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Prediction of protein–protein interactions and co-complex models with deep learning

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Protein–protein interactions (PPIs) are fundamental to cellular processes, and essential for understanding biological function and disease mechanisms. In this review, we emphasize recent deep learning-based methods for protein interaction study. Focusing on three closely related tasks of proteome-wide PPI prediction, PPI interface prediction, and PPI co-complex structure prediction, we discuss how emerging concepts and computation approaches have evolved to shape these fields We categorize recent approaches according to their methodological paradigms, summarize their strengths and limitations, and further explore diverse biological and biomedical applications, highlighting how computational methods in PPI prediction, PPI interface prediction, and PPI structure prediction jointly contribute to understanding of complex biological systems.

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Introduction

Protein–protein interactions (PPIs) are fundamental to almost all cellular processes, governing signal transduction, metabolic regulation, immune responses, and pathogen-host interactions [1]. Rather than acting in isolation, proteins typically exert their functions through coordinated interactions within complex molecular networks. As a result, systematic characterization of protein interactions is essential for understanding biological mechanisms, disease etiology, and therapeutic intervention [2].

Over the last forty years, diverse experimental assays for detecting protein interactions and solving complex structures have produced a large amount of PPI data [3]. These resources have driven rapid progress in computational methods that filter and curate PPI databases [4–7], predict new interactions [8–13], identify binding interfaces [14–18], and model protein complex structures [16,19,20]. Our review focuses on three closely related and highly active problems in studying PPIs at the whole proteome scale. The first is proteome-wide PPI prediction, which aims to distinguish interacting protein pairs from non-interacting ones at scale and to construct global interaction networks [21,22]. The second is PPI interface prediction, which seeks to identify the specific residues mediating binding, providing mechanistic insight into molecular recognition and enabling downstream applications such as mutagenesis analysis and drug design [16,18,23,24]. The third is protein co-complex structure prediction, which focuses on generating three-dimensional complex structures directly from sequence information, revealing detailed binding modes and enabling de novo protein design. Together, these three tasks help form a large part of the methodological backbone of many modern PPI studies.

Progress in PPI research has been driven fundamentally by advances in deep learning and representation learning. Conceptually, the field has moved from global protein-level embeddings to residue- and atom-level encodings: protein-level features underpin binary PPI prediction, residue-level representations enable interface and binding-site localization, and atom-level, geometry-aware representations coupling with generative models make co-complex structure generation and design feasible. These representations integrate multiple modalities, including sequence, predicted or experimental structure, co-evolutionary signals, and physico-chemical context; even within a single modality, information can be instantiated as different data structures, such as graphs, surfaces, and point clouds, giving rise to both unimodal and multimodal methods. At the same time, progress is bounded by foundational data

limitations: existing protein and interaction databases are imbalanced and heterogeneous, with biases, missingness, and uncertain negatives, which complicate training, benchmarking, and practical application.

In this review, we survey recent computational methods for these three tasks, with a particular focus on deep learning based approaches (see Table 1). We organize the discussion by methodological paradigms and summarize their respective strengths and limitations. We also discuss representative applications of these methods in diverse settings, illustrating how PPI prediction, PPI interface prediction, and PPI co-complex structure prediction jointly contribute to mechanistic understanding across different biological problems.

Proteome-wide protein–protein interaction prediction

Proteome-wide protein interaction prediction aims to identify physically interacting protein pairs from non-interacting ones across the entire proteome of an organism, thereby enabling the construction of a comprehensive "interactome". A wide range of computational approaches have been developed to predict protein interactions, which can be grouped into the following categories based on their data sources: (1) sequenced-based methods that exploit amino acid compositions and underlying patterns, (2) co-evolution based methods that investigate evolutionary signals by constructing multiple sequence alignments (MSAs), (3) structure-based methods that utilize experimentally resolved or predicted complex structures, and (4) hybrid approaches which leverage complementary information from diverse data types (Figure 1). Recent advances in machine learning including graph neural networks (GNNs), protein language models (PLMs) have accelerated the progress across these categories, enabling prediction tools with improved accuracy and proteome-wide coverage.

Sequence-based approaches have emerged as the dominant paradigm for protein–protein interaction prediction, primarily due to the transformative impact of protein language models (PLMs) [25,26] and the practical advantages of sequence data availability, as evidenced by their integration into most state-of-the-art PPI prediction models. For example, models like D-SCRIPT [8] demonstrate the power of transforming PLM-encoded protein sequence embeddings [27] into the inter-protein contact maps before aggregating interaction probabilities. TUnA [28] applies transformer layers to process ESM-2 [25] sequence embeddings for individual proteins and employs a Gaussian process prediction module that outputs an uncertainty-adjusted interaction probability. PLM-interact [11] challenges the conventional paradigm of independent protein encoding by fine-tuning ESM-2 [25] on concatenated protein pairs, effectively adapting natural language processing concepts like "next sequence" prediction to protein interaction prediction contexts.

