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Deep learning for RNA structure prediction

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Predicting RNA structures from sequences with computational approaches is of vital importance in RNA biology considering the high costs of experimental determination. AI methods have revolutionized this field in recent years, enabling RNA structure prediction with increasingly higher accuracy and efficiency. With an increase in the number of models proposed for this task, this review presents a timely summary of the applications of AI, particularly deep learning, in RNA structure prediction, highlighting their methodology advances as well as the challenges and opportunities for further work in this field.

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Introduction

RNAs' role in a wide range of biological processes, such as transcription regulation and cell signaling, is deeply related to their structures [1]. RNA molecules, represented by their nucleotide sequences (1D), first fold into secondary (2D) structures through base interactions, which then organize into complex functional tertiary (3D) conformations. Various approaches have been developed to experimentally determine RNA structures, such as X-ray crystallography, nuclear magnetic resonance (NMR), cryogenic electron microscopy (cryo-EM) for the tertiary structures, and chemical probing experiments [2] for the secondary structures.

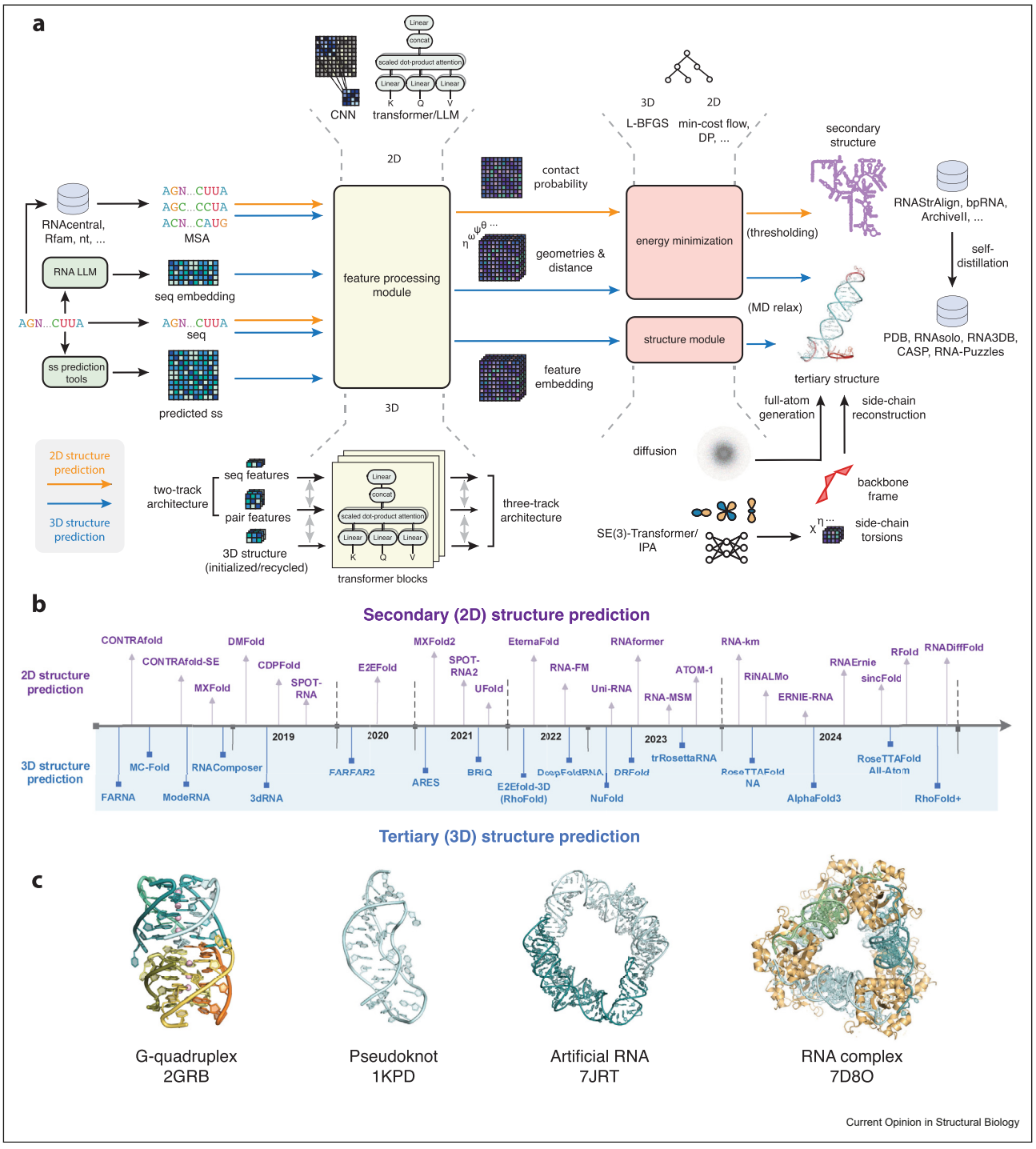
Considering the high costs and resolution limits of experimental methods, computational models have arisen as a promising alternative to determine RNA structures from sequences at scale. However, RNA structure prediction has been a challenging topic. In the early approaches, RNA structures are determined by templated-based modeling, relying mostly on known homologous structures [3]. A complementary approach is conformation sampling with physics- or knowledge-based energy constraints, which could be performed with dynamic programming [4], Monte Carlo sampling [5], fragment assembly [6], and molecular dynamic simulation [7], followed by the sampled structures being filtered with decoy selection methods [8]. Recently, inspired by the success of artificial intelligence (AI), especially deep learning, in many similar biological tasks like protein folding [9], various deep learning methods have been developed to model RNA folding. Some works aim to evaluate the quality of candidate structures folded by others [10], while the majority of methods focus on generating RNA structures directly from sequences.

