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siRNADesign: A Graph Neural Network for siRNA Efficacy Prediction via Deep RNA Sequence Analysis

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Abstract

With the growing attention on siRNA silencing efficacy prediction, many methods have been proposed recently ranging from traditional data analysis methods to advanced machine learning models. However, previous works fail to explore complex but vital information, e.g., the RNA sequence interactions and related proteins. To alleviate this issue, we propose siRNADesign, a GNN model that innovatively analyzes both non-empirical and empirical-rules-based features of siRNA and mRNA sequences. This comprehensive approach allows siRNADesign to capture the nuanced dynamics of gene silencing effectively, achieving unprecedented state-of-the-art results across various datasets. Furthermore, we introduce a novel dataset-splitting methodology to mitigate the issues of data leakage and the shortcomings of traditional validation techniques in prior works. By considering siRNA and mRNA sequences as separate entities for dataset segmentation, this method guarantees a more accurate and unbiased evaluation of the model's performance. Through extensive evaluation on both widely used and external datasets, siRNADesign has demonstrated exceptional predictive accuracy and robustness under diverse experimental conditions. This work not only provides a robust foundation for the advancement of predictive models in gene silencing but also proposes a new dataset-splitting approach that aims to redefine the standards for future research, promoting more thorough and realistic assessment methodologies.

Key words: siRNA Efficacy Prediction, Gene Silencing Efficacy, RNA Sequence Analysis, Graph Neural Network, Deep Learning

Introduction

RNA interference (RNAi) serves as a critical regulatory mechanism in cells, utilizing short double-stranded RNA (dsRNA) molecules to guide the homology-dependent regulation of gene expression [1]. This process involves small interfering RNAs (siRNAs), which are RNAi-based regulators consisting of 21-22 nucleotides. These siRNAs are processed from longer dsRNA precursors by Dicer, an RNase III-like enzyme, and then incorporated into the RNA-induced silencing complex (RISC). Within RISC, siRNA strands are separated, and the strand with a more stable 5' end guides the complex to bind homologously to the target mRNA. The catalytic component of RISC, a member of the argonaute family (AGO2), then cleaves the mRNA, leading to its degradation and the silencing of the gene [2].

Thousands of potential siRNAs can target a gene, and identifying the most effective siRNA is currently a focus of study. Researchers have developed various algorithms to predict siRNA activity, categorized into two generations. The **first**-generation algorithms rely on validated small siRNA datasets and consider several features, such as GC content, base preference at specific positions, thermodynamic stability, internal structure, and the secondary structure of the target mRNA [3]. However, first-generation methods are shown to have low accuracy, with experiments indicating that about 65% of the predicted active siRNAs failed to achieve 90% knock-down efficacies. Furthermore, approximately 20% of the siRNAs are found to be inactivated [4]. This issue could be attributed to the limited size of early datasets, which likely did not encompass all critical features [5]. With the expansion of siRNA data, the **second**-generation algorithms improve predictions of siRNA knockout efficiency by utilizing advanced data mining techniques. Huesken constructed a dataset containing 2431 siRNAs using a high-throughput analysis technique, and developed a tool named Biopredsi to predict siRNA

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efficiency, based on artificial neural networks [6]. Subsequently, with the contribution of Huesken dataset, numerous machine learning-based siRNA activity prediction tools emerged. DSIR used simple linear LASSO model combining basic features of siRNA sequences for siRNA efficacy prediction with a good performance [7]. Additionally, i-Score algorithm predicted active siRNAs by applying a linear regression model, only comprised of nucleotide (nt) preferences at each position without other parameters [8]. However, the performance of second-generation algorithms exhibits instability, potentially stemming from the heterogeneity of siRNA datasets, which are assessed by various groups across different protocols. Moreover, the performance of these artificial intelligence methods also largely depends on feature selection [9].

To address the issue of biased and incomplete feature engineering, [10] introduces deep learning scenarios, i.e., Convolutional Neural Network (CNN), into this field to achieve a more powerful siRNA efficacy predictor. Specifically, they utilize convolution and linear layers to extract hidden features from sequence context and thermodynamic properties, followed by a deep architecture for efficacy prediction. This CNN method is proven to be more stable and efficient than previous works [8, 8, 11] for the prediction of siRNA silencing efficacy.

Meanwhile, there is another issue that the numerous interactive factors and nonlinear characteristics of biological characteristics of biological processes significantly challenge the construction and optimization of traditional models and CNN models, since these models struggle to consider the intrinsic relationships within datasets. However, Graph neural networks (GNNs) excel in analyzing data represented in graphical structures, including biological networks [12]. GNNs are able to analyze the hidden relationships and information within the data, offering a straightforward and intuitive representation of heterogeneous and complex biological processes [13]. In biological molecular networks, siRNA and mRNA constitute a complex interaction network, where each mRNA molecule may interact with numerous different siRNA molecules. Thus, GNNs offer a potent solution for constructing a siRNA-mRNA interaction network to predict their interaction ability based on the features of siRNA and mRNA. Massimo et al. [14] introduced the first GNN-based model named GNN4siRNA for siRNA inhibition prediction, featuring three node types: siRNA nodes with 3-mer counts, mRNA nodes with 4-mer counts, and siRNA-mRNA interaction nodes with features from thermodynamic parameters. The features of interaction edges are attributed to interaction nodes, while the weights of the original interaction edges server as labels for these nodes. Utilizing the proposed graph topology enables the prediction of siRNA efficiency through node regression tasks.