Given the vast availability of protein sequences relative to experimentally determined protein structures, sequence-based approaches are inherently broadly applicable and are particularly valuable for proteins that are intrinsically disordered or lack resolved structure. However, sequence data alone captures limited spatial information, and its predictive performance often declines when sequence information is insufficient to infer structure compatibility. Moreover, large pretrained models may exhibit implicit memorization and homology bias, potentially leading to overestimated performance on proteins with close training set relatives. As a consequence, these approaches often show reduced effectiveness when applied to orphan proteins that lack close evolutionary homologs, highlighting the need for more rigorous benchmark train-test splits to avoid data leakage and achieve accurate assessment of model generalization capabilities.

Beyond inference relying solely on primary sequences, many methods instead exploit co-evolutionary signals, inspired by the observation that residues in interacting proteins tend to coevolve. Co-evolutionary based machine learning frameworks typically begin with constructing multiple sequence alignments (MSAs) for each pair of proteins, and then apply neural network layers to compute interaction probability. However, paired MSAs are often limited by shallow depth especially for rare gene families, leading to unreliable predictions. To tackle this challenge, Zhang et al. [13] introduced omicMSAs, which assemble protein sequences from unannotated draft eukaryotic genomes and unassembled genomic reads. Using omicMSAs together with a domain–domain interaction augmented training set, they developed the deep learning framework RoseTTAFold2-PPI (RF2-PPI), and performed systematic interaction screening across 200 million human protein pairs. Co-evolution signals capture evolutionary coupling and constraints between residues, reflecting the evolutionary pressure to maintain physical contacts between proteins. Nonetheless, these methods often require substantial computational resources in generating MSAs.

When individual protein structures are available, structure-based approaches offer substantial advantages by incorporating rich spatial and physicochemical information beyond sequence data. In particular, two methodological paradigms have proven particularly effective in leveraging structural information for PPI prediction, i.e. geometric deep learning (GDL) and topological deep learning (TDL). GDL approaches [29–31] demonstrate exceptional capability in modeling proteins as graph structures, where nodes

Table 1

Summary of representative methods for PPI prediction, interface prediction, and PPI structure generation, categorized by their primary data sources, modeling strategies, and prediction tasks.

Name	Task	Input Features	Sequence Representation	Structure Representation	Core Model Components	Evaluation Data	Open Source
MINT Ullanat et al., 2026	Binary PPI prediction, binding affinity prediction	Sequences	Learned embeddings	–	ESM-2, cross-chain attention	Bernett dataset, HumanPPI, YeastPPI from PEER benchmark, PDB-Bind, SKEMPI, MutationalPPI, FLAB benchmark	Yes
PLM-interact Liu et al., 2025	Binary PPI prediction	Sequences	Learned embeddings	–	ESM-2	STRING cross-species dataset (Sledzieski et al.); Bernett dataset, virus-human PPI dataset	Yes
AlphaPulldown2 Molodenskiy et al., 2025	Binary PPI prediction	Sequences	–	–	An automated Snakemake pipeline, structure modeling backends (AlphaFold2, etc.)	–	Yes
SWING Siwek et al., 2025	Binary PPI prediction	Sequences	Interaction embeddings via Doc2Vec	–	XGBoost	NetMHCpan v.4.1, NetMHCpan v.4.2, MixMHCpredv2.2, MixMHC2predv2.0	Yes
RoseTTAFold2-PPI (RF2-PPI) Zhang et al., 2025	Binary PPI prediction, complex structure prediction	Sequences, MSAs	Learned MSA embeddings	–	RoseTTAFold2, Evoformer-like blocks	STRING, PDB, UniProt, BioGRID	Yes
LLaPA Zhou et al., 2025	Multi-type interface prediction, multi-sequence affinity prediction	Sequences, known PPI network	ESM-2 pretrained embeddings	–	Graph-based retrieval augmented prompt preparation, multimodal fusion framework	SHS27K, SHS148K	Yes
TUnA Ko et al. , 2024	Binary PPI prediction	Sequences	ESM-2 pretrained embeddings	–	Transformer encoders, Spectral-normalized neural Gaussian process (SNGP)	STRING cross-species dataset (Sledzieski et al.); Bernett dataset	Yes
TT3D Sledzieski et al., 2023	Binary PPI prediction	Sequences, structures, known PPI network	Bepler & Berger pretrained embeddings		CNNs, GLIDE, Foldseek	STRING cross-species dataset (Sledzieski et al.)	Yes
PASNVGA Luo et al., 2023	Binary PPI prediction	Sequences, known PPI network	Learned embeddings	–	Variational graph autoencoder	STRING	No
SGPPI Huang et al., 2023	Binary PPI prediction	Sequences, structures, MSAs	Preprocessed sequence/co-evolution features	Residue-level graphs	Graph convolutional networks, siamese network	Profppikernel, HuRI dataset and Pan's dataset	Yes
HIGH-PPI Gao et al., 2023	Multi-type PPI prediction	Sequences, structures, known PPI network	Residue physicochemical properties	Residue-level graphs	Hierarchical graph neural networks	SHS27k, PDB	Yes
				–			Yes

(*continued on next page*)

Table 1. (*continued*)

Name	Task	Input Features	Sequence Representation	Structure Representation	Core Model Components	Evaluation Data	Open Source
AFTGAN Kang et al., 2023	Multi-type PPI prediction	Sequences, known PPI network	ESM-1b pretrained embeddings		Attention free transformer, graph attention networks	SHS27K, SHS148K, human PPIs in STRING (tSTRING)	
SidechainDiff Liu et al., 2023	Sidechain conformation prediction, PPI binding affinity prediction	Sequences, structures	Learned embeddings	Residue-level graphs	Riemannian diffusion models	SKEMPI, PDB-REDO, SARS-CoV-2	Yes
Topsy-Turvy Singh et al., 2022	Binary PPI prediction	Sequences, known PPI network	Bepler & Berger pretrained embeddings	–	CNNs, GLIDE	STRING cross-species dataset (Sledzieski et al.)	Yes
D-SCRIPT Sledzieski et al., 2021	Binary PPI prediction, residue contact map prediction	Sequences	Bepler & Berger pretrained embeddings	–	CNNs	STRING cross-species dataset (Sledzieski et al.)	Yes
TopNetTree Wang et al., 2020	PPI binding affinity prediction	Sequences, complex structures	Atom and residue-level physiochemical properties	Point clouds	Convolutional neural networks, gradient boosting tree	SKEMPI, AB-Bind	Yes
PIONEER2.0 Xiong et al., 2025*	Protein–protein interface prediction	Sequences, structures, MSAs	Residue physicochemical properties	Atom-level and residue-level graphs	Graph transformers, convolutional neural networks, transformer encoders, structural homology	PDB, BioGRID, DIP, IntAct, MINT, iRefWeb, HPRD, MIPS	Yes
PIONEER Xiong et al., 2024	Protein–protein interface prediction	Sequences, structures, MSAs	Residue physicochemical properties	Residue-level graphs	Ensemble learning, GCNs, GRUs	PDB, CAPRI, BioGRID, DIP, IntAct, MINT, iRefWeb, HPRD, MIPS	Yes
ElliDock Yu et al., 2024	Rigid-body protein docking	Sequences, structures	Learned embeddings	Residue-level graphs	Pairwise-independent SE(3)-equivariant GNNs, graph transformers, elliptical paraboloid modeling	DB5.5, SAbDab	Yes
PeSTo Krapp et al., 2023	Multi-type interface prediction	Structures	–	Atom-level graphs	Translation-invariant and rotation-equivariant geometric transformer	PDB, PPDB5	Yes
DiffDock-PP Ketata et al., 2023	Rigid-body protein docking	Sequences, structures	ESM-2 pretrained embeddings	Heterogeneous residue-level graphs	Diffusion generative models, SE(3)-equivariant convolutional networks	DIPS	Yes
ScanNet Tubiana et al., 2022	Protein–protein interface prediction, antibody binding site prediction	Sequences, structures, MSAs (optional)	One-hot encoding	Point clouds	Geometric deep learning, Gaussian mixture model, neighborhood attention	Dockground, SAbDab	Yes
Boltz-2 Passaro et al., 2025	Complex structure generation	Sequences, MSAs	Learned embeddings	–	Atom attention encoder, PairFormer modules	PDB, Polaris-ASAP challenge benchmark, mdCATH, ATLAS, OpenFE, CASP16	Yes

AlphaFold 3 Abramson et al., 2024	Complex structure generation	Sequences, MSAs	Learned embeddings	–	Diffusion modules, PairFormer modules	PDB, PoseBusters (v.1, August 2023 release), CASP15 RNA	Yes
RoseTTAFold2 Baek et al., 2024	Complex structure generation	Sequences, MSAs	Learned embeddings	–	Three-track architecture, SE(3)-Transformer	PDB, CASP14, CAMEO	Yes
DeepAssembly Xia et al., 2023	Assemble protein complex structures	Sequences, MSAs	One-hot encoding	–	Convolutional neural networks, attention-based triangle interaction module	PDB, CASP14, CASP15	No, web-server available