This review aims to present a timely and comprehensive overview of the recent advances in deep learning methods for RNA structure prediction (Fig. 1b). We first introduce the datasets driving these AI models, followed by summarizing the cutting-edge approaches for predicting both secondary and tertiary structures. Lastly, we discuss the frontier opportunities as well as challenges that lie ahead for AI-based RNA structure prediction methods. For additional technical details and progress on experimental approaches for structure determination, we recommend the respective method papers and some other recent review articles [11,12].

Structure datasets driving the machine learning-based RNA folding models

The development of machine learning-based RNA structure prediction methods heavily relies on the availability of high-quality datasets for training and evaluation at all 1D, 2D, and 3D levels (Table 1). Community-wide competitions, including Critical Assessment of Structure Prediction (CASP) [13] and RNA-Puzzles [14], provide new RNA targets for blind tests, but they are typically limited in their sizes.

Figure 1



Overview of the progress of deep learning for RNA structure prediction. **(a)**. Pipelines and datasets of recent AI methods for RNA structure prediction. Shorthand *ss* stands for secondary structure. **(b)**. Timeline of progress in RNA structure prediction including AI methods and representative traditional methods. **(c)**. Examples (with PDB IDs) of some challenging structures for AI-based RNA structure prediction methods.

Protein Data Bank (PDB) [15] currently contains the most experimentally determined RNA structures, which are commonly used for training both 2D and 3D structure prediction models. Recently, curated datasets, such as RNAsolo series [16], and RNA3DB [17], have been introduced, containing cleaned and clustered RNA structures from PDB and the related BGSU representative set [18], enabling more rigorous and reproducible

Table 1

Datasets and resources used by recent AI methods for RNA secondary (2D) and tertiary (3D) structure prediction as of 2024−11-27. *Size* refers to the number of structures in the datasets, with the numbers in the parentheses indicating the number of representative samples. *Structure source* refers to whether the structures in the datasets are obtained with experimental approaches, comparative analysis, computational prediction, or a mixed approach; we also mark in the parenthesis whether this database is derived from another. *Purpose* refers to whether the datasets are mainly used for training or testing in practice by the AI-based methods for RNA structure prediction in the literature. *Redundancy removal criteria* refers to any control measure to remove redundant structures during data curation, such as sequence similarity (*seq sim*) and structure similarity (*structure sim*) controls.

Name	Type	Size	Last updated	Structure source	Purpose	Redundancy removal criteria
RNAcentral	1D	35,400,760	2024	–	train	–
nt	1D	~96,000,000	2024	–	train	–
RNAStrAlign	2D	30,451	2017	*mostly* comparative analysis	train/test	–
bpRNA-1m	2D	102,318	2018	*mostly* comparative analysis	train/test	seq sim
bpRNA-new	2D	5,401	2021	*mostly* comparative analysis (from Rfam)	train/test	seq sim
ArchiveII	2D	3,975	2022	*mostly* comparative analysis	test	–
Rfam	2D	20,322,187 (4,178 families)	2024	*mostly* comparative analysis	train/test	–
CASP15	3D	13	2022	experimental	test	–
RNA-Puzzles	3D	42	2023	experimental	test	–
PDB	3D	23,021	2024	experimental	train/test	–
BGSU representative set	3D	18,580	2024	experimental (from PDB)	train/test	seq sim, structure sim
RNAsolo	3D	16,208 (3,622 classes)	2024	experimental (from BGSU)	train/test	–
RNAsolo2	3D	16,171 (3,475 classes)	2024	experimental (from BGSU)	train/test	–
RNA3DB	3D	1,720 (118 components)	2024	experimental (from PDB)	train/test	seq sim, structure sim
CASP16	3D	70	2024	experimental	test	–

benchmarking. For 2D structures, besides extracting secondary base pairing from experimentally determined 3D structures, there are also datasets like RNAStrAlign [19], ArchiveII [20], bpRNA-1m [21], and bpRNA-new [22] that are annotated mainly based on comparative modeling.

Currently, the amount of experimentally determined RNA 3D structures is still limited compared with that for proteins. As a countermeasure to data scarcity, many methods [23−27] have adopted a self-distillation set, where the model trained with experimentally determined structures is used to predict structures from large sequence databases like bpRNA-1m [21], and high-confidence predictions are used to augment the training data. Some methods [24,28−32] have also incorporated RNA language models pre-trained on large-scale sequence-only databases without structure information [33,34] as an enhancement (Table 1). However, as the field advances, there is a growing need for larger and more diverse high-quality RNA structure datasets, which could be a potential limiting factor for the development of machine learning-based structure prediction models, especially for those deep learning methods with high model capacity.

Advances in deep learning methods for RNA structure prediction

Predicting RNA secondary structures with deep learning

Current secondary structure prediction models typically predict the contact probabilities or folding scores with a deep learning module, then further optimize them for the final structure (Table 2). Due to the 2D image-like structure of contact maps, most methods (*e.g.* refs. [22,35−37]) adopt convolutional neural networks (CNNs) to predict the sparse RNA structures. To further improve the structure prediction accuracy, there has been a growing trend of integrating CNNs with transformer-based pre-trained RNA language models in the prediction modules, such as RNA-FM [29] and RNAErnie [38]. After the deep learning modules predict the contacts, methods generally apply either a simple probability threshold [36,39,40] or various optimization algorithms like dynamic programming [22,38,41,42] and minimum-cost flow [28,32] to

Table 2

Summary of AI methods in RNA secondary structure prediction. The model can receive sequence (*seq*), MSA, or initially processed pairing information (*pair*), and output either contact probability (*contact prob*) or folding scores. *Post-processing* records the method for converting the AI model output to the final secondary structure, where *cutoff* represents directly applying a threshold to the contact probability or distance map; *DP* represents applying dynamic programming optimization. For the *Loss* column, *CE* stands for cross-entropy loss; *SSVM* stands for structured support vector machine; *diffusion* refers to the standard diffusion loss [43]. Model size is measured by the number of parameters.

Name	AI model input	AI model output	Prediction module	Post-processing	Loss	Model size
SPOT-RNA [35]	seq	contact prob	CNN + LSTM	cutoff	CE	10^6
DMfold [41]	seq	contact prob	LSTM + FCN	DP	CE	10^6
CDPfold [42]	seq, pair	contact prob	CNN	DP	CE	10^5
E2Efold [36]	seq	contact prob	Transfomer + CNN	cutoff	geometry constraint	10^5
MXfold2 [22]	seq	folding scores	LSTM + CNN	DP	SSVM	10^5
SPOT-RNA2 [44]	seq, MSA	contact prob	CNN	cutoff	CE	10^6
UFold [37]	seq, pair	contact prob	CNN	constrained optimization	CE	10^6
GCNfold [39]	seq	contact prob	GCN + CNN	cutoff	CE	10^6
Sincfold [40]	seq	contact prob	CNN	cutoff	CE, ℓ_1 loss	10^6
KnotFold [28]	seq	contact prob	Transfomer + FCN	min-cost flow	CE	10^6
RNA-FM [29]	seq	contact prob	Transfomer + CNN	cutoff	CE	10^7
RNA-MSM [30]	seq, MSA	contact prob	Transfomer + CNN	cutoff	CE	10^7
UNI-RNA [31]	seq	contact prob	Transfomer + CNN	cutoff	CE	10^8
RNAErnie [38]	seq	folding scores	Transfomer + CNN	DP	CE	10^7
RNA-km [32]	seq	contact prob	Transfomer + FCN	min-cost flow	CE	10^8
RNADiffFold [43]	seq	contact prob	Transformer + diffusion	cutoff	diffusion	10^7

produce the contact map representation of the 2D structure.

Predicting structure distance and geometries with deep learning

RNAs' nucleotide pairwise distances and 3D spatial geometries, such as orientations, are important constraints for their 3D structures. Traditional optimization methods have been well established for modeling RNA structures using the energy function derived from those features. Consequently, many deep learning methods have been proposed to predict those distance and geometry features for RNA structure modeling.

Here, these 2D distance and 3D geometries can be inferred with a deep learning model, and 3D backbones can then be solved using them as constraints (Table 3). For instance, DeepFoldRNA [27] first predicts the distance maps and geometries like torsions and orientations with transformers; then it converts those geometries into energy potentials and adopts L-BFGS to optimize backbone torsion angles for constructing the final structure. Similarly, trRosettaRNA [25] also predicts geometries and generates full-atom coordinates based on them. Another method, DRFold [45], uses two deep learning networks to predict geometry and local frames and then optimizes their combined energy potential for the final structure. Despite their focus on distance and geometry prediction, these methods generally adopt feature processing modules similar to AlphaFold2 [9]. Additionally, while not differentiable end-to-end, predicting distance and geometries allows for the incorporation of physics-based constraints and prior knowledge about RNA structure, leading to more physically realistic and biologically plausible predictions.

Optimizing RNA 3D structures end-to-end

Many current RNA 3D structure prediction methods, including the RhoFold series [23,24] and RoseTTAFold (RF) series [46,47], model RNA folding as inspired by AlphaFold2 [9]. In contrast to modeling the distance and geometry constraints, these methods output torsion-based parametrization of the structure with fully differentiable end-to-end pipelines (Table 3). These modern methods typically use sophisticated architectures like graph neural networks and attention layers for feature processing. Additionally, these models are invariant to rotation and translation by considering the equivariance of RNA 3D structures in their structure modules.

Table 3

Recent AI methods for RNA 3D folding. *AI input* and *AI output* refer to the direct input and output of the AI model, where *seq* represents sequence and *ss* represents secondary structure. *Structure module* refers to the model that outputs the final structure representation; *IPA* stands for invariant point attention. In the *Post-processing* column, *Reconstruction* refers to building full-atom structures from backbone frames and torsions; *relaxation* refers to the use of molecular dynamics optimization. *Loss* records the type of objective functions used by the methods on RNA data, where *1D* refers to sequence-level objectives such as MSA recovery loss, *2D* refers to secondary structure-related objectives such as distance and base pairing, *3D* refers to tertiary structure-related objectives such as 3D structure alignment, measured by frame-aligned point error (FAPE) [9] or mean square error (MSE) [26], and various orientation angles, and *Confidence* refers to other objectives related to confidence estimation, including predicted local distance difference test (pLDDT) scores, pairwise atom−atom aligned error (PAE), predicted distance error (PDE), and if the atom is experimentally resolved in the training data (resolved) [26]. Of note, some methods like RFNA [46] do not apply certain loss terms when modeling RNA structures. *CASP15-RNA RMSD* shows the average RMSD with standard deviations shown in the parenthesis for each method when applied on six CASP15 natural single-chain RNA targets; methods without inference code or currently does not support single-chain RNA prediction are marked as empty; results were retrieved from CASP15 official data [13] and more recent papers' benchmarks [24,25]. *Complex modeling* records whether the method is capable of predicting RNA complex structures.