However, GNN4siRNA's mining of sequence characteristics is not in-depth enough, which does not account for several key factors that critically influence the efficacy of siRNA-mediated gene silencing. First, it overlooks the importance of siRNA's position on the mRNA, and the spatial alignment of siRNA with its target mRNA site is essential for accessibility and binding potential [15]. Second, using k-mer counts to represent siRNA and mRNA sequences inadequately captures the complexity of nucleotide interactions. Additionally, the model neglects the role of AGO2, a crucial component of the RNA-induced silencing complex (RISC), and AGO2 is key to RISC assembly for effective gene silencing. Considering the role of these characteristics in practice, we believe that GNN4siRNA, along with some other models [11, 8, 10], has overlooked much useful information, which could effectively help siRNA efficiency prediction. Therefore, we ask the question: Can we utilize the advantages of GNN and deeply grasp the RNA sequences' characteristics and semantics, achieving better and robust prediction performance of siRNA efficiency?

Dataset Name	siRNA Num.	mRNA Num.	Pairs Num.
Dataset_HUVK [14]	2816	44	2816
Simone [16]	322	6	322
In-house($ACON$)	102	1	102

Table 1. Dataset Information. We summarize the information of the three datasets utilized in our experiments, including mRNA number, siRNA number, and siRNA-mRNA pair number.

To this end, we propose **siRNADesign**, a GNN framework that thoroughly explores the sequence features of siRNA and mRNA with a specific topological structure, enhancing siRNA efficacy prediction performance. Our siRNADesign firstly extracts two distinct-type features of RNA, i.e., non-empirical features and empirical-rules-based ones, and integrates them into GNN training. Specifically, the non-empirical features include one-hot sequence encodings, position encodings, base-pairing probabilities, and RNA-protein interaction probabilities. The empirical-rules-based features include the thermodynamic stability profile, nucleotide frequencies, the G/C percentages, and the rule codes (siRNA). Compared with other existing methods, the experiment results on all datasets demonstrates that our model achieves *state-of-the-art* performance.

Upon that, we notice a data leakage issue within the cross-validation setting, i.e., a siRNA from the test set might have been included in the training phase, thereby compromising the integrity of the evaluation. What's more, existing methods [14, 11, 8, 10] employ the same subset for both validation and testing, which does not make sense and is impractical for real-world applications. Therefore, we further propose **a new splitting method** that randomly divides the dataset into three subsets for training, evaluation, and testing. To avoid data leakage or deviation from randomness, we respectively treat siRNA and mRNA as the basis of division and repeat multiple times. This evaluation setting enables a more rational and effective evaluation of models' predictive performance and robustness. We conduct extensive experiments and verify that our siRNADesign shows superior performance on all datasets under different splitting settings.

The main contributions of our paper are summarized as follows:

- We propose **siRNADesign**, a GNN framework that thoroughly explores the sequence features of siRNA and mRNA, and boosts siRNA efficacy prediction performance.
- To tackle the data leakage issue in the previous evaluation settings, we propose **a new evaluation method** (data-splitting manner) that achieves a more rational and effective evaluation of models' predictive performance and robustness, serving as a new benchmark for future works.

- We conduct experiments on the common-used dataset [14] under our proposed evaluation settings, where siRNADesign achieves *state-of-the-art* performance.
- We also verify siRNADesign on two external datasets, i.e., Simone and our in-house dataset, and our model shows superior performance compared with others across all metrics.

Materials and Methods

In this section, we elaborate on the proposed data splitting manner and details of siRNADesign.

Data sources

Public data

In this work, we collect the siRNA datasets with 2816 siRNA-mRNA pairs and related efficacy values from [14], which come from the original studies of Huesken [6], Harborth [17], Ui-Tei [18], Vickers [19], and Khovorova [20]. We term this dataset as 'Dataset_HUVK'. Besides Dataset_HUVK, Simone dataset [16] is used to test our models with 322 siRNA.

In-house data

The procedures were followed to test the silencing efficiency of designed siRNA on the target SERPINC1. On day 0, 293A cells (ATCC, Manassa, VA, US) were cultured in 96-well cell culture plate with 100 μL culture medium. The initial seeding was 12,000 cells per well. On day 2, medium was replaced with 150 μL. 0.4 μL of Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, US) was used as plasmid transfection agent for the cells. Four different concentrations (10 nM initial, 10-fold dilution) of designed siRNA (in-house synthesized by ACON Pharmaceuticals, Cranbury, NJ, US), the negative control wells (transfection with luciferase plasmid only) and the background control wells (wells containing cells) were used. On day 2, after 24-hour transfection, double luciferase detection was performed, and silencing efficiency was calculated accordingly.

We conducted multiple trails for each siRNA and obtained 298 siRNAs. Those demonstrating negative knockdown efficiency were removed from further consideration. For the siRNAs retained in our dataset, we omit those siRNAs demonstrating significant variability across their trials due to experimental errors. By calculating the average knockdown efficacy of siRNAs across various trials within the same experiment, we refined our dataset to include 102 siRNAs with consistent results as the final validation set.

In Table 1, we summarize the information of the three datasets, including mRNA number, siRNA number, and siRNA-mRNA pair number, utilized in our experiments.

Dataset Splitting

Prior works [14, 11, 8, 10] evaluate the models via 10-fold cross-validation. Specifically, each dataset is divided into ten equal parts, with nine parts used for training and the remaining one part for testing. This cycle is executed ten times, allowing each segment to serve as the test set once. However, we observe a data leakage issue within the cross-validation setting, i.e., a siRNA from the test set might have been included in the training phase, thereby compromising the integrity of the evaluation. What's more, existing methods [14, 11, 8, 10] employ the same subset for both validation and testing, which does not make sense and is impractical for real-world applications. Consequently, we introduce a **new** evaluation setting. Here, we randomly divide the dataset into a training set, an evaluation set, and a test set with a ratio of 70:15:15. To avoid data leakage or deviation from randomness, we respectively treat siRNA and mRNA as the basis of division, and repeat the splitting process 10 times with different random seeds. This approach yields 20 distinct splits, offering a more robust and realistic evaluation. Note that we denote the split datasets divided based on siRNA and mRNA as 'siRNA-split data' and 'mRNA-split data' respectively.