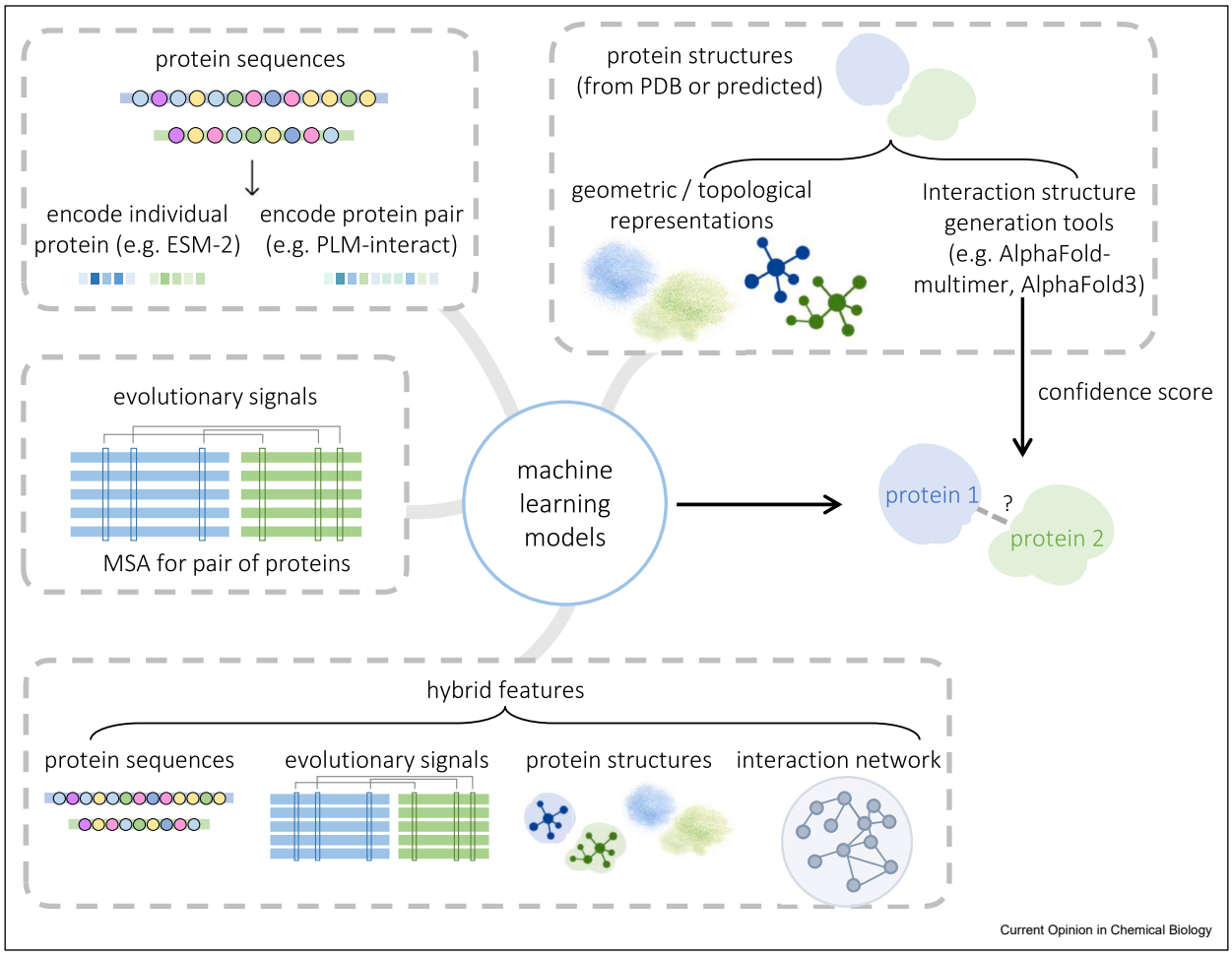
represent atoms or residues and edges encode covalent bonds, non-covalent interactions, or spatial distances. Methods such as graph convolutional networks (GCNs) [29], graph autoencoders [30], and graph transformers [31] utilize message passing mechanisms to integrate diverse properties, generating comprehensive graph representations that effectively inform interaction predictions through downstream classification layers. By integrating persistent homology with deep learning architectures, TDL methods [32] encode complex structural relationships into topological invariants that remain stable under continuous deformations. Notably, approaches like TopNetTree [33], which combines topological representations with hybrid neural networks, have shown particular promise in predicting mutation-induced binding free energy changes ($\Delta\Delta G$), contributing to discoveries in viral evolution mechanisms including SARS-CoV-2 mutation analysis.

The advent of AlphaFold [18,34] provides proteome-wide, highly accurate 3D structure predictions for individual proteins, facilitating development of structure-informed interaction prediction methods. Computational tools such as AlphaFold-multimer [35] are further developed to predict structures for multimeric protein. AlphaPulldown [36] and its later implementation AlphaPulldown2 [37] provide streamline solutions for interaction screening which take sequences of proteins of interest as inputs and output summary of each pair including the model confidence scores. Many new statistical metrics (e.g. pDockQ [34], pDockQ2 [38], interface similarity (IS) score [39]) are designed to improve the evaluation of predicted interfaces, thereby better distinguishing interacting from non-interacting protein pairs.

This evolution highlights the emerging tension between sequence-only scalability and structure-dependent accuracy. While sequence-only models enable general application to large protein datasets, structure-dependent models provide enhanced accuracy at the cost of requiring additional computational costs and introducing coverage limitations as novel proteins may lack high-quality structures in structural databases. The field increasingly recognizes the need for hybrid approaches that can achieve a trade-off between structure availability and sequence characteristics.