Name	End-to-end	Model size	AI model input	Structure module	AI model output	Post-processing	Loss				CASP15-RNA RMSD (Å)	Complex modeling
							1D	2D	3D	Confidence		
E2Efold-3D (RhoFold) [23]	Yes	10^8	seq, MSA	IPA	backbone frame, torsion	reconstruction, relaxation	MSA recovery	distance, base pair	FAPE, clash violation	pLDDT	8.62 ± 5.53	No
DeepFoldRNA [27]	No	10^7	seq, MSA, ss	–	3D geometry	L-BFGS	MSA recovery	–	FAPE, planar angles	–	9.25 ± 4.96	No
DRFold [45]	No	10^7	seq, ss	IPA	backbone coord.	Arena, relaxation	–	distance	FAPE, torsion angles	–	13.70 ± 6.90	No
trRosettaRNA [25]	No	10^7	seq, MSA, ss	–	distance, base pairing, 3D geometry	pyRosetta	–	distance, base pair	planar angles, torsion angles	–	11.85 ± 5.58	No
RoseTTAFold-NA [46]	Yes	10^7	seq, MSA	SE(3)-Transformer	backbone frame, torsion	reconstruction	–	–	FAPE, planar angles, bond geometry	pLDDT	15.41 ± 7.82	Yes
RoseTTAFold All-Atom [47]	Yes	10^7	seq	SE(3)-Transformer	backbone frame, torsion	reconstruction	–	–	FAPE, planar angles, bond geometry	pLDDT	–	Yes
AlphaFold3 [26]	Yes	10^8	seq	diffusion	full atom coord.	–	–	distance	Structure alignment, bond length	pLDDT, PAE, PDE, resolved	11.03 ± 7.25	Yes
RhoFold+ [24]	Yes	10^8	seq, MSA	IPA	backbone frame, torsion	reconstruction, relaxation	MSA recovery	distance, base pair	FAPE, clash violation	pLDDT	7.74 ± 5.23	No

E2Efold-3D (RhoFold) [23] was the first to use a similar architecture as AlphaFold2 [9] and ranked the best among deep learning methods in the CASP15 competition [13,26], proving the effectiveness of transferring the AlphaFold2 model architecture to RNA structure prediction. Notably, it performs well on natural RNA targets, but current deep learning methods, including RhoFold, typically have a reduced performance on artificial RNAs. In addition to the sequence and multiple sequence alignment (MSA) input, RhoFold also incorporates a large language model that enhances the representation and implicitly augments the training dataset. Its successor, RhoFold+ [24], further improves the prediction by enabling clustering and sampling from the input MSAs to allow for potentially multi-conformational predictions of the same query sequence.

RoseTTAFoldNA (RFNA) [46] adopts a relatively different approach to the RhoFold series [23,24], following their previous work on protein folding [48]. While the RhoFold series uses a two-track architecture that operates on the extracted 1D and 2D features, RFNA enhances it to three tracks by simultaneously updating representations at 1D, 2D, and 3D levels, which are then integrated with an SE(3)-Transformer to predict the final 3D structure (Fig. 1a). RoseTTAFold All-Atom (RFAA) [47] inherits this three-track model and further includes the prediction of ions and small molecules. Yet, in terms of model architecture, it is essentially the same as RFNA.