Preliminaries: GNNs and GCNs

In this section, we elaborate on the design of classical Graph Neural Networks (GNNs) and Graph Convolutional Networks (GCNs) as preliminaries of our model.

Graph Neural Networks (GNNs)

Different from traditional machine learning models that primarily operate within Euclidean spaces, GNNs serve as specialized deep learning frameworks adept at handling graph-structured data. GNNs are somehow motivated by the growing prevalence of data that naturally form networks or graphs. This is particularly evident in the realm of biological research, where complex biological mechanisms are often conceptualized as networks, such as protein interactions and metabolic pathways [12]. Consequently, GNNs have proven to be instrumental in biological analysis. Specifically, graphs are mathematically represented as $G = (V, E)$, where V denotes the vertices or nodes of the graph, with $|V| = n$, and E represents the edges, with $|E| = e$. Each edge $e_{ij} \in E$ connects nodes v_i and v_j. The structural properties of a graph are further detailed by its adjacency matrix $A \in \mathbb{R}^{n\times n}$, while node and edge attributes are captured through feature matrices $X_v \in \mathbb{R}^{n\times d}$ and $X_e \in \mathbb{R}^{e\times c}$, respectively. Heterogeneity in graphs arises when nodes represent distinct entities, each characterized by unique features.

Graph Convolutional Networks (GCNs)

Graph Convolutional Networks (GCNs) stand as a pivotal extension within the GNNs framework, specifically engineered to leverage the convolutional concept for graph-structured data analysis. The inception of GCNs is largely attributed to the necessity of integrating the powerful feature extraction capabilities of Convolutional Neural Networks (CNNs), traditionally used in image processing, into the domain of graph data. Specifically, GCNs adapt the convolutional mechanism to function on graphs by aggregating feature information from a node's local neighborhood, effectively capturing the topological structure of the graph. This aggregation is mathematically formulated as a function of the adjacency matrix A and the feature matrices X_v and X_e, facilitating the learning of node representations that reflect both their features and the influence of their adjacent nodes. The ability of GCNs to encode both node and structural information into dense vector representations makes them highly effective for tasks such as node classification, link prediction, and graph classification, wherein the spatial relationships among data points play a crucial role.

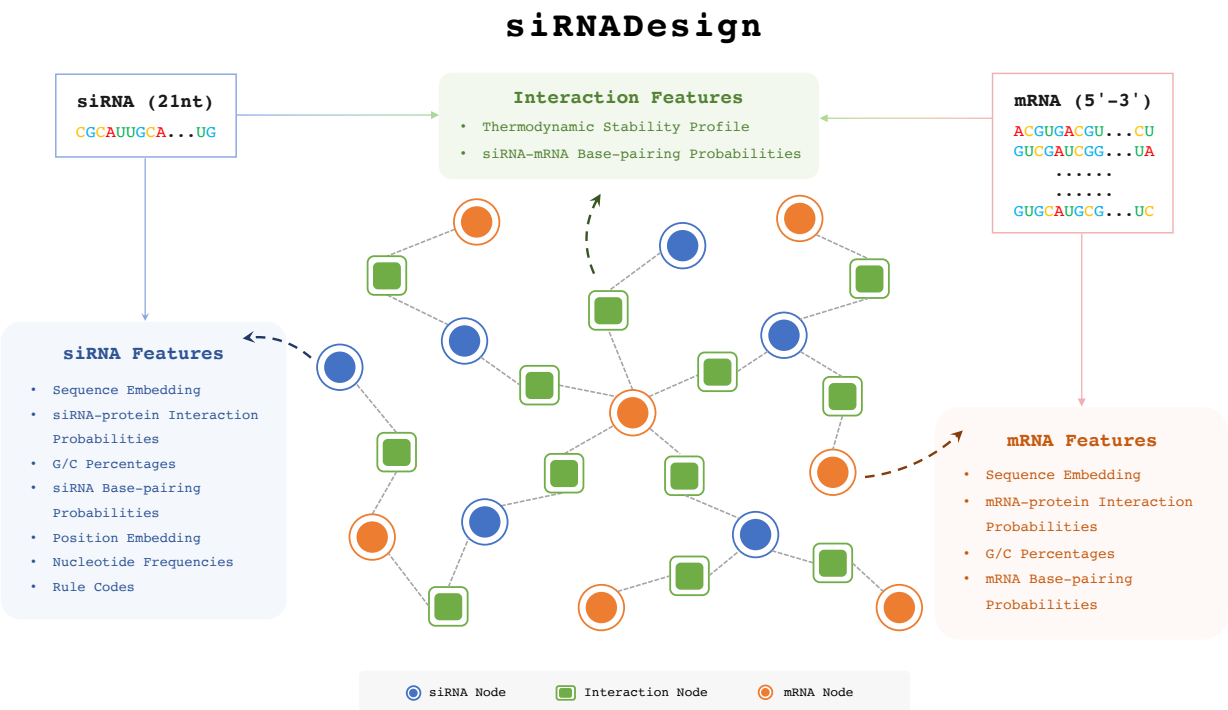

Fig. 1. Overall Works of siRNA-DESIGN. Our model contains two modules, i.e., a **feature extraction module** for deep sequence characteristics exploration, and a **GNN prediction module** for feature fusion and efficacy regression.

Node Type	Assigned Features
mRNA	Sequence Embedding mRNA-protein Interaction Probabilities G/C Percentages mRNA Base-pairing Probabilities
siRNA	Sequence Embedding siRNA-protein Interaction Probabilities G/C Percentages siRNA Base-pairing Probabilities Position Embedding Nucleotide Frequencies Rule Codes
Interaction	Thermodynamic Stability Profile siRNA-mRNA Base-pairing Probabilities

Table 2. Assigned Features for Different Nodes. We show the features assigned to each kind of node. 'mRNA', 'siRNA', and 'Interaction' represent the mRNA node, siRNA node, and the siRNA-mRNA interaction node respectively.

Model Structure

As shown in Fig 1, given the input siRNA and mRNA sequences, our model first extracts sequence characteristics deeply, and then assigns the features to the corresponding GNN nodes for feature fusion and efficacy regression. In the following paragraphs, we introduce the RNA sequence features and model structure in detail.

GNN Node Feature Assignment

To fully grasp the information embedded within mRNA and siRNA sequences, we encompass the initial extraction of **two** types of features from the input RNA sequences and then assign the features to the nodes of our GNN model. **The first type comprises non-empirical rule features**, which include sequence embeddings, positional embeddings, siRNA-mRNA base-pairing probabilities, and RNA-protein interaction probabilities. **The second type is constituted by empirical rule features**, which are thermodynamic stability profile, nucleotide frequencies, G/C percentages, and siRNA rule codes. To further explore the intrinsic properties of mRNA/siRNA and their interaction, we employ advanced methodologies [21, 22, 23, 24] to extract the aforementioned features. These methodologies collectively form the feature extraction phase of our method.

Our model contains three kinds of nodes, i.e., the mRNA nodes, siRNA nodes, and the siRNA-mRNA interaction nodes, which are introduced in Section 2.4 in detail. In Table 2, we show the features assigned to each kind of node respectively. Below, we present the non-empirical rule features and empirical rule features in detail.

For the non-empirical rule features:

- ***Sequence Embedding.*** We employed one-hot encoding to represent biological sequences. Each nucleotide of the siRNA and mRNA sequences, corresponding to the four DNA bases A, C, G, T, is encoded by a unique four-dimensional binary vector: A = <1, 0, 0, 0>, C = <0, 1, 0, 0>, G = <0, 0, 1, 0>, T = <0, 0, 0, 1>. For ambiguous nucleotides, we denoted them as 'N' with a distinct vector <0, 0, 0, 0>.
- ***Positional Embedding of siRNA.*** The positional context of siRNA along the mRNA strand is crucial for its gene silencing efficacy [15]. Each positional information of siRNA along the related mRNA is encoded into a higher-dimensional space of dimension D, then a positional

encoding matrix $\mathbf{P}$ of a dimension $n \times D$ is calculated in an element-wise manner. $\mathbf{P}_{ij}$ is defined as below:

$$\mathrm{P}_{ij} = \begin{cases} \sin\left(\frac{i}{10000^{\frac{2k}{D-1}}}\right), & \text{if } j = 2k \\ \cos\left(\frac{i}{10000^{\frac{2k}{D-1}}}\right), & \text{if } j = 2k+1 \end{cases}$$

Where i denotes the position of siRNA on the mRNA strand and j is the channel dimension.

- ***siRNA-mRNA Base-pairing Probabilities.*** The previous research reports that the efficacy of RNA interference(RNAi) approaches is primarily determined by the sequence of siRNA or the structure of mRNA [25]. Both siRNA and mRNA strands are capable of folding into secondary structures independently, a process largely by nucleotide base pairing including canonical (A-U, C-G), non-Watson-Crick (G-U), and non-canonical interactions, establishes its secondary structure [26]. Previous studies indicated that the base-pairing secondary structure is more crucial for RNA's proper function than the primary sequence itself in many cases [27]. Additionally, siRNA and mRNA can form complexes through intermolecular base pairing. Consequently, we used the RNAfold tool to obtain the base-pairing probability matrix for siRNA and mRNA, and RNAcofold for the siRNA-mRNA interaction base-pairing probability matrix. Both tools are part of the ViennaRNA package [21]. Due to the prevalence of zero probabilities in each matrix, truncated singular value decomposition (SVD) is employed to reduce dimensions and serve as a feature of the model.
- ***RNA-protein Interaction Probabilities.*** In the process of RISC assembly, the guide strand binds to the Argonaut protein AGO2 and directs RISC to its complementary target RNA, which is subsequently cleaved and destroyed by the RNase activity within AGO2 [28]. RNA-protein interations are significant in RNAi process, a factor previously overlooked in earlier studies for siRNA efficacy prediction. RPISeq [22] is a machine learning-based tool designed to predict RNA-protein interactions. It requires sequences of an RNA and a protein as input, and provides interaction probability classifiers through two methods: RPISeq-SVM(Support Vector Machine) and RPISeq-RF (Random Forest). We utilized this tool to predict the interaction between siRNA-AGO2 and mRNA-AGO2, obtaining probabilities using RPISeq-RF as a feature of our model.

For the empirical rule features:

- ***Thermodynamic Stability Profile.*** The prevailing view shows that the thermodynamic stability profile of the siRNA duplex strongly influences the efficacy of siRNA by reflecting a guide strand selection mechanism [29]. The thermodynamic stability profile of the siRNA antisense strand includes the calculation of Watson–Crick pair free energy (ΔG) between every two adjacent nucleotides, the total energy of the siRNA and the difference of energy at the 5' and 3' end of siRNA, all these computations followed the work [23].
- ***Nucleotide Frequencies.*** Certain short motifs can influence the function of siRNA. For instance, sequences containing AAAA or TTTT are likely to be terminated by RNA polymerase III transcriptions, and CCCC or GGGG motifs may impact RNAi function by forming a nucleotide quartet [30]. Nucleotide frequency represents the count of each nucleotide within a siRNA sequence, and it is widely adopted in existing research [31, 32, 9]. Given the constant length of siRNAs, we calculated the frequencies of 1-mer (A, U, C, G), 2-mer (e.g., AU, CG, etc), 3-mer (e.g., AUC, UCG, etc), 4-mer (e.g., AUCG, UCGA, etc) and 5-mer (e.g., AUCGA, UCGUA, etc) segments with 4, 16, 64, 256, 1024 possible motifs, respectively.
- ***G/C Percentages.*** GC content is a critical factor in the efficacy of siRNAs. Low GC content leads to weak and non-specific binding, conversely, high GC content can hinder the unwinding of the siRNA duplex by RSIC complex and helicase [33]. Moreover, the past study indicates that lower GC content in both the global and local flanking regions of siRNA binding sites leads to siRNA inhibition. Thus, we compute the GC content of siRNA and mRNA sequences [29].
- ***Rule Codes of siRNA.*** Previous studies have shown that certain nucleotides at specific positions can either enhance or impair the function of siRNA. Initially, the impact of nucleotides at each position within siRNA is cataloged in the paper [34] and subsequently simplified by He et al [24]. This effort is made to clarify the situation, as different rules explain preferences for specific positions in siRNA. In the simplified rules proposed by He et al., an encoding of 1 indicates a nucleotide's preference for enhancing siRNA efficiency, whereas an encoding of -1 denotes a preference for reducing efficiency. If no rule specifies such a preference, the encoding defaults to 0. Here, we represented these rules using a unique three-dimensional binary vector: 1 = <0, 0, 1>, 0 = <0, 1, 0>, -1 = <1, 0, 0>.

GNN Model of siRNADesign

We model the graph of siRNADesign as an undirected heterogeneous one with three distinct node types, i.e., siRNA, mRNA, and their interactions. We approach efficacy prediction as a node regression task, where outputs from the feature extraction module are aptly assigned to corresponding nodes. The strategy for assigning extracted features is detailed in Table 2. As shown in the Figure 1, $M_j, j \in [1, m]$ and $S_i, i \in [1, s]$ are involved within the constructed graph, and m, s are the total number of mRNA and siRNA respectively. Also, node $I_{S_i}^{M_j}$ appears in the graph, indicating known interaction features to the GNN module. Following [14], we adopt the Heterogeneous GraphSAGE platform [35], which utilizes a dual-layer structure to aggregate each node's feature with features of its neighbors. For any given node v, we utilize $N(v)$ to represent its neighborhood, facilitating the aggregation process. The feature vector of node v at layer l is denoted by $h_v^{(l)}$, while $h_{N(v)}^{(l)}$ represents the aggregated feature vector from its neighborhood, the update rule for node v is formalized as:

$$h_v^{(l+1)} = \sigma\left(W^{(l)} \cdot \mathrm{CONCAT}\left(h_v^{(l)}, h_{N(v)}^{(l)}\right)\right) \tag{1}$$

where $W^{(l)}$ is a layer-specific weight matrix, σ is a non-linear activation function, and CONCAT denotes the concatenation of the node's current features with the aggregated neighborhood features.

Hyperparameter Tuning

To avoid overfitting, we employ Optuna [36], a Bayesian optimization library, for efficient hyperparameter tuning and benchmarking. Optuna is an advanced framework specifically designed to facilitate the automatic exploration

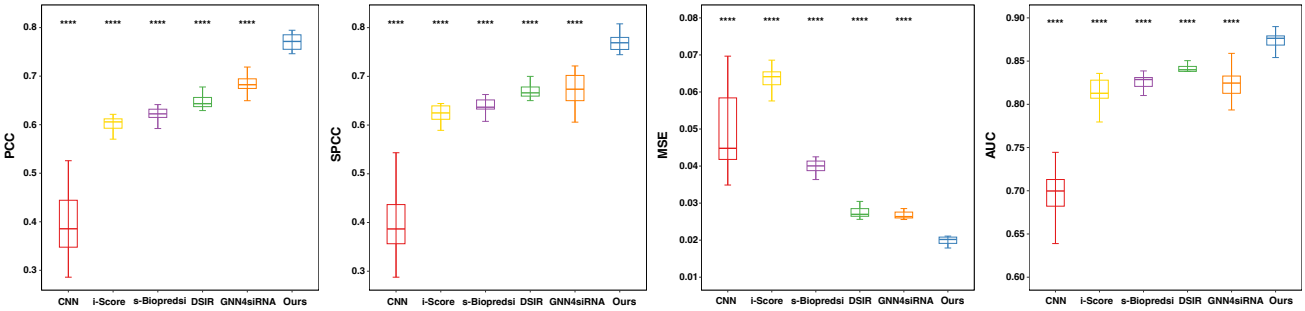

Fig. 2. Performance of siRNADesign on siRNA-split Data (Dataset_HUVK). Our method achieves superior performance and remarkable stability across multiple metrics over other models. PCC: Pearson correlation coefficient; SPCC: Spearman correlation coefficient; AUC: Area Under Curve; MSE: Mean squared error. P values are calculated using paired t-test to compare the siRNA_design with the metric of other models. $* * * * P \leq 0.0001$.

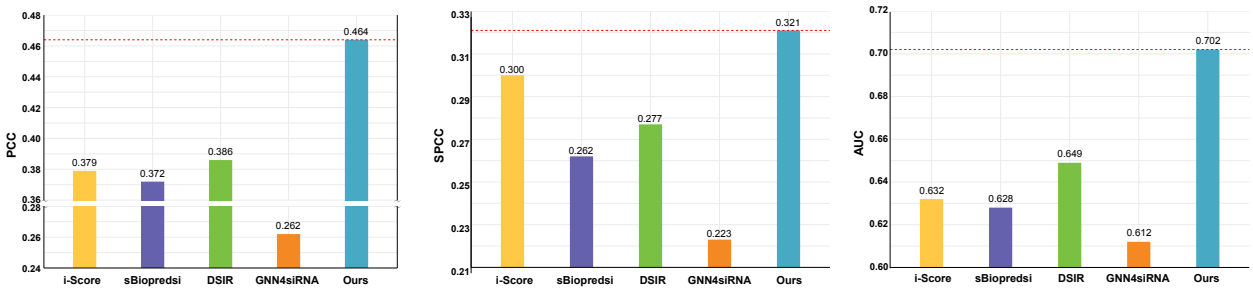

Fig. 3. Performance of siRNADesign on an External Dataset, i.e., Simone [16]. Notably, our siRNADesign significantly surpasses other methods by large performance gains, demonstrating the strong efficacy prediction capacity of our model.

Name	Values
Training Hyperparameter	
Batch Size	64
Learning Rate	1e-3
Loss Function	MSE
Epoch Number	26
Model Hyperparameter	
HinSAGE Layer Size	[64, 32]
Hop Neighbor Sample	[12, 6]
Dropout Rate	0.1
Dim. of Positional Embeddings	6
Dim. of mRNA Reduced Matrices	500
Dim. of siRNA Reduced Matrices	6
Dim. of Pair Reduced Matrices	50

Table 3. Trainging Hyperparameters and Model Hyperparameters of siRNADesign on siRNA-split data. We list the best combination of the training and model hyperparameters among all trails. 'Dim.' denotes feature dimension.

of hyperparameter spaces, aiming to identify the most effective combinations of hyperparameters through a systematic approach. In our study, we perform 100 trials of Bayesian optimization, adopting the Pearson Correlation Coefficient (PCC) as the primary metric to be maximized.

Model Evaluation

To evaluate the efficacy of our model, we employ three key metrics: the Pearson Correlation Coefficient (PCC), the Spearman Correlation Coefficient (SPCC), and the Area Under the receiver operating characteristic Curve (AUC). Both PCC and SPCC serve to measure the correlation between the actual and predicted efficacies, with PCC applied to continuous data and SPCC to ordinal rankings. The formulas for these coefficients are as follows:

$$PCC = \frac{1}{n-1} \sum_{i=1}^{n} \left(\frac{X_i - \bar{X}}{\sigma_X} \right) \left(\frac{Y_i - \bar{Y}}{\sigma_Y} \right), \tag{2}$$

$$SPCC = 1 - \frac{6 \sum d^2}{n\left(n^2 - 1\right)}, \tag{3}$$

where X and Y denotes the predicted values and observed labels, and n is their common size. d represents the difference between the X rank and Y rank for each pair of data. In addition to these correlation coefficients, the AUC metric is employed to measure the overall predictive performance of the model. AUC values range from 0 to 1, where higher values indicate better performance.

Results

Training Settings and Model Settings

We adopt Oputna [36] to conduct hyperparameter tuning. We encompass a series of trials focusing on various parameters, including the sizes of HinSAGE layers, hop neighbor samples, batch sizes, dropout rates, dimensions of positional embeddings, and the dimensions of reduced matrices for both RNA base-pairing probabilities and siRNA-mRNA base-pairing probabilities. The final settings and configurations of

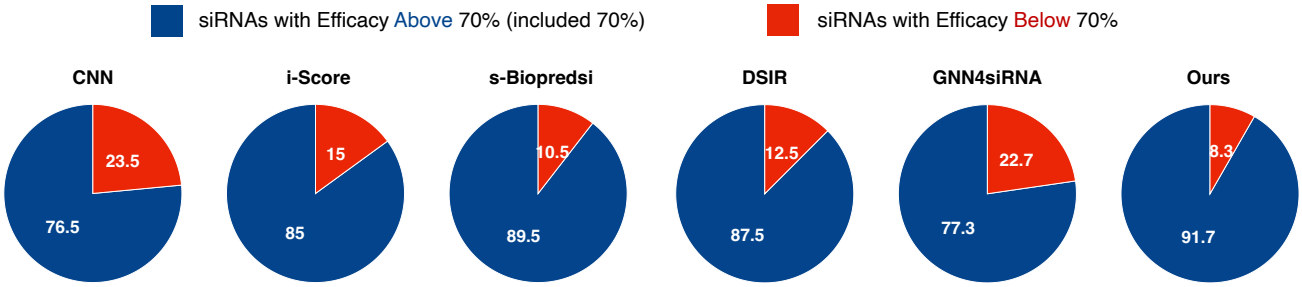

Fig. 4. Proportion of Top-200 siRNAs with True Efficacy Above/Below 70%. We filter the siRNAs from various models based on their top-200 predicted values **trained on siRNA-split data**. We calculate and display the percentage of these siRNAs whose actual efficacy surpasses or falls below the 70% threshold.

