m6AGE: A Predictor for N6-Methyladenosine Sites Identification Utilizing Sequence Characteristics and Graph Embedding-Based Geometrical Information

Yan Wang¹², Rui Guo¹, Lan Huang¹, Sen Yang¹*, Xuemei Hu¹ and Kai He¹

¹ Key Laboratory of Symbol Computation and Knowledge Engineering of Ministry of Education, and College of Computer Science and Technology, Jilin University, Changchun, China, ² School of Artificial Intelligence, Jilin University, Changchun, China

N⁶-methyladenosine (m⁶A) is one of the most prevalent RNA post-transcriptional modifications and is involved in various vital biological processes such as mRNA splicing, exporting, stability, and so on. Identifying m⁶A sites contributes to understanding the functional mechanism and biological significance of m⁶A. The existing biological experimental methods for identifying m⁶A sites are time-consuming and costly. Thus, developing a high confidence computational method is significant to explore m⁶A intrinsic characters. In this study, we propose a predictor called m6AGE which utilizes sequence-derived and graph embedding features. To the best of our knowledge, our predictor is the first to combine sequence-derived features and graph embeddings for m⁶A site prediction. Comparison results show that our proposed predictor achieved the best performance compared with other predictors on four public datasets across three species. On the A101 dataset, our predictor outperformed 1.34% (accuracy), 0.0227 (Matthew’s correlation coefficient), 5.63% (specificity), and 0.0081 (AUC) than comparing predictors, which indicates that m6AGE is a useful tool for m⁶A site prediction. The source code of m6AGE is available at https://github.com/bokunoBike/m6AGE.

Keywords: m⁶A, machine learning, graph embedding, feature fusion, CatBoost

INTRODUCTION

N⁶-methyladenosine (m⁶A) is one of the most prevalent RNA post-transcriptional modifications. It was first found in mammalian RNA in 1974 (Desrosiers et al., 1974). Subsequently, m⁶A modification was observed in various species, such as Saccharomyces cerevisiae (Schwartz et al., 2013), Arabidopsis (Luo et al., 2014), humans and mouse (Dominissini et al., 2012). Research shows that m⁶A sites are enriched in long internal exons and 3’UTRs around stop codons rather
than randomly distributed in the genome (Dominissini et al., 2012; Meyer et al., 2012; Wan et al., 2015). It has been reported that m6A modification is associated with many biological processes, including but not limited to protein translation and localization (Meyer and Jaffrey, 2014), mRNA splicing and stability (Nilsen, 2014), RNA localization and degradation (Meyer and Jaffrey, 2014). Therefore, precisely identifying m6A sites contributes to understanding the regulatory mechanism and biological significance of m6A modification.

High-throughput techniques have enabled locating the m6A sites in genomes. MeRIP-Seq (or m6A-Seq), a combination of immunoprecipitation and next-generation sequencing technology, has successfully mapped m6A in several species genomes (Dominissini et al., 2012; Schwartz et al., 2013; Wan et al., 2015). In 2015, Chen et al. developed photo-crosslinking-assisted m6A-sequencing (PA-m6A-seq) which produced a high-resolution (about 23nt) mammalian map (Chen et al., 2015a). MeRIP-Seq and PA-m6A-seq can only locate the high methylation regions of m6A rather than the exact positions. In the same year, Linder produced a single-nucleotide resolution map of m6A sites using a new technology termed miCLIP (Linder et al., 2015). However, the current experimental methods face a lot of limitations and expensive costs. With the rapid development of computational methods, it is possible to use machine learning algorithms to predict m6A. Hence, building advanced models to predict m6A sites is significant for the following research of m6A.

Chen et al. (2015b) proposed the first predictor named iRNA-Methyl for m6A sites in Saccharomyces cerevisiae, using three physical-chemical properties of dinucleotide and SVM classifier. WHISTLE (Chen et al., 2019) integrates genomic features besides the sequence features to train a predictor with SVM classifier. Liu and Chen (2020) developed a computational method called iMRM for detecting different RNA modifications simultaneously with XGBoost classifier. Recently, deep learning methods show better performance trend in bioinformatics problems. DeepM6Aseq (Zhang and Hamada, 2018), BERMP (Huang et al., 2018), Gene2vec (Zou et al., 2019), DeepPromise (Chen et al., 2020), and im6A-TS-CNN (Liu et al., 2020) establish deep learning frameworks by using convolutional neural network (CNN) layers and gated recurrent unit (GRU) to seek the m6A sites on DNA/RNA sequence level on the same dataset as SRAMP (Zhou et al., 2016). In this study, seven kinds of sequence-derived features are employed to encode RNA sequences, including CTD (Tong and Liu, 2019), Pseudo k-tuple Composition (PseKNC) (Guo et al., 2014), nucleotide pair spectrum (NPS) (Zhou et al., 2016), nucleotide pair position specificity (NPPS) (Xing et al., 2017), nucleotide chemical properties and density (NCP-ND) (Golam Bari et al., 2013), electron-ion interaction pseudopotentials (EIIP) (Nair and Sreenadhan, 2006), and bi-profile Bayes (BBP) (Shao et al., 2009). Besides, graph embedding methods are innovatively introduced to distill the potential structure information. Firstly, a network is constructed by mapping each sample of the dataset to a node. Secondly, the three graph embedding methods SocDim (Tang and Liu, 2009), Node2Vec (Grover and Leskovec, 2016), and GraRep (Cao et al., 2015) are used to learn the distributed representation of the sample in an unsupervised manner. At last, all the feature vectors are merged as the input of model. The predictive results show that m6AGE improves the performance of identifying m6A sites.

**MATERIALS AND METHODS**

**Datasets**

The m6A sites of different species share different consensus motifs. The adenosines lying within the consensus motif are considered to be the potential methylation sites. The samples in the dataset are RNA sequence segments with the potential methylation sites at their center. The samples with the m6A sites experimentally annotated are put into the positive dataset, whereas the other samples are put into the negative dataset.

There have been many datasets across multiple species for training m6A site predictors. We have collected four datasets that involve three species: Saccharomyces cerevisiae, Arabidopsis thaliana, and human. The following are details of these datasets.

**A101.** Wang extracted *A.thaliana* m6A sites from the m6A peak data of Luo et al. (2014) and Wan et al. (2015). The dataset (Wang and Yan, 2018) Wang built contains 2,518 positive samples and 2,518 negative samples. Every sample in the dataset is a 101nt RNA sequence segment.

**A25.** Luo obtained 4,317 m6A peaks detected both in Can-0 and Hen-16 strains. After removing the sequences with more than 60% sequence similarity, Chen et al. (2016) obtained 394 positive samples. The same number of negative samples were selected randomly from sequences without the m6A site. The length of every sample is 25nt.

**S21.** Chen further constructed this dataset (Chen et al., 2015c) based on the previous work (Chen et al., 2015b). They selected 832 RNA sequence segments as the positive samples in the training set whose distances to the m6A-seq peaks are less than 10nt. Then, 832 of 33,280 RNA sequence segments with non-methylated adenosines were selected randomly as negative samples in the training set. The rest 475 RNA sequences with methