Effectiveness and efficiency: label-aware hierarchical subgraph learning for protein-protein interaction (laruGL-PPI)

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Abstract

Protein-Protein interactions (PPIs) are crucial in various biological processes and their study has significant implications for drug development and disease diagnosis. Existing deep learning methods for PPIs prediction, suffer from remarkable performance degradation under complex real-world scenarios due to various factors, e.g., label scarcity and domain shift. Among these factors, topological shortcut of PPIs is considerable and limits model to capture the pattern of protein-protein structure, which makes generalization terrible. In addition, huge data growth of PPIs challenges computing device. In this paper, we propose a ***l***abel-***a***ware hie***r***archical s***u***b***g***raph ***l***earning framework (laruGL-PPI) that can effectively infer PPIs while being both interpretable and generalizable. In laruGL-PPI, we not only model the inner-outer connection of PPIs as a hierarchical graph but explore the label dependencies by constructing a label graph. Importantly, edge-based subgraph sampling is adopted to effectively alleviate the problems as topological shortcut and high computing cost. Extensive experiments on PPI datasets of different scales with various evaluation settings demonstrate that laruGL-PPI outperforms state-of-the-art PPI prediction methods, particularly in testing of unseen proteins. In addition, our model can recognize crucial sites of proteins, such as surface sites for binding and active sites for catalysis.

Keywords: protein-protein interaction, multiple graph learning, subgraph sampling, deep learning

1 Introduction

Biological functions are accomplished by interactions and chemical reactions among biomolecules. Among them, protein-protein interactions(PPIs) are undoubtedly one of the most essential molecular events in the human body and are an important source of therapeutic interventions against diseases. A comprehensive dictionary of PPIs can help connect the dots in complicated biological pathways and expedite the development of therapeutic[1, 2]. In past decades, high-throughput experimental methods, e.g. yeast two-hybrid screens (Y2H)[3], and mass spectrometric protein complex identification (MS-PCI)[4] have been developed to identify PPIs. Nevertheless, genome-scale experiments are expensive, tedious, and time-consuming while suffering from high error rates and low coverage[5]. Therefore, it is urgent to establish an interpretable computational approaches with high accuracy to identify potential PPIs.

More recently, a large variety of computational approaches have been developed to detect PPIs, consisting of classic machine learning (ML)-based [6-8] and deep learning (DL)-based methods[9-12]. Compared with classic ML methods, DL algorithm has advantage in processing complicated and large-scale data and extracting useful features automatically, achieving a huge success in computational biology[13], including PPI prediction[14]. Previously, DL-based methods treated interactions as independent instances, ignoring protein connections. To improve performance of models, recent works construct a PPIs graph with proteins as nodes and connections as edges. And various graph neural networks (GNN) architectures[15, 16] had been utilized to explore the PPIs graph to facilitate inference of type of interactions[12, 17, 18]. Among these graph-based methods, the HIGH-PPI is notable because of improvement in interpretability and accuracy. However, HIGH-

PPI suffer from intensive computational cost due to hierarchical graph with protein structures and PPI network. More importantly, emergence of topological shortcut in PPI task, which is similar to CPI[19], causes DL-based methods difficult to learning from protein itself. This topological shortcut stemming from the PPIs annotation imbalance prompts DL models to leverage degree information (positive and negative annotations) in making binding prediction instead of learning binding patterns from the intrinsic protein structures.

Protein-Protein Interaction Prediction. Early works leverage machine learning (ML) techniques to map pairs of handcrafted sequence features of proteins to interaction patterns[6, 8, 20, 21]. With the development of deep learning (DL), recent works have applied deep neural networks to automatically extract features from protein sequences for enhancing feature representation[10, 11, 22]. Furthermore, the latest works utilize graph neural networks (GNN) to model PPI network[12]. Among these works, the SemiGNN-PPI[17] establish a multi-graph learning framework to improve the model performance and the HIGH-PPI[18] organize the structure-based internal graph for each protein to enhance interpretability of model. However, all the PPI prediction models face a topological shortcut problem due to annotation imbalance. Also, the framework using a hierarchical graph with protein structure exert pressure on computational devices because of huge memory cost.

Topological Shortcut. In drug-target interaction (DTI) prediction, similar to PPI, DL models learn a map from features to interaction labels in the training data. Features refer to the chemical structures of proteins and ligands, which determine their physical and chemical properties. In an ideal scenario, the ML model learns the patterns characterizing the features which drive the protein-ligand interactions, capturing the physical and chemical properties of a protein and of a ligand that determine the interaction. Nevertheless, multiple state-of-the-art deep learning models ignore the

features and depend largely on annotations, i.e., the degree information for each protein and ligand in the drug-target interaction (DTI) network, as a shortcut to make new binding inference. In protein-protein interaction prediction, the topological shortcut problem also exists in the PPI network.

Multi-Label Learning. MLL addresses the problem in assigning multiple labels to a single instance. It has been applied successfully in various domains, e.g., computer vision[23, 24]. Traditional MLL methods typically train independent classifiers for all labels but fail to pay attention to the potential label interdependence, leading to suboptimal performance. Recent trends in MLL incorporate deep learning to capture the label dependencies[25, 26]. As an example, CNN-RNN[25] make use of recurrent neural networks (RNNs) to transform the label vectors into an embedded space to learn label correlations implicitly. More recently, graph-based MLL approaches have gotten great attention from scientific communities[27, 28]. Especially, the SemiGNN-PPI[17] successfully makes use of Graph Convolutional Network (GCN) by constructing a directed graph over labels to explicitly model the protein-protein interaction dependencies adaptively. In this regard, we add graph-based MLL to hierarchical graph learning for more accurate PPI prediction.

Subgraph Sampling. In a GCN, data to be gathered for one output node comes from its neighbors in the previous layer. Each of these neighbors in turn, gathers its output from the previous layer, and so on. Clearly, the deeper we back track, the more multi-hop neighbors to support the computation of the root. The number of support nodes (and thus the training time) potentially grows exponentially with the GCN depth. To mitigate such “neighbor explosion”, state-of-the-art methods use various layer sampling techniques[29-31]. While these methods significantly speed up training, they face challenges in scalability, accuracy or computation complexity. Recently, the GraphSAINT[32] was presented to efficiently train deep GCNs. Instead of building a GCN on the full training graph and

then sampling across the layers, GraphSAINT samples the training graph first and then build a full GCN on the subgraph, which is graph sampling based. In this work, we leverage edge-based subgraph sampling to alleviate the topological shortcut problem in PPI network and computational cost.

In this work, to tackle the above challenges and limitations, we proposed an interpretable and generalizable PPI prediction framework with edge-based subgraph sampling, referred to as ***l***abel-***a***ware hie***r***archical s***u***b***g***raph ***l***earning framework (laruGL-PPI). Firstly, we propose leveraging graph structure to model protein correlations and label dependencies for multi-graph learning. In detail, we derive an inter-dependent classifier to extract information from the label graph, which are then employed to the protein representations aggregated by neighbors in the PPI network for multi-type PPI prediction. Secondly, we also exploit graph to depict protein structure, represented with nodes as residues and edges as contacts between them. Thirdly, we adopt edge-based subgraph sampling to alleviate the topological shortcut and the pressure on computational devices. To the best of our knowledge, this is the first study to investigate emergence of topological shortcut and to mitigate it in PPI predictive task. Precisely, the main contributions of the work can be summarized as follows:

- For multi-type PPI prediction, we first investigate phenomenon called the topological shortcut, which limits learning ability of model. In order to this problem, we propose an effective ***l***abel-***a***ware hie***r***archical s***u***b***g***raph ***l***earning-based PPI prediction (laruGL-PPI) framework
- In laruGL-PPI, we construct multiple graphs to learn protein structure, connections between proteins and label dependencies simultaneously. We further advance subgraph sampling to train model for enhancing generalization ability of model and reducing computational

pressure on devices.

- Extensive experiments on three PPI datasets with different settings demonstrate that laruGL-PPI outperforms other state-of-the-art methods for multi-label PPI prediction under various challenging scenarios and has a high interpretability for PPI type prediction.

2 Materials and methods

2.1 Task definition

Given a set of protein $P=\{p_0, p_1, ..., p_n\}$ and a set of PPIs $E=\{e_{ij}=\{p_i, p_j\} \mid i \neq j, p_i, p_j \in P, I(e_{ij}) \in 0,1\}$, where $I(e_{ij})$ is a binary PPI indicator function that is 1 if the PPI between proteins p_i and p_j has been confirmed, and 0 otherwise, the types of PPI can be represented by the label space $C=\{c_1, c_2, ..., c_t\}$ with t different types of interactions, and the labels for a confirmed PPI e_{ij} can be represented as $y_{ij} \subseteq C$. The goal of multi-type PPI learning is to learn a function $f: e_{ij} \rightarrow \hat{y}_{ij}$ from the training set such that for any PPI $e_{ij} \in E^s_{test}$, $\hat{y}_{ij}$ is the set of predicted labels for e_{ij}. To confirm the emergence of topological shortcut in PPI prediction, we analyze annotations in three common PPI datasets (i.e., SHS27k, SHS148k, and STRING).

2.2 Network architecture design of laruGL-PPI

Figure 1 depicts the overview of our proposed laruGL-PPI framework. We first construct the multi-graph encoding module to effectively predict PPI types, consisting of a protein graph encoding network for capture binding patterns, a PPI network graph encoding network for modeling protein-protein correlations, and a label graph encoding network for learning label dependences. To mitigate

topological shortcut problem and computational pressure, we make use of edge-based subgraph sampling approach to train the laruGL-PPI model.

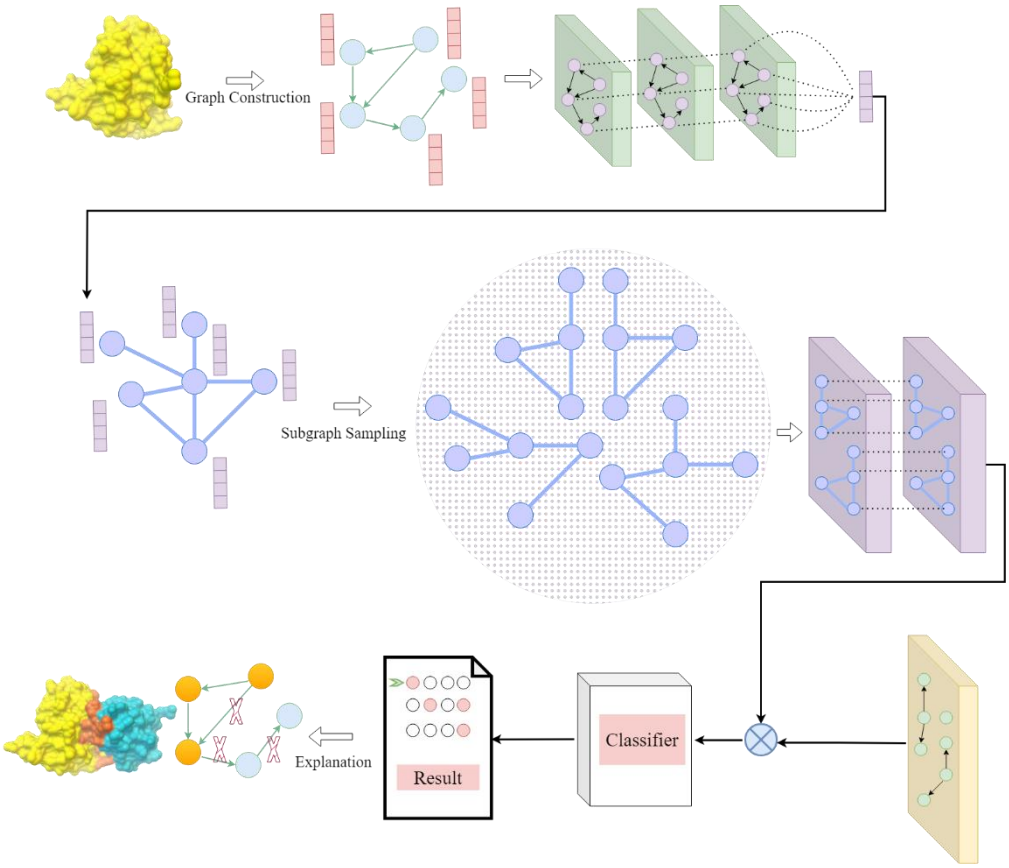

Figure 1: The overall framework of laruGL-PPI. First, we construct protein graph with residues as nodes and contacts as edges. And we treat physical and chemical properties of amino acid as features of nodes. Then, GCN blocks are employed to characterize protein graph and all the output features of nodes are aggregated to a feature vector as feature of protein node in the PPI network graph, which is characterized by proteins as nodes and correlations as edges. Afterwards, we use GIN blocks to model PPI network, leading a network-aware protein features. We arrange GCN blocks on the label graph to learn a classifier, which is applied to the products of two protein features for the protein-protein interaction prediction. Later on, laruGL-PPI is interpreted by GNNExplainer.

2.2.1 Multi-Graph Encoding

Protein-Graph Encoding. We denote a set of amino acid residues in a protein as

$prot = \{r_1, r_2, ..., r_n\}$. Each residue is described with t kinds of physicochemical properties. For the bottom inside-of-protein view, a protein graph $g_b = (V_b, A_b, X_b)$ is built to model the relationship between residues in Prot, where $V_b \subseteq prot$ is the set of nodes, A_b is an $n \times n$ adjacency matrix representing the connectivity in g_b, and $X_b \in R^{n \times \theta}$ is a feature matrix containing the properties of all residues. The contact map is exactly equivalent to the adjacency matrix $A_b \in \{0,1\}^{n \times n}$ in the protein graph. Contact maps are obtained with atomic level 3D coordinates of proteins. First, we retrieve the predictive structures from the AlphaFold Protein Structure Database. Then we represent the location of each residue by the 3D coordinates of its C_α atom. The presence or the absence of contact between a pair of residues is determined by their $C_\alpha - C'_\alpha$ physical distance. In this work, we choose the cutoff distance of 12 A. For a feature matrix X_b, each row stands for a set of properties for one amino acid residue. In this context, seven residue-level properties are considered: isoelectric point, polarity, acidity and alkalinity, hydrogen bond acceptor, hydrogen bond donor, octanol-water partition coefficient, and topological polar surface area. We apply variant graph neural networks to learn protein representations. Specifically, four GCN blocks are employed. Given the adjacency matrix $A_b \in \{0,1\}^{n \times n}$ and the feature matrix $X_b \in R^{n \times \theta}$ of an arbitrary protein graph g_b, we update residue representations with the neighbor aggregations based on the work of Kipf and Welling:

$$H^{(l+1)} = BN(\mathrm{Re}\,LU(\tilde{D}^{-1/2}(A_b + I_n)\tilde{D}^{-1/2}H^{(l)}W^{(l)}))$$

where $I_n \in R^{n \times n}$ is the identity matrix, $\tilde{D} \in R^{n \times n}$ is the diagonal degree matrix with entries $D_{ii} = \sum_j (A_b + I_n)_{ij}$, $W^{(l)}$ is a learnable weight matrix for the GCN layer l, ReLU, BN denotes the ReLU activation function and batch normalization, respectively. In the first GCN layer, $H^{(0)} = X_b$. After four rounds of iteration, we perform the readout operation with a self-attention graph

pooling layer and average aggregation to obtain the entire graph representation.

PPI-Graph Encoding. The PPI network structure determines the adjacency matrix $A_t \in \{0,1\}^{m\times m}$, in which the i-th row and j-th column elements is 1 if the i-th protein interacts j-th. The i-th row of the feature matrix X_t describes the representation vector for the i-th protein graph g_b.We use graph isomorphism network (GIN) to learn PPI network information. Formally, we are given the PPI graph $g_t = (V_t, A_t, X_t)$, where $X_t \in R^{m\times d}$ is defined as the feature matrix whose row vector is a representation from protein graph. In the k-th GIN layer,

$$x_v^{(k)} = \mathrm{MLP}^{(k)}((1+\varepsilon)\bullet x_v^{(k-1)} + \sum\nolimits_{u\in \mathrm{N}(v)} x_u^{(k-1)})$$

where $x_v^{(k)}$ denotes the representation of protein v after the k-th GIN layer, $\mathrm{N}(v)$ is a set of proteins adjacent to v, and ε is a learnable parameter. After two GIN layers, we feed $x_v^{(2)}$ in a linear layer with the ReLU activation to obtain final protein representation. For an arbitrary query pair containing the i-th and j-th proteins, we adopt element-wise product to combine protein pair information. Then the generated vector goes through a fully connected layer (FC) to come by protein-protein representation.

Label-Graph Encoding. In multi-label PPI prediction, correlations exist among different types of interactions, i.e., some PPI types may appear together frequently while others rarely appear together. Following Chen et al, we model the interdependencies between different PPI types(labels) using a graph and make model learn an inter-dependent classifier with the GCN, which is directly applied to protein-protein representation for multi-type PPI prediction. The GCN aims to learn a function $f(\cdot,\cdot)$ on the graph with t nodes. With the convolutional operation, $f(\cdot,\cdot)$ can be expressed as:

$$C = \mathrm{BN}(\mathrm{ReLU}(\tilde{D}^{-1/2}(A_c + I_n)\tilde{D}^{-1/2} X_c W_c))$$

where $I_n \in R^{n\times n}$ is the identity matrix, $\tilde{D} \in R^{n\times n}$ is the diagonal degree matrix with entries $D_{ii} = \sum_j (A_c + I_n)_{ij}$, $A_c \in R^{t\times t}$ and $X_c \in R^{t\times d_c}$ denotes adjacency and feature matrix in label

graph individually, W_c is a learnable weight matrix for the GCN, ReLU, BN denotes the ReLU activation function and batch normalization, respectively. Considering that PPI type names are semantic, we utilize the BioWordVec model pre-trained on the biomedical corpus for generating word embedding X_c to better capture their semantics. To construct the label correlation matrix, we compute the conditional probability of different labels within the dataset. To avoid noises, we binarize the correlation matrix with a threshold τ to obtain A_c.

2.2.2 Multi-Graph Learning with Subgraph Sampling

By applying the learned classifier W from label graph encoding(LGE) to the learned representations from hierarchical graph encoding (HGE) for PPI e_{ij}, we can obtain the predicted scores $\hat{y}_{ij} = W(h_{p_i} \cdot h_{p_j})$. We use the traditional multi-label classification loss function to update the whole network in an end-to-end manner. The loss function can be written as:

$$L = \sum_{c=1}^{t} (y^c \log(\sigma(\hat{y}^c)) + (1 - y^c) \log(1 - \sigma(\hat{y}^c)))$$

where $\sigma(\cdot)$ is the sigmoid function. Our model learns the aggregated features by combining protein neighbors and models the label correlations by learning inter-dependent classifiers simultaneously to improve the model generalization. With multi-graph learning, the learned classifiers are expected to be neighborhood-aware at both feature and label levels. We select edge-based sampler to build subgraph G_s due to the nature of protein-protein interaction. In detail, Given training PPI-network graph G and edge budget m. Firstly, sampling probability $P((u,v))$ is calculated by the following formula:

$$P((u,v)) := \left(\frac{1}{\deg(u)} + \frac{1}{\deg(v)} \right) / \sum_{(u',v') \in \varepsilon} \left(\frac{1}{\deg(u')} + \frac{1}{\deg(v')} \right),$$

where $\deg(\cdot)$, ε are referred to as the degree of a protein node and a set of all edges in training graph. Secondly, m edges are randomly sampled from ε according to P and sets of nodes that are end-points of edges in ε_s are determined as V_s. Finally, subgraph G_s is derived from V_s with node inducing.

2.2.3 Residue importance computation

We employ the method called GNNExplainer[33] to generate explanations for laruGL-PPI. By taking the well-trained GNN model and its predictions as inputs, GNNExplainer returns the most important subgraph by maximizing the mutual information *MI* between the model prediction and possible subgraphs. Given protein graphs G_1 and G_2 that connect in the PPI network, our goal is to identify a subgraph $G_s \subseteq G_1$ that is important for our model's prediction $\hat{y}$. The mutual information MI is formulated as:

$$MI(Y, G_s) = H(Y) - H(Y \mid G = G_s)$$

MI_s represents importance of G_s, Y is a variable indicating the probability of PPI presence of G_1 and G_2, and $H(\bullet)$ is the entropy term. In effect, MI quantifies the change in the probability of prediction $\hat{y} = \Phi(G_1, G_2)$ when computation graph is limited to explanation subgraph G_s. A computationally efficient version of GNNExplainer's objective, which we optimize using Adam, is as follow:

$$\min_M -\sum_{c=1}^{C} \mathbb{1}[y = c] \log P_\Phi(Y = y \mid G = A \odot \sigma(M))$$

Lastly, we compute the element-wise multiplication of $\sigma(M)$ and adjacency matrix A and set a threshold to arrive at the explanation G_s for our model's prediction $\hat{y}$ at protein graph G_1.

2.2.4 Datasets and metrics

We perform extensive analyses and experiments on three benchmark datasets, i.e., SHS27k,

SHS148k, and STRING. Both SHS27k and SHS148k are subsets of the STRING datasets. These are multi-label PPI data of Homo sapiens from the STRING database. We retrieve protein structures from AlphaFold Protein Structure Database. In this work, SHS27k has 1553 proteins and 6660 protein-protein multi-type labels. SHS148k consists of 4072 proteins and 30067 labels. The PPIs are annotated with 7 types, i.e., Activation, Binding, Catalysis, Expression, Inhibition, Post-translational modification (Ptmod), and Reaction. Each PPI is labeled with at least one of them. The micro-F1 score was applied to evaluate and compare the predictive performance of laruGL-PPI and existing methods. We also use area under the precision-recall curve (AUPR) to estimate model with regard to specific interaction type.

3 Results and discussion

3.1 Topological Shortcut Analysis.

In context of the PPI predictive task, ML models learn a map from features to labels as annotations and then make inference for the likelihood of each node (protein) to bind to other nodes. Annotations capture protein-protein interactions. Normally, known protein-protein interaction is denoted as 1, while the unknown interaction is labeled as 0. Features refer to the chemical structures of proteins, which determine their physical and chemical properties, and are expressed as amino acid sequences or 3D structures for proteins. With success of network inference in various fields, many ML models have considered PPI network and regard it as one of features externally. In an ideal scenario, the ML model learns the patterns characterizing the features which drive the protein-protein interactions, capturing the physical and chemical properties of protein pairs that determine the mutual binding action. However, as we show next, multiple state-of-the-art deep learning models, such as HIGH-

PPI rely largely on the degree information for each protein in the protein-protein interaction network, as a shortcut to make new binding predictions. We begin by noticing that the number of annotations linked to a protein follows a fat-tailed distribution, indicating that the vast majority of proteins have only a small number of annotations, which then coexist with a few hubs, nodes with an exceptionally large number of binding records.

Distributions of the number of annotations in the SHS27k and SHS148k are shown in **Figure 2.** As an example, the number of annotations for proteins follows a power law distribution with degree exponent $\gamma = 1.46$, maximum degree $k_{\max} = 182$, minimum degree $k_{\min} = 1$ on SHS27k dataset. For the degree exponent, the second moment of the distribution diverges, implying that the expected uncertainty in the binding information is highly significant, limiting our ability to predict the binding between proteins in terms of intrinsic protein characteristics. Furthermore, it is found that the number of annotation k and the average interaction per k, calculated as the average across all links stemming from nodes of degree k, are associated with each other. In this work, there are seven interaction types between proteins, consisting of reaction, binding, posttranslational modification, activation, inhibition, catalysis and expression. For catalysis, they are positively correlated with $r_{Spearman} = 0.37$ ($p < 0.01$), indicating stronger catalysis propensity for proteins with more annotations. Whereas, they are negatively correlated with $r_{Spearman} = -0.34$ ($p < 0.01$) for expression, implying stronger expression tendency for proteins with less annotations (Table 1). As the annotations follow fat-tailed distributions, the observed correlation drives the hub proteins to have disproportionately more interaction or non-interaction on average, whereas proteins with fewer annotations have both interaction and non-interaction examples. This annotation imbalance prompts the ML models to leverage degree information (positive and negative annotations) in

making interaction prediction instead of learning interaction patterns from the protein intrinsic structures.

To investigate the emergence of topological shortcuts, for each node i with number of annotations k_i, we quantify the balance of the available data information via the degree ratio, $\rho_i = \frac{k_i^+}{k_i^+ + k_i^-} = \frac{k_i^+}{k_i}$, where, k_i^+ is the positive degree, corresponding to the number of known binding annotations in the training data, and k_i^- is the negative degree, or the number of unknown annotations in the training data. As most proteins lack either binding or unknown annotations for interaction types (Table 1), the resulting p_i are close to 1 or 0 (See Figure 3), which represent the annotation imbalance in the prediction problem. As many state-of-the-art deep learning models, such as HIGH-PPI, uniformly sample the available positive and negative annotations, they tend to assign higher binding probability to proteins with higher ρ. Consequently, their binding predictions are driven by topological shortcuts in the protein-protein network, which are associated with the positive and negative annotations present in the dataset rather than the structural features characterizing proteins.

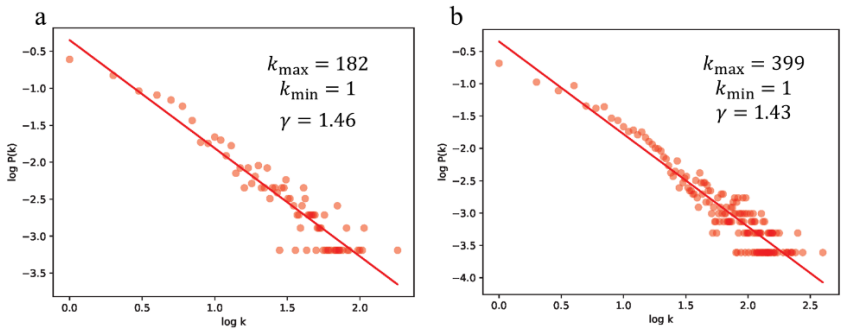

Figure 2 Distributions of the number of annotations in the SHS27k (a) and SHS148k (b)

Class	Has only positive labels	Has only negative labels	Has both labels	correlation
Reaction	275	737	541	0.06
	541	1903	1628	-0.09
Binding	502	370	681	-0.12
	1186	895	1991	-0.14
Ptmod	24	1216	313	0.10
	84	2854	1134	0.22
Activation	232	672	649	0.12
	455	1667	1950	0.12
Inhibition	48	1150	355	0.16
	72	2863	1137	0.10
Catalysis	206	781	566	0.37
	372	2044	1656	0.23
Expression	82	1151	320	-0.34
	189	2905	978	-0.45

Table 1 Statics of SHS27K and SHS148K. Most proteins in common datasets have either positive or negative labels, which creates imbalance in the degree ratio.

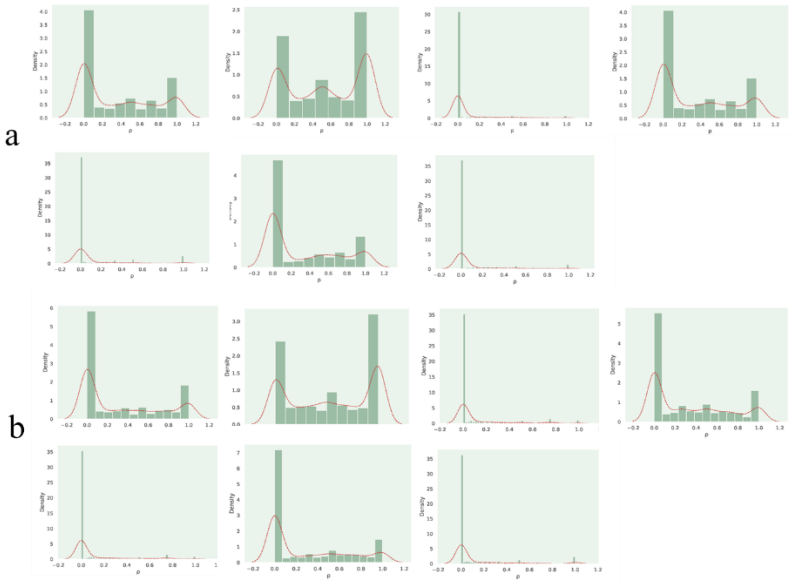

Figure 3 The distribution of degree ratios for the proteins in SHS27K and SHS148K.

3.2 Comparison with competitive methods

In this section, we display the performances achieved by our laruGL-PPI and competitive baselines on SHS27k and SHS148k datasets. Here, we ran each model five times independently to eliminate

bias caused by randomness and examine the robustness of laruGL-PPI. In table 2, we compare our methods with other baseline methods under different partition schemes and various datasets. It is observed that graph-based methods, i.e., GNN-PPI, SemiGNN-PPI, and HIGH-PPI exceed other ML and DL models, even under more difficult BFS and DFS partitions with more unseen proteins. It can be ascribed to graph learning, which can model correlations between proteins despite the existence of more unknown proteins. Furthermore, our proposed method, which incorporates multiple graph learning in conjunction with subgraph sampling, achieves competitive performance in multi-type PPI prediction in common transductive setting. It is worth mentioning that our method reaches state-of-the-art level in difficult data partition, i.e., DFS and BFS. In realistic application of biology, models tend not to have seen predicted protein pairs in training process. In order to conform with the reality, it is the first time to consider inductive setting in multi-type PPI prediction. Apparently, when facing unseen protein, model cannot utilize degree information to conduct prediction, leading to decreasing accuracy. The inductive setting requires models to more focus protein structures instead of degree information. By subgraph sampling, our model improves predictive performance and outperform state-of-the-art HIGH-PPI under the more difficult inductive setting with SHS27k, regardless of partition mode (Table 2).

3.3 Performance on specific PPI types

For each of the six PPI types, we offer a separate performance analysis in terms of AUPR. It can be found that our presented model with lower learning cost parallels with HIGH-PPI as shown in Figure 4. Notably, laruGL-PPI consistently beats previous state-of-the-art model in the most challenging setting (i.e. inductive setting with BFS). This is reasonable as laruGL-PPI learns spatial-biological

patterns as possible as it can by sampling subgraph in multiple graph framework. When facing unknown protein pairs, it is able to exploit these patterns to predict better than other.

Model	SHS27K			SHS148K		
	Random	DFS	BFS	Random	DFS	BFS
RF	0.7845	0.3555	0.3767	0.8210	0.4326	0.3896
	(0.0088)	(0.0222)	(0.0157)	(0.0020)	(0.0343)	(0.0194)
LR	0.7155	0.4851	0.4306	0.6700	0.5109	0.4745
	(0.0093)	(0.0187)	(0.0505)	(0.0007)	(0.0209)	(0.0142)
DPPI	0.7399	0.4612	0.4143	0.7748	0.5203	0.5212
	(0.0504)	(0.0302)	(0.0056)	(0.0139)	(0.0118)	(0.0870)
DNN-PPI	0.7789	0.5434	0.4890	0.8849	0.5842	0.5740
	(0.0497)	(0.0130)	(0.0724)	(0.0048)	(0.0205)	(0.0910)
PIPR	0.8331	0.5780	0.4448	0.9005	0.6398	0.6183
	(0.0075)	(0.0324)	(0.0444)	(0.0259)	(0.0076)	(0.1023)
GNN-PPI	0.8791	0.7472	0.6381	0.9226	0.8267	0.7137
	(0.0039)	(0.0526)	(0.0179)	(0.0010)	(0.0085)	(0.0533)
HIGH-PPI	0.8797	0.7884	0.6749	0.8909	0.7567	0.6798
	(0.0089)	(0.0052)	(0.0211)	(0.0245)	(0.0103)	(0.0397)
SemiGNN-PPI	0.8951	0.7832	0.7215	0.9240	0.8545	0.7178
	(0.0046)	(0.0315)	(0.0287)	(0.0022)	(0.0117)	(0.0356)
laruGL-PPI	0.8762	0.7972	0.7407	0.9069	0.8061	0.7307
	(0.0053)	(0.0160)	(0.0672)	(0.0039)	(0.0096)	(0.0259)
HIGH-PPI*	0.8480	0.7351	0.6665	0.8572	0.7127	0.6674
	(0.0067)	(0.0080)	(0.0452)	(0.0211)	(0.0277)	(0.0416)
laruGL-PPI*	0.8635	0.7657	0.7097	0.9013	0.7852	0.7311
	(0.0048)	(0.0371)	(0.0394)	(0.0035)	(0.0110)	(0.0374)

Table 2 Prediction accuracy of laruGL-PPI and other competing methods. The accuracy is represented with micro-F1 score (mean±std). The optimal value in settings has been emphasized in bold. The symbol * stands for inductive setting and the model without * means transductive.

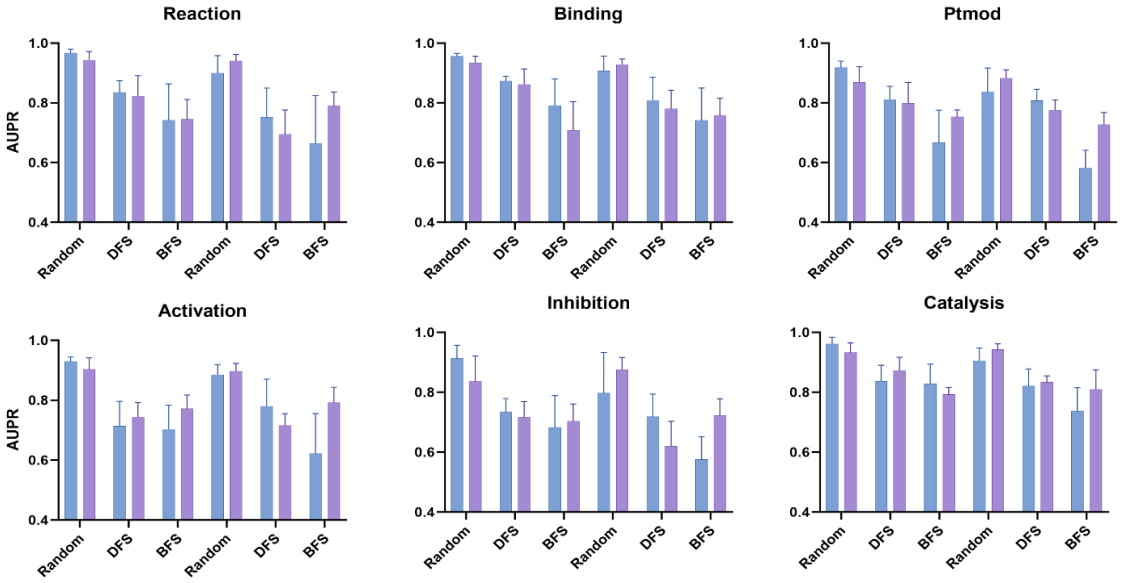

Figure 4 Performance of laruGL-PPI in predicting specific PPIs on SHS27k, compared with HIGH-PPI. Blue and purple represents HIGH-PPI and laruGL-PPI, respectively. Error bars express standard deviation of the mean. Source data are provided as a Source Data file.

3.4 The results of training with various subgraph size

In this section, we display the results of comparison of HIGH-PPI and our laruGL-PPI with various subgraph size. Beginning by subgraph size of 200, we observe that our model has attained competing effectiveness in SHS27k under the transductive setting. From results of effect of subgraph size, using subgraph sampling is capable of reducing the memory cost of Graphics Processing Unit (GPU) significantly and having a comparable outcome at the same time (Table S1). It is indicated that taking size of 300, 400 or 500 can reach a greater performance and request a relatively smaller memory cost in SHS27k dataset (Figure 5).