Hybrid approaches are proposed to leverage complementary information from different feature types, for example, to integrate geometric information from protein structures with sequence-derived biological information. Multimodal PPI prediction frameworks, such as AFTGAN [31], leverage the integration of PLMs with geometric deep learning to jointly encode sequence and structural features. Su et al. [40] introduced structure-aware vocabulary that enables PLM to explicitly incorporate protein structural information. They proposed

Figure 1



Overview of protein–protein interaction prediction. We categorize PPI prediction methods by their primary data sources. Machine learning-based approaches utilize input features derived from (1) protein sequences (upper left), (2) evolutionary signals from MSAs (middle left), (3) protein structures with geometric or topological representations (upper right), or (4) hybrid representations that integrate complementary information from sequences, evolution, structures, and interaction networks (bottom). In a parallel line of work, some methods instead estimate interaction likelihood directly from confidence metrics produced by interaction structure generation tools.

SaProt [40], a protein language model trained using structural tokens derived from Foldseek [41] encoded 3D structures, which outperforms sequence-only PLMs in protein–protein interaction prediction. Beyond SaProt and Foldseek, various works in the protein structure tokenization field [42,43], which encodes local 3D structural contexts into discrete or continuous representations, have explored the effect of different protein structure tokenization (PST) strategies on downstream interaction prediction task. For instance, the scaling study of AminoAseed [44] highlights that the performance of downstream supervised tasks exhibits a U-shaped dependency on token vocabulary size, underscoring the importance of defining an optimal alphabet size to maximize PPI prediction performance.

Beyond the sequence-structure integration challenge, protein interaction data exhibits complex, heterogeneous characteristics. This inherent heterogeneous nature of protein interaction data necessitates advanced approaches to effectively leverage diverse representations and complementary information. To tackle this, some studies take into account topological properties of the global network of known interactions. Topsy-Turvy [9] and its later implementation TT3D [10] extend the previous D-SCRIPT [8] model by employing network-based prediction with GLIDE [45], therefore predict protein interactions through the combined information of protein-level characteristics and the global interaction network. The HIGH-PPI [46] constructs hierarchical graphs for interaction prediction by first

encoding residue-level protein graphs with graph convolutional networks to generate protein-level embeddings, which are then propagated through a top−level interaction graph connecting known interacting protein pairs. For each pair of query proteins, the resulting embeddings are combined to predict the interaction likelihood.

Recent foundation models have revolutionized protein interaction prediction through innovative architectural designs. SidechainDiff [47] employs Riemannian diffusion models to generate side-chain conformations from unlabeled experimental structures, eliminating binding free energy dependencies. SWING [48] encodes biochemical differences between residue pairs for accurate protein-peptide interaction prediction. MINT [49] introduces cross-chain attention mechanisms to capture interdependencies between interacting sequences, outperforming ESM-2 and ProtT5 by 14−29% in PPI tasks. LLaPA [50] incorporates external memory mechanisms using the full interactome as a knowledge base for multi-chain complex analysis. These specialized foundation models have significantly advanced the field of protein interaction prediction.

One major problem of proteome-wide PPI prediction is raised by combinatorial explosion of possible protein pairs. For example, the human genome contains approximately 20,000 protein coding genes [51] that lead to approximately 200 million possible protein pairs, creating high demands for computational and storage resources. Another critical limitation of current PPI prediction models is their lack of proper calibration and poor uncertainty quantification, meaning that predicted confidence scores often do not reflect true prediction reliability. In addition, proteome-wide interaction prediction tasks inherently face extreme class imbalance issues, as the number of experimentally verified interactions is on the order of 10^6 as curated in the BioGRID database [5] in 2025, which is considerably less frequent than non-interactions [52]. Although many studies [8−11,13] partially mitigate this problem by training models on datasets with positive-to-negative ratios of 1:10, the application to real proteomes may still yield a large number of false positives despite reported high accuracy and precision on test sets. Moreover, issues of data integration and reliability require careful consideration. Interactions collected in databases such as BioBRID [5], IntAct [4], STRING [6] are reported by different experimental systems. While some entries are supported by evidence for direct physical interactions, a vast majority may only correspond to co-occurrence within the same protein complex. Not to mention, the quality of the PPIs reported in the literature can vary drastically [53]. Ignoring these distinctions can lead to inflated performance estimates and misleading biological interpretations. More robust integration benefits from systematic assessments of evidence provenance and confidence, protein-space coverage and cross-modality overlap, consistency checks across sources, and statistical diagnostics to flag outliers and batch effects.