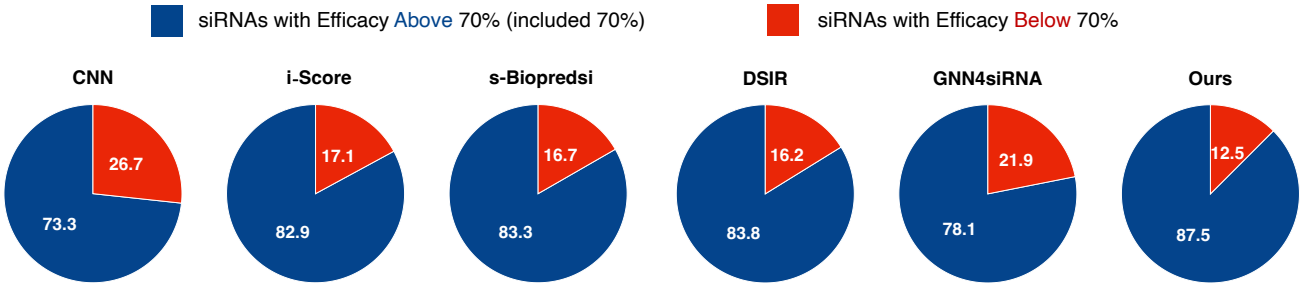

Fig. 5. Proportion of Top-300 siRNAs with True Efficacy Above/Below 70%. We filter the siRNAs from various models based on their top-300 predicted values **trained on siRNA-split data**. We calculate and display the percentage of these siRNAs whose actual efficacy surpasses or falls below the 70% threshold.

siRNADesign on siRNA-split data are comprehensively outlined in Table 3.

Performance

To verify the effectiveness of our proposed method, we compare siRNADesign with five previous works, i.e., GNN4siRNA [14], DSIR [11], s-Biopredsi [8], i-Score [8] and CNN model [10]. We obtain the predicted results of DSIR, s-Biopredsi, and i-Score from the i-Score's website. In addition, we reproduce the results of the CNN model [10] according to the introduction and details in its paper.

Performance on Dataset_HUVK

We conduct experiments and report the performances on siRNA-split data in Figure 2. Experiment results under mRNA-splitting settings are presented in Supplementary Materials. As presented in Section 2.2, to avoid deviation from randomness, we conduct experiments under 10 distinct splits divided under different random seeds and report the average metrics and variance values. As shown in Figure 2, our **siRNADesign outperforms the other five methods with small variation**, reaching an average PCC of 0.770, SPCC of 0.771, MSE of 0.020, and AUC of 0.874. Meanwhile, GNN4siRNA achieves an average PCC of 0.679, SPCC of 0.673, MSE of 0.026, and AUC of 0.821. Results of DSIR, s-Biopredsi and i-Score are worse than GNN4siRNA, while the CNN model reached the worst with an average PCC, SPCC, MES, and AUC of 0.393, 0.399, 0.050, and 0.703, respectively.

Performance on Simone Dataset

Furthermore, we evaluated the effectiveness and robustness of these models on an external dataset, Simone [16], which contains 322 siRNAs. We first train all models on the training set of siRNA-split data and utilize the pre-trained models to conduct inference on Simone. In Figure 3, we show the evaluation results, where our siRNADesign shows the best performance across all metrics, achieving a PCC of 0.464, SPCC of 0.321 and AUC of 0.702. However, the CNN model's predictions exhibited significantly lower performance, with a PPC of -0.017, SPCC of -0.054, and AUC of 0.4999, which are therefore omitted from the figure. It further demonstrates the superior robustness of our method even for external datasets compared with other methods.

Result Analysis for Dataset_HUVK and Simone

The poor performance of DSIR, s-Biopredsi, and i-Score can be attributed to their reliance on traditional machine learning methods, which utilize feature engineering and depend on empirical rules. Furthermore, important features like the location of the siRNA within the mRNA and the information about interactions between the siRNA and mRNA are absent from the GNN4siRNA model. Its capacity to identify underlying patterns among the features is hampered by this omission. Meanwhile, the inferiority of the CNN model may stem from a difference in focus. The convolutional kernels prioritize understanding local information, whereas comprehending RNA sequences requires an exploration of the entire sequence's global content and meaning, as well as the overarching relationship between siRNA and mRNA.

Performance and Analysis on Our In-house Dataset

We evaluate the robustness and generalization of all six models on our in-house dataset. As shown in Fig 6, our siRNADesign outperforms the other models and achieves a PCC of 0.565, SPCC of 0.626 and AUC of 0.815.

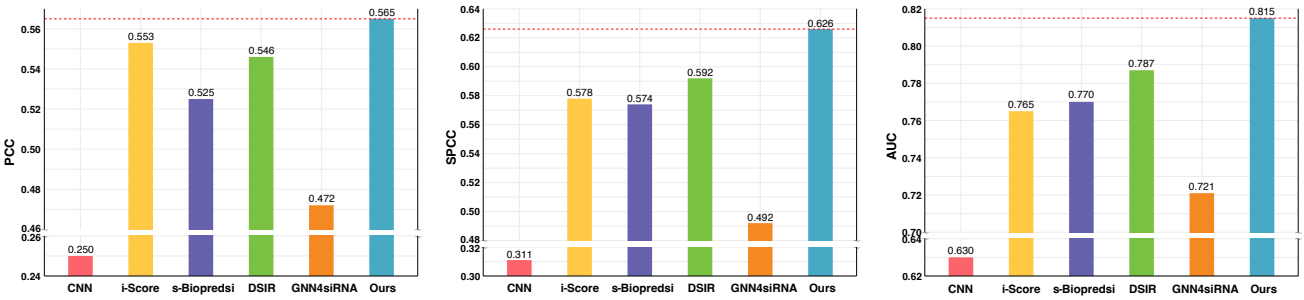

Fig. 6. Performance of siRNADesign on our In-house Dataset. Notably, our siRNADesign significantly surpasses other methods by large performance gains, demonstrating the strong efficacy prediction capacity of our model.

Additionally, due to the limitation on the number of siRNAs, we selected top-ranked siRNAs from predictions of each method. We found that our siRNADesign had the highest proportion of siRNAs with truth efficacy above 70% (included 70%), reaching 91.7% in the top 200 and 87.5% in top 300, respectively shown in Figure 4 and Figure 5.