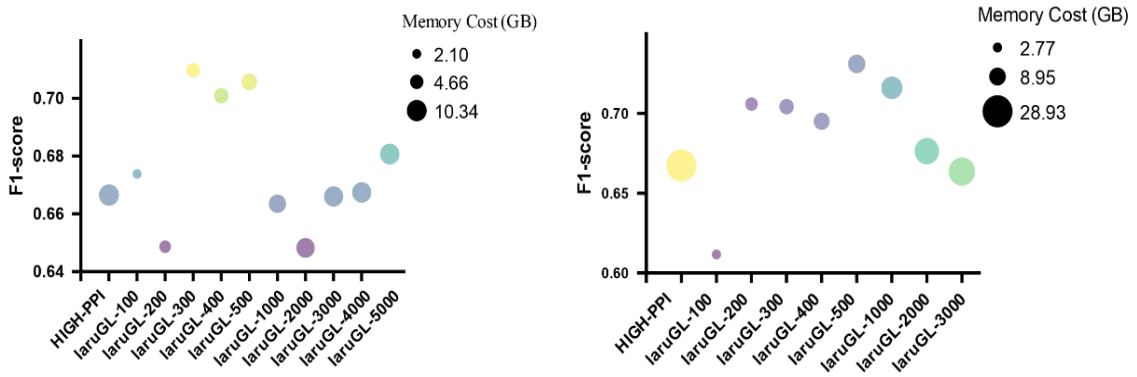

Figure 5 F1-score and Memory cost of HIGH-PPI and laruGL-PPI with various size subgraphs under inductive setting (BFS) on SHS27k(left) and SHS148k(right).

3.5 Ablation study

We investigate the effectiveness of different components in laruGL-PPI in Table 3. It can be seen that model improves a lot (from 0.6749 to 0.7238) under relatively difficult circumstance (i.e. transductive setting with BFS) when considering adjacent residues in sequence. This implies that sequence information plays an important role on determining PPI types. What's more, label graph encoding brings plenty profits in a majority of scenarios, particularly under the most arduous condition (i.e. inductive setting with BFS, from 0.6652 to 0.7072). Finally, our model is getting more powerful when adopting edge-based sampling strategy on PPI graph. In particular, under transductive setting, performance in terms of DFS achieves 0.7972. While under inductive setting, more challenging scene, laruGL-PPI reaches the best F1-score in the light of all the partitions.

Model	Transductive setting			Inductive setting		
	Random	DFS	BFS	Random	DFS	BFS
HGL-origin	0.8797 (0.0089)	0.7884 (0.0052)	0.6749 (0.0211)	0.8480 (0.0067)	0.7351 (0.0080)	0.6665 (0.0452)
HGL	0.8873 (0.0059)	0.7743 (0.0149)	0.7238 (0.0383)	0.8369 (0.0058)	0.7400 (0.0165)	0.6652 (0.0797)
laHGL	0.8894 (0.0073)	0.7819 (0.0226)	0.7545 (0.0399)	0.8405 (0.0040)	0.7350 (0.0470)	0.7072 (0.0543)
laruGL	0.8762 (0.0053)	0.7972 (0.0160)	0.7407 (0.0672)	0.8635 (0.0048)	0.7657 (0.0371)	0.7097 (0.0394)

Table 3 Results of ablation studies on different components of laruGL-PPI using the SHS27k dataset.

3.6 Interpretability of our laruGL-PPI

3.6.1 Analysis of feature importance in residue-level （inductive random）

The feature importance in residue-level for overall (left-most column) and type-specific (right six columns) PPI prediction calculated as the average z-score resulting from dropping each individual feature dimension from our model and calculating changes of AUPR before and after.

For node features in protein graphs, we select seven important features in terms of laruGL-PPI. Here, we list the selected seven residue-level physicochemical properties in Figure 5 and discuss their importance for different types of PPIs to both better interpret our model and discover enlightening biomarkers for PPI interface. The average z-score, which results from deleting each feature dimension and analyzing changes in metrics before and after, is calculated to determine the importance of a feature. From the results, laruGL-PPI regards topological polar surface area (TPSA) and octanol-water partition coefficient (KOW) as dominant features in multiple types of PPIs. This finding supports the conventional wisdom that TPSA and KOW play a key role in drug transport process[34], protein interface recognition[35, 36], and PPI prediction[37].

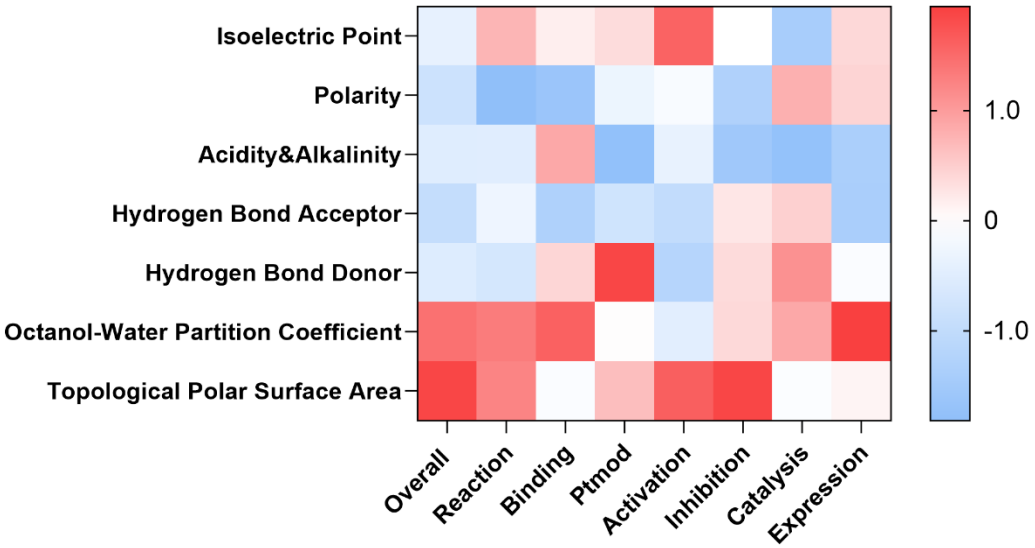

Figure 5 The feature importance in residue-level for overall (left-most column, F1-score) and type-specific (right seven columns, AUPR) PPI prediction.