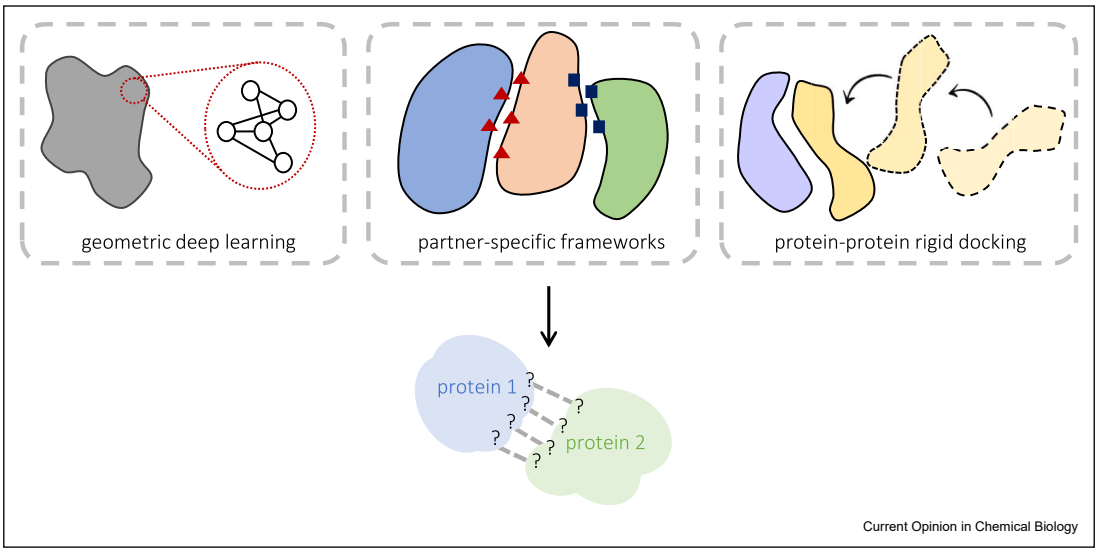
Protein−protein interaction interface prediction

Accurate prediction of protein interaction interfaces is fundamental for understanding molecular recognition, guiding mutagenesis experiments, and aiding rational drug design. Over the past decade, computational strategies for interface prediction have diversified, reflecting distinct perspectives on the determinants of binding specificity. These strategies can be broadly classified into three categories: (1) geometric deep learning approaches that learn interface residues from structural and sequence-based representations, (2) partner-specific frameworks that incorporate interaction context, and (3) rigid docking methods that search and score possible binding orientations (Figure 2). Each class introduces different assumptions about the binding process, offering unique advantages and limitations for interface prediction.

Geometric deep learning methods identify interface residues by capturing spatial and chemical patterns surrounding each residue and correlating them with binding likelihood. By combining local atomic geometry and global fold information, these models detect complex interface signatures. Representative work includes ScanNet [54], an end-to-end model that constructs atom- and residue-level representations from the spatial and chemical context of neighboring elements. By integrating structural features across multiple scales with sequence-derived information, ScanNet achieves accurate binding site prediction and enables meaningful interpretation of the structural motifs it learns. Similarly, the Protein Structure Transformer (PeSTo) [14] method predicts binding interfaces by modeling residue-level geometry using a translation-invariant and rotation-equivariant geometric transformer. However, these methods predict the binding sites of a single protein without taking its interacting partners into account. In reality, it is common for different interactors to bind to the same protein using completely distinct, non-overlapping interfaces.

To address this limitation, PIONEER [17] advances to predict a protein's binding surface in the context of its interaction partner. Its input features incorporate information such as sequence co-evolution, docking-derived signals, and pairwise potentials, enabling the model to discern interface residues of a given protein when bound to different partners. PIONEER includes four deep learning models (sequence-to-sequence, sequence-to-structure, structure-to-sequence, and structure-to-structure) covering diverse scenarios of

Figure 2



Overview of protein–protein interaction interface prediction. Existing approaches can be broadly categorized into three groups: (1) geometric deep learning approaches that capture residue neighborhood information (upper left), (2) partner-specific frameworks that incorporate signals from interacting partners to provide interaction-aware predictions (upper center) and (3) rigid docking methods that model the physical process of complex formation (upper right).

structural data availability. As a continuation of this study, PIONEER2.0 [55] strengthens partner-context modeling by incorporating structural homologs of protein–protein interactions. The structural homologs and their related features are derived using PrePPI [12], a structural template-based PPI prediction algorithm. Notably, the PIONEER series is lightweight and computationally efficient, making it deployable in environments with limited GPU resources. The evolution to PIONEER2.0 demonstrates the increasing importance of partner-specific prediction and contextualized structural modeling in the field. By explicitly modeling how the same protein adopts different binding conformations with distinct partners, these methods move beyond generic interaction predictions toward more biologically relevant, context–aware interaction prediction.

Rigid docking methods link interface prediction to the physical process of complex formation by exploring possible relative orientations of proteins and identifying residues at feasible binding interfaces. Traditional rigid docking exhaustively searches binding poses and empirically scores them [56–58]. More recent methods such as DiffDock-PP [15] and ElliDock [59] introduce deep learning to reduce the numerous computational costs. Here, the docking problem is formulated as a regression task: given two protein structures obtained from sources such as the Protein Data Bank (PDB) [7] or via homology modeling [60], the neural network predicts the final binding pose by estimating the rotation and translation parameters of the rigid-body transformation. Although interpretable, rigid protein–protein docking assumes that the conformations of the interacting proteins remain fixed during binding, which neglects the intrinsic flexibility often present at the interface. As a result, it may fail to capture side-chain rearrangements, induced-fit effects, and complex surface geometry.