Recently, AlphaFold3 [26] has extended its predecessor work [9] in protein folding to predict the structures of all biomolecules. Compared with AlphaFold2, one of the key innovations in AlphaFold3 is to directly model the positions of all atoms while discarding the equivariance structural module employed by most of the other end-to-end models today.

Diffusion models for predicting RNA structures

AlphaFold3 [26] implements the aforementioned direct modeling of all atoms with a diffusion model. Diffusion models, which have shown remarkable performance in generating high-quality images and audio, are generative models trained to model a data distribution by learning a denoising process. In the context of RNA folding, the diffusion model characterizes the RNA conformation distribution at atomic level. As a result, AlphaFold3 directly outputs full-atom positions, eliminating the need for the equivariant frame prediction plus side-chain reconstruction procedure that has been widely adopted by other methods. Moreover, as the model captures RNA's structure distribution, this framework is able to generate diverse and realistic samples, potentially enabling the prediction of multiple conformations and dynamic behavior of RNA molecules. For secondary structures, RNADiffFold [43] adopts the standard diffusion scheme conditioned on the sequence information and embeddings from an RNA language model [29]. As the diffusion framework has been first applied to RNA structure modeling, its performance on challenging targets like non-canonical base pairing, pseudoknots, and complexes could potentially be limited. We anticipate further exploration of diffusion models in more future works on RNA structure prediction.

Enhancing RNA structure prediction with additional information

Incorporating evolutionary information for structure prediction

Evolutionary information derived from MSAs has been shown to significantly improve the accuracy of RNA structure prediction. Recent methods like RhoFold [23] and RhoFold+ [24], DeepFoldRNA [27], and trRosettaRNA [25] incorporate MSAs as additional inputs to their deep learning models. These raw MSAs are typically generated using tools like rMSA [49] from large RNA sequence databases like Rfam [50] and RNAcentral [33], then processed through clustering, ranking, stacking, and encoding steps. The encoded MSAs provide valuable co-evolutionary information that helps the models better capture long—range interactions and conserved structural motifs.

MSAs have also been used to improve RNA secondary structure prediction. For instance, SPOT-RNA2 [44] extends the original SPOT-RNA [35] by incorporating MSA-derived features such as position-specific scoring matrix (PSSM) and direct coupling analysis (DCA) alongside the sequence information. These additional evolutionary features have been shown to enhance the prediction accuracy, particularly for sequences with deep alignments. Another example is RNA-MSM [30], which employs a transformer-based language model pretrained on MSAs to generate rich sequence embeddings. These embeddings are then used as inputs to a convolutional neural network for predicting base-pairing probabilities.

Compared with methods that rely solely on single sequences, the incorporation of MSAs can provide valuable additional information for the prediction of the RNA structure. However, quickly generating high-quality MSAs remains a challenge, particularly for novel or divergent RNA sequences. Many of the current state-of-the-art 3D structure prediction methods, such as RhoFold+ [24] and RFNA [46], were inspired by AlphaFold2 [9], which heavily relies on MSA representation and processing. The more recent AlphaFold3 [26], however, significantly reduces the role of MSAs and combines their processing with other features like pair representation. This shift in approach may be attributed to the lack of rich alignments for many RNA sequences and the need to process a wider range of

biomolecules like ligands without MSAs. Nonetheless, despite the reduced emphasis, AlphaFold3 still leverages MSA information to achieve high-accuracy predictions. Based on current empirical evidence, MSAs remain important for RNA structure modeling, and it is not yet fully clear whether AlphaFold3 can maintain comparable accuracy without substantial MSA input. We anticipate future methods could further investigate the relationship between models' performance and their dependency on MSAs, as well as explore novel ways to integrate evolutionary information while predicting RNA complex structures.

Large language models for structure prediction

As ESMFold first demonstrated the biological large language model's success in modeling protein structures [51], RNA language models have also been increasingly developed and applied for structure generation. For RNA secondary structures, methods like RNA-FM [29] and RNA-MSM [30] have demonstrated the power of integrating such large language models with conventional deep learning modules like CNNs. Typically, these models are first pre-trained on large-scale unlabeled sequence datasets and then fine-tuned alongside the prediction modules for the structure prediction objective. Notably, although RNA-FM was pre-trained on non-coding RNAs while RNA-MSM was on MSA data, they can both generate informative sequence embeddings that can be further processed by downstream modules like CNNs or be used for conditional generation in diffusion frameworks [43].