3.6.2 laruGL-PPI identifies key residues constituting functional sites

Typically, functional sites are spatially clustered sets of residues. They control protein functions and are thus important for PPI prediction. As our proposed model has the capacity to capture spatial-biological arrangements of residues in the protein graph learning, this characteristic can be used to explain the model's decision. It is meaningful to notice that laruGL-PPI can automatically learn the residue importance without any residue-level annotations. In this section, we provide a case study of predicting important residues for the binding surface and two cases of estimating key residues for catalytic function.

First, a binding example between the query protein (PDB id: 2B6H) and its partner (PDB id: 2REY) is investigated, whose complex structure is generated by ZDOCK[38]. Subsequently, we apply the GNN explanation approach on the laruGL-PPI model. As can be seen from Figure 6a.

LaruGL-PPI can accurately and automatically identify the residues belong to the binding domain.

Second, in order to evaluate the prediction of crucial sites of catalysis for PPIs, we utilize the same GNN explanation approach in our model. The ground truth key sites, normally including binding sites and active sites, are retrieved from the Universal Protein Knowledgebase (UniProt)[39]. We explore the prominent residues of catalytic function for query proteins (UniProt id: P62714, Q9UMX6). As seen in Figure 6b, our proposed laruGL-PPI infers that ASN117 is important for catalysis of P62714 known as catalytic subunit of protein phosphatase 2A, in terms of Q9Y4X3. The coordinate bond between ASN117 and Mn^{2+} activates the enzyme to play a highly efficient catalytic role. For Guanylyl cyclase-activating protein 2 (Q9UMX6)**,** model can correctly predict that Asp66 and Asp102 are key to perform catalysis on Q16270.

Protein functional site prediction sheds light on the model decisions and how to conduct additional wet experiments for PPI investigation. Excellent model interpretability also demonstrates that our approach can accurately describe biological evidence for proteins.

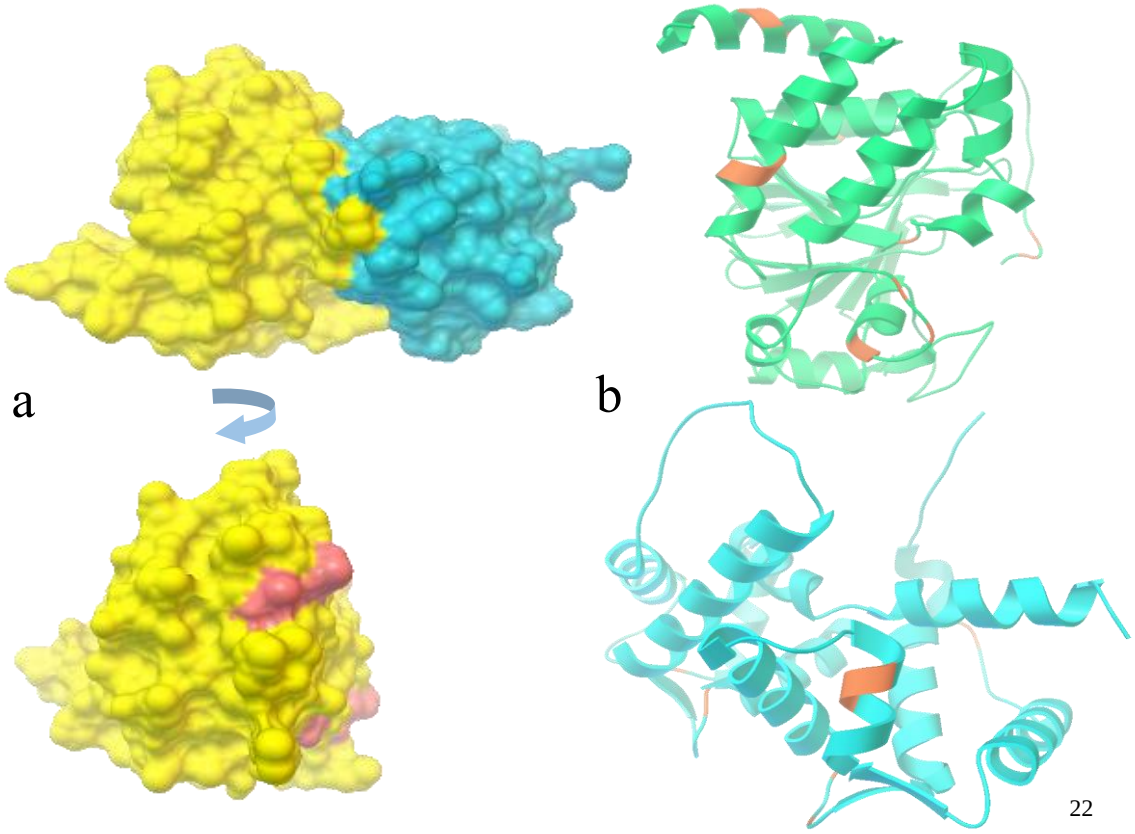

Figure 6 Automatic explanation for key residue without supervision. **a** Top: Depiction of a complex protein (left, query protein, PDB id: 2B6H; right, interacted protein, PDB id: 2REY) modeled in surface representation. Bottom: important residues of the query protein learned from laruGL-PPI with pink. **b** Depiction of two proteins (top, UniProt id: P62714; bottom, UniProt id: Q9UMX6) modeled in cartoon representations. Residues inferred by model, which are colored with brown, are important for catalysis.

4. Conclusions

In this study, it was found that conventional machine learning is subject to the problem of topological shortcuts. A new framework based on subgraph sampling was proposed to address this issue, enabling the model to focus more on the protein structure itself. This framework improved prediction accuracy and generalization, especially in challenging settings, where it exhibited significant improvements in encountering new proteins. Additionally, subgraph sampling saved computational resources. Through interpretable analysis of the model, it was found that the model can identify key sites, binding surfaces, or active sites. This may also be attributed to the subgraph sampling algorithm. Nevertheless, there is still room for improvement. For instance, the model does not explicitly consider protein sequence information, and future research may consider sequence-structure co-modeling. Another potential direction is to extend the model to protein all-atom graphs, extracting more microscopic information about proteins and learning the essence of PPI, thereby further enhancing the model's generalization ability and interpretability.

Data and code availability

The preprocessed data and model code associated with the study are available at https://github.com/xxx.

Acknowledge

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