, or conformational transitions.

10.5 Interaction Does not Always Mean Biological Interaction

A model may generate a plausible protein complex. This does not prove that the proteins interact inside a living cell.

Expression pattern, concentration, cellular location, cofactors, modifications, and competition with other molecules can determine whether an interaction actually occurs.

10.6 Experimental validation remains essential

Predicted structures are hypotheses.

Important conclusions should still be supported by experimental methods whenever possible. These may include X-ray crystallography, cryo-electron microscopy, NMR, mutagenesis, biochemical assays, binding experiments, cross-linking, or other structural and functional approaches.

11. Applications in Biology and Medicine

Deep-learning structure prediction already has many applications.

It can help researchers identify possible protein domains and catalytic residues. It can support interpretation of newly discovered genes. It can help study proteins from organisms that are difficult to culture.

Predicted structures are also useful in drug discovery. They can provide starting models when experimental structures are unavailable.

AlphaFold3 and related systems may be particularly useful for predicting protein-ligand and protein-nucleic-acid complexes (Abramson et al., 2024).

Structural predictions are also valuable in agriculture. They can help researchers study plant enzymes, disease-resistance proteins, pathogen proteins, and interactions between plant and viral proteins.

Another important application is protein engineering. Deep-learning systems can help design enzymes with improved stability or altered substrate specificity. They may also support the design of antibodies, vaccines, biosensors, and therapeutic proteins.

However, computational prediction should normally be used to reduce the number of experimental candidates. It should not be considered a replacement for laboratory testing.

12. The Next Frontier

The next major advance may not come from simply making static structure prediction slightly more accurate.

Future models will need to answer more complex biological questions.

12.1 Predicting Conformational Ensembles

A protein should ideally be represented by several biologically relevant states rather than one structure.

Models may need to predict the probability of each state and the transitions between them.

12.2 Predicting Molecular Interactions

Future systems will increasingly model complete molecular assemblies.

This includes proteins, nucleic acids, metabolites, membranes, ions, and drugs.

The expansion of AlphaFold3 and the AlphaFold Database toward complexes already shows this direction (Abramson et al., 2024; Callaway, 2026).

12.3 Predicting Binding Strength

Predicting where two molecules bind is only part of the problem.

Researchers also need to know how strongly they bind.

Reliable prediction of binding affinity would greatly improve drug discovery and protein engineering.

12.4 Combining AI with Physics

Purely data-driven models can learn powerful structural patterns. However, biological molecules must still obey physical laws.

Future models may combine deep learning with molecular mechanics, molecular dynamics, thermodynamics, and quantum chemical calculations.

Such hybrid systems may improve the prediction of difficult interactions and conformational changes.

12.5 Multimodal Biological Foundation Models

Models such as ESM3 suggest that future AI systems may learn protein sequence, structure, function, evolution, and experimental data together (Hayes et al., 2025).

The same model might eventually predict a structure, explain its function, suggest mutations, and design an improved protein.

12.6 From Individual Molecules toward Cellular Modelling

Cells contain thousands of interacting molecules.

A long-term goal is therefore to move from modelling a single protein toward modelling large molecular systems.

Such systems may eventually connect structural prediction with gene regulation, metabolism, signalling, and cellular phenotype.

This remains a very difficult goal. However, progress in multimolecular prediction makes it increasingly realistic as a research direction.

13. Conclusion

Deep learning has transformed protein structure prediction.

AlphaFold2 demonstrated that highly accurate protein structures can often be predicted directly from sequence and evolutionary information. RoseTTAFold provided another powerful architecture. ColabFold improved accessibility. ESMFold showed that protein language models can learn structural information directly from very large sequence datasets.

AlphaFold3 expanded the problem from individual proteins to interactions among proteins, nucleic acids, small molecules, ions, and other biomolecular components. RFdiffusion, ProteinMPNN, and ESM3 are moving the field from prediction toward design.

However, protein biology is much more than a static three-dimensional structure.

Proteins move. They interact. They change conformation. Their behaviour depends on their cellular environment.

The next frontier will therefore involve prediction of **structure, dynamics, interactions, function, and molecular design within a unified framework**.

Deep learning will be central to this progress. Experimental biology will remain equally important. The strongest future approach will combine artificial intelligence, physical modelling, structural biology, and laboratory validation.

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