Meanwhile, pre-trained models have also been used for 3D structure prediction. RhoFold [23] and RhoFold+ [24] both adopt the RNA foundation model, RNA-FM [29], to implicitly augment the scarce RNA 3D structure training data. This approach has led to significant improvements in structure prediction accuracy, particularly for sequences with limited homology, complementing the shallow MSAs. In contrast to secondary structure prediction, the RNA-FM model here is not fine-tuned together with the rest of the network, possibly due to the limitations of computational resources. Future work could further enhance the incorporation of language models like RNA-FM into the structure prediction pipeline, improving the folding accuracy especially for novel RNAs. As the field progresses, we anticipate that RNA language models will play an increasingly important role in both secondary and tertiary structure prediction.

Frontiers in AI-based RNA structure prediction

Predicting RNA complex structures

Current state-of-the-art AI methods for RNA structure prediction primarily focus on folding individual RNA sequences. However, RNA molecules frequently form complexes with other macro-biomolecules, ligands, and ions, which can alter the RNAs' isolated conformations. The inclusion of RNA–protein, RNA–nucleic acid, and RNA–ligand complexes in the recent CASP16 competition also highlights the necessity for AI approaches that can predict the structures of RNA-containing complexes.

Building upon the success of single-sequence structure prediction methods, several recent works have begun exploring the computational modeling of RNA complex structures. The RF series, including RFNA [46] and RFAA [47], have demonstrated the capability of predicting the structures of RNA-protein complexes. RFNA extends the protein-centric three-track RF architecture [48] to handle RNA components by also incorporating relevant RNA-specific features, while RFAA further involves small molecule inputs to the three-track model, enabling the prediction of ligand-involving compound structures.

AlphaFold3 [26] is also capable of generating structures for a diverse range of biomolecules, including RNA, protein, and ligands. Its diffusion module generates the full-atom structures of different compounds together conditioned on the input and processed representations of each component. Future research could build upon these efforts to further improve the prediction performance on RNA complexes and develop more comprehensive frameworks to predict the structure of a wider range of biomolecules beyond RNA.

RNA structure prediction for *de novo* RNA design

RNA structure prediction and structure-based RNA design (*i.e.*, inverse folding) are complementary reverse processes. Accurately predicting the structures of RNA molecules from their sequences can enable the rational design of many functional RNA devices, such as RNA aptamers, small interfering RNAs (siRNAs), and guide RNAs. In the most straightforward case, accurate predictions of 3D structures can be leveraged to enhance the training dataset for structure-based RNA design models, as several recent design methods [52,53] have adopted the RhoFold series [23,24] for data augmentation.

Deep learning methods for structure prediction can also guide the rational design of those functional RNAs by controlling the sampling process from the structure space. For example, diffusion models offer an iterative sampling framework where a pre-trained, accurate structure prediction method can constrain sequence sampling, enabling the joint optimization of both sequence and structure to design protein-binding RNAs [54].

In addition, accurate AI-based structure prediction methods can be used to facilitate the development of design methods. For instance, in protein engineering, RFdiffusion All-Atom [47] has been proposed based on the RFAA structure generation model [47] to design functional proteins as small molecule binders, which could potentially inspire the development of similar RNA-centric design frameworks by adapting and reversing the current RNA structure prediction models.

Despite the rapid advance of deep learning methods for RNA structure prediction, novel and artificially designed sequences remain a challenging type of targets for current AI-based methods to predict, limiting their applicability for *de novo* design efforts. Moreover, experimental verification of these structure-based designs remains a crucial step [52,55]. As a result, future works could further enhance the generalizability of the structure prediction models and enable iterative design refinement, therefore unlocking the full potential of structure-based RNA design and accelerating the development of RNA therapeutics.