Ablation study

In our ablation study, we conducted a systematic evaluation to ascertain the impact of various features on performance metrics, specifically focusing on the PCC across eight distinct categories of features on siRNA-split data. These categories encompass sequence embeddings, thermodynamic stability, interaction probabilities, RNA-protein interactions, positional embeddings, nucleotide frequencies, rule-based codes, and G/C content percentages. As illustrated in Figure 4, the integration of all features yielded the highest performance compared to other configurations. We observe a progressive enhancement in PCC as additional features are incrementally incorporated into the model. Notably, the inclusion of nucleotide frequencies resulted in the most significant improvement, with a PCC increase of 0.03.

Discussion

In this section, we delve into further discussions on our design choices, explorations, and directions for future research.

The Importance of Position Embeddings

A significant oversight in many methodologies is the neglect of encoding the position of siRNAs on the mRNA within their models. However, research [37] has demonstrated that the location of siRNA target sites on the mRNA can significantly influence siRNA efficacy, with siRNAs exhibiting higher mismatch tolerance at 3'-UTR locations and greater repression in coding regions when targeted regions are structurally stable and require unraveling during translation processes [38]. Some existing methods have considered siRNAs' positional effects but are limited to simplistic one-hot encoding strategies or sparse-semantic feature matrices with low dimensions. In contrast, our approach, siRNADesign, adopts high-dimensional transformation for positional embeddings, providing effective semantics that enhances the learning capabilities of Graph Neural Networks (GNNs).

Incorporating RNA-AGO2 Interaction Features

We pioneer the inclusion of RNA-AGO2 interaction features to enhance siRNA efficacy prediction. The rationale behind this is that the binding between RNA and AGO2 is a critical component of the siRNA knockdown process. By integrating RNA-AGO2 interaction features, we aim for the model to grasp a more comprehensive understanding of the biological process, thereby improving prediction accuracy. Our future endeavors could explore molecular docking techniques to further consider the binding affinities between nucleic acids and proteins, which could enhance the model's predictions of siRNA knockdown efficiency.

Incorporating RNA Base-pairing Probabilities

We first consider the impact of siRNA-mRNA co-fold binding matrices, along with the self-fold binding matrices of siRNA and mRNA, on the silencing effect of siRNAs. This consideration reflects the influence of both intrinsic and mutual interactions between siRNA and mRNA on silencing efficiency. Future work could explore novel feature engineering techniques, such as employing Convolutional Neural Networks (CNNs) for dimensionality reduction or integrating these features more seamlessly into the model.

Performance on mRNA-split Data

We conduct a rigorous evaluation on mRNA-split data through grid searches and ablation studies, where our mode displays comparable performance to several existing methods and surpasses that of both CNN [10] and GNN algorithms [14]. The results, **shown in Supplementary Materials**, reveal no significant performance disparity between our model and established methods such as DSIR [11] or s-Biopredsi [8], all of which outperform CNN and GNN4siRNA. Notably, our model maintains consistent stability across both siRNA-split and mRNA-split data.

Significance of Proposed siRNA-split and mRNA-split Data Evaluation

As presented in Section 2.2, we address the issue of data leakage observed in previous evaluations, where siRNAs from the test set might inadvertently be included in the training phase, thus undermining the evaluation's integrity. Moreover, we critique the impracticality of employing the same subset for both validation and testing as seen in existing methods. To counter these issues, we propose and will release new data-splitting methodologies based on siRNA and mRNA, previously unconsidered by researchers. This novel approach aims to set a new benchmark for future research, encouraging more rigorous and realistic evaluations.

Sequence Embeddings	Thermodynamic Stability	Interaction Probabilities	RNA-protein Interaction	Positional Embeddings	Nucleotide Frequency	Rule Codes	G/C Percentages	PCC
✓	✓	-	-	-	-	-	-	0.731
✓	✓	✓	-	-	-	-	-	0.735
✓	✓	✓	✓	-	-	-	-	0.736
✓	✓	✓	✓	✓	-	-	-	0.738
✓	✓	✓	✓	✓	✓	-	-	0.768
✓	✓	✓	✓	✓	✓	✓	-	0.769
✓	✓	✓	✓	✓	✓	✓	✓	**0.770**

Table 4. Ablation Study on Different Features Components. We report the PCC of siRNADesign on siRNA-split Data. Clearly, the combination of all the proposed extracted features performs the best.

Directions for Future Work

In the future, we plan to explore the chemical modification of siRNAs and the integration of transformer models and other advanced deep-learning architectures. These avenues hold the promise of further enhancing the predictive accuracy and understanding of siRNA efficacy.

Conclusion

In this paper, we introduce **siRNADesign**, a Graph Neural Network (GNN) framework that marks a significant advancement in the prediction of siRNA efficacy. To alleviate the limitations observed in previous models [14] that lack the comprehensive exploration of RNA sequence and related proteins, our model delves deeply into both the non-empirical and empirical-rules-based features of siRNA and mRNA sequences. In this way, our siRNADesign comprehensively captures the intricate features and semantics of gene silencing and achieves *state-of-the-art* results on all datasets. What's more, to address the critical issue of data leakage and the impracticality of conventional validation methods, we propose an innovative dataset-splitting methodology that enhances the evaluation's integrity and realism. This methodology, by treating siRNA and mRNA as separate entities for dataset division, ensures a robust and unbiased assessment of model performance across various datasets. Extensive experiments, encompassing widely used and external datasets, demonstrate the superiority of our **siRNADesign**, showcasing unparalleled predictive performance and robustness across different experimental settings. We hope our model offers a solid foundation for the development of more reliable predictive models in the field of gene silencing, and hope our proposed dataset-splitting methodology sets a new benchmark for future research, encouraging more rigorous and realistic evaluations.

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