PPI interface prediction faces several fundamental methodological challenges that limit current approaches. First, current interface prediction methods generally do not consider the possibility that two proteins may be non-interacting. When given such protein pairs, these methods often still produce binding site predictions, which can lead to misleading interpretations. Developing strategies that integrate binding site prediction with reliable PPI screening remains a significant challenge. Architectures that jointly score pair-level interaction likelihood and residue-level contacts, such as siamese or graph-based multi-task models, may help separate non-interacting pairs from interface predictions.

The second major obstacle in PPI interface prediction is the limited and imbalanced nature of available training datasets. High-quality, experimentally determined complex structures remain scarce compared to the vast diversity of PPIs that occur *in vivo*. Existing datasets are frequently biased toward certain protein families or interaction types. This structural bias in training data,

together with potential overfitting and data leakage, contributes to poor generalizability of PDB-trained models to diverse interaction types, including transient interactions, interspecies PPIs, and IDR-mediated binding events that are underrepresented in crystallographic datasets. What's more, current AI models do not merely inherit these biases: they appear to actively learn and amplify them, becoming highly confident on interaction types that resemble their training distribution while systematically underperforming on these underrepresented interfaces. For instance, PIONEER2.0 [55] showed that AlphaFold 3 assigns ranking scores above 0.5 preferentially to PPIs that are already represented in the PDB and achieves substantially better interface accuracy on this high-confidence subset than on PPIs receiving lower scores, indicating that the model has internalized, rather than corrected for, the experimental bias of its training corpus. This pattern suggests that benchmark improvements driven solely by PDB-trained models may overstate real-world performance on the underrepresented interaction regimes that matter most for biological discovery. For example, public repositories such as the PDB [7] contain far fewer interspecies PPIs than intraspecies PPIs, resulting in limited capacity to predict interspecies binding sites, an ability that is essential for advancing host-pathogen studies. The challenge is also particularly acute for intrinsically disordered regions (IDRs). More than 70% of human proteins contain IDRs, and many cellular interactions involve a folded domain engaging an IDR or contacts between two IDRs [61]. IDR-mediated binding spans two regimes: sequence-specific interactions that yield structured interfaces, and chemically specific interactions that produce ensembles of bound configurations rather than a single, well-defined interface. This heterogeneity and dynamism, together with the lack of a stable three-dimensional structure, make such interfaces difficult to delineate, leaving accurate prediction of IDR-mediated binding sites a persistent hurdle for PPI interface prediction. As a possible solution, repurposed parameters from coarse-grained molecular mechanics force fields can serve as sequence-level descriptors of inter-residue interactions, offering approximate estimates of attractive and repulsive contributions in disordered regions.

Protein–protein interaction structure generation

The field of protein–protein interaction study has undergone a paradigmatic transformation, marking a decisive shift from classification models that determine whether two proteins interact to end-to-end structural generative models that directly generate three-dimensional atomic coordinates of protein complexes, providing detailed structural insights into how proteins interact at the molecular level. This evolution represents a fundamental leap from binary prediction tasks, where computational methods primarily focused on answering whether proteins interact, to comprehensive structural modeling that addresses the mechanistic question of how these proteins physically associate and what their interaction interfaces entail.

Among the most prominent examples are AlphaFold3 [18] and Boltz-2 [16]. Both AlphaFold3 and Boltz-2 employ an encoder-generator architecture. Given the sequences of two proteins, the models first embed the sequence pair into high-dimensional representations. These embeddings are then fed into a diffusion module, which generates 3D protein structures from the learned features. The diffusion process gradually refines random noise into well-organized atomic coordinates, ultimately yielding a physically plausible structure.

RoseTTAFold2 [62] represents another significant contribution to end-to-end protein structure modeling, utilizing a three-track neural network architecture that iteratively refines protein structure predictions. The model simultaneously processes 1D sequence features, 2D distance maps, and 3D coordinate representations, enabling it to capture both local and global structural constraints for accurate complex structure generation. Building upon this foundation, the RFdiffusion series methods [63,64] have extended the paradigm from structure prediction to de novo protein design, leveraging diffusion models to generate novel protein structures that satisfy specific functional constraints or design objectives. Complementing these diffusion-based approaches, DeepAssembly [19] employs a hierarchical strategy that predicts individual domain structures and optimizes their spatial arrangement through learned inter-domain interactions. The method integrates MSA, templates, and domain boundaries into a self-attention network, utilizing iterative optimization to assemble complete multi-domain structures.

While AlphaFold series and related diffusion-based models present unprecedented opportunities for advancing PPI understanding, several limitations constrain their practical application. These models exhibit a notable dependence on accurate MSA pairing and high-quality evolutionary information, with performance being considerably affected when evolutionary signals are inaccurate, sparse, or misaligned across protein families. While AlphaFold3 has made significant progress in reducing the amount of MSA processing compared to AlphaFold2 to mitigate this dependence, this limitation can still become evident in challenging scenarios such as interspecies interactions, where evolutionary coupling between proteins from different organisms may be weak or entirely absent [65]. Additionally, while these models demonstrate impressive capabilities on moderately-sized protein complexes, they may experience computational breakdowns once sequence length surpasses certain thresholds, as GPU

memory requirements increase exponentially rather than linearly with longer sequences, creating practical barriers for large multi-domain proteins or extensive protein assemblies. Furthermore, an analysis of 317 AF3 complexes [66] reveals that the model consistently struggles with intrinsically flexible regions and disordered domains, demonstrating systematically higher prediction errors in regions characterized by high B-factors, which correspond to areas of high conformational mobility that are inherently difficult to capture in static structural predictions.

Applications

Protein–protein interface prediction has broad applications in understanding molecular function and disease. Xu et al. [67] integrate protein–protein interaction networks with GWAS and brain x-QTL data to identify disease risk genes for Alzheimer's disease. Zhang et al. [68] apply AlphaFold to obtain structure predictions for 1798 interactions involving cancer driver proteins and show that somatic cancer mutations frequently exert their effects by perturbing protein–protein interactions and their interfaces. PIONEER [17] demonstrates enrichment patterns of disease-associated mutations in their predicted interaction interfaces and explores their impact on disease prognosis and drug responses.

Identification of binding interfaces facilitates drug discovery by enabling the targeting of specific disease-related PPIs rather than inhibiting global function of individual proteins. Advances in interaction and interface prediction tools have improved identification of PPI hotspots [61,69], druggable pockets [16–18], thereby accelerating discovery and design of PPI-targeted drugs. For example, Trepte et al. [70] integrates binary PPI assays with AlphaFold–Multimer complex predictions to guide large scale drug screening. The pipeline successfully identifies a compound that inhibits NSP10-NSP16 interaction, thereby disrupting the methyltransferase activity of the complex and suppressing SARS-CoV-2 replication. These advances illustrate how interface-resolved structural prediction is transforming PPI-targeted drug discovery from heuristic screening toward mechanism-driven, interaction-specific therapeutic design.

Improved understanding of protein–protein interactions and binding interfaces has increasingly enabled de novo design of protein binders [71]. By combining AlphaFold-multimer and sequence redesign by ProteinMPNN [72], BindCraft [71] demonstrates its capability in designing binders against 12 challenging targets, including cell-surface receptors, common allergens and multi-domain nucleases, such as CRISPR-Cas9. More importantly, AI-designed de novo protein binders have shown promising translational potential in practical applications, including toxin neutralization [73], and modulation of immune responses [74]. In structure-guided antibody design, for example, Bennett et al. [63] show that a fine-tuned RFdiffusion model can generate de novo antibodies from scratch with atomic-level precision entirely in silico, targeting user-specified epitopes. They performed cryo-EM experimental validation on computational designed antibody variable heavy chains (VHHs) to influenza haemagglutinin, TcdB and SARS-CoV-2. Collectively, these studies highlight the growing impact of interface-aware design frameworks in advancing therapeutic protein engineering and rational binder development.

Identifying residues on the interaction interface provides critical insights into predicting effects of variants to protein–protein binding affinity. Many machine learning models focus on predicting changes in binding affinity ($\Delta\Delta G$) between wild-types and mutants, typically using the SKEMPI v2.0 [75] database. To obtain informative protein representations, many methods adopt the pre-training strategy on large collections of unlabeled protein complex structures, and subsequently apply the learned knowledge to $\Delta\Delta G$ prediction. For example, RDE-Network [76] and DiffAffinity [47] both pre-train deep probabilistic models to learn protein side-chain conformations that provide structural context of mutations on the protein interfaces, and then utilize these learned representations to predict $\Delta\Delta G$. However, most existing methods do not adequately account for potential data leakage arising from sequence and structural homology at both protein and interaction levels, which may lead to overestimation of predictive performance.

Viral immune evasion is largely mediated through protein–protein interactions that rewire host signaling pathways essential for innate immunity. By engaging or disrupting key host proteins, viral factors can suppress interferon production, alter transcriptional programs, and promote viral replication [77,78]. Structural characterization of viral–host interfaces has revealed how viruses hijack signaling hubs, exemplified by the interaction between SARS-CoV-2 spike protein and human ACE2 receptor, which enabled rapid therapeutic development [79]. Integrating protein interaction screening with interface-level analysis thus provides critical mechanistic insight into viral modulation of host immune responses [80].

Conclusions

Deep learning approaches for proteome-scale PPI prediction, PPI interface prediction, and PPI structure generation have enabled a wide range of biological applications, from dissecting virus–host interactions to interpreting disease-associated variants and guiding therapeutic design. The integration of sequential, evolutionary, and structural information has

substantially expanded the applicability of PPI modeling across diverse contexts. At the same time, key challenges remain, including extreme class imbalance in large-scale screening, limited availability of high-quality interface annotations, and the reliable interpretation of predictions in complex biological systems. Addressing these challenges will be critical for translating the methodological advances in PPI prediction into robust insights in future biological and biomedical research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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