Challenges of current AI methods for RNA structure prediction

Generalizability

As AI models for RNA structure prediction aim to approach experimental accuracy, generalizability remains a significant challenge for these methods, as they are prone to overfitting the training data, which can limit their ability to accurately predict structures for novel sequences. Additionally, compared with protein structure modeling, the scarce availability of RNA 3D structures also reduces the robustness of their prediction models. Below we highlight some of the generalization aspects that are challenging current RNA structure prediction models.

Sequence and structure identity

Sequence and structure identity is the most general requirement for generalizability. Such capability can be evaluated by applying multiple sequence or structure identity cutoffs to control the level of similarity between the datasets, demonstrating the model's performance change with respect to different similarity thresholds as low as 50 % identity in some works [24].

Target release time

Target release time is another perspective on generalizability, which evaluates the model's performance on structures that are experimentally resolved after the training is complete. This can be achieved by joining community-wide challenges like CASP or conducting a train-test split based on the release time of the target structures. Despite their small data sizes, this approach can ensure that the model has not seen these structures during training, delivering a rigorous evaluation of its generalizability.

Family and type

As RNAs can be categorized into families and types, some models have been tested for the ability to generalize to new families or types by holding out certain RNA families or types during training for testing purposes. This can be an important perspective as the majority of the PDB dataset consists of riboswitches, rRNAs, and tRNAs, leading to many models underperforming on other small but important families, such as introns and CRISPR RNAs, compromising the model's robustness.

Sequence length

Furthermore, evaluating how the performance of AI methods correlates with the query sequence length can provide insights into their generalization capabilities. As it is more challenging to experimentally determine the structures of longer RNA sequences, there has also been an imbalance of the sequence length distribution, leading to weaker performance on long sequences. Additionally, the model's processing of long targets could also be affected by the computation resources available, whose demand grows quickly with increasing sequence length and could be a limiting factor to the performance.

Challenging structures

Compared with proteins, RNA structures can display more complex topologies, which challenge the computational modeling of them at both 2D and 3D levels.

Challenging substructures

At substructure level, two representative and commonly studied examples are G-quadruplexes, which involve non-canonical base pairing, base stacking, and interaction with ions, and pseudoknots, which form non-nested base pairings (Fig. 1c). The models can be assessed on those challenging substructures in terms of both global accuracy of samples containing these motifs and local accuracy of the specific regions using metrics like local distance difference test (LDDT).

Artificial RNAs

The accurate prediction of artificial RNAs has proven to be even more challenging due to their possible novel structure motifs different from training data or lack of MSA. In the CASP15 competition, while many AI-based methods performed well on natural RNA targets, their accuracy for artificial ones remained significantly lower than human experts (Fig. 1c).

RNA complexes and conformational flexibility

As discussed, RNAs frequently form complexes, which requires modeling different molecules' individual

folding mechanisms as well as their full interaction dynamics, making it inherently more challenging than modeling single sequences alone. Moreover, certain RNAs, like riboswitches, can transition between multiple conformational states in response to environmental changes (Fig. 1c). While recent diffusion-based methods like AlphaFold3 [26] offer opportunities by learning the conformational distribution, current AI methods still struggle to effectively model this inherent flexibility.

Essentially, RNA's complex and flexible conformations require the models to search through a more extensive conformational space more efficiently, so more robust models could be developed to address this problem with additional information, such as secondary structure constraints, and more carefully designed modules to process these inputs. Additionally, expanding the training datasets to include a greater diversity of complex RNA structures and artificial sequences may help improve the generalization capabilities of AI-based methods in handling these challenging cases.

Conclusion

AI methods have demonstrated remarkable accuracy and efficiency in both secondary and tertiary structure prediction and have been gradually approaching experimental accuracy. The integration of evolutionary information, large language models, and diffusion-based generative models into the prediction pipeline has further extended the capabilities of current RNA structure prediction methods. While challenges remain, with continually evolving model architectures and increasingly available data, AI-based RNA structure prediction methods will not only accelerate our understanding of RNA biology but also open up new opportunities for rational RNA design, paving the way for novel RNA therapeutics development and biotechnology applications.

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

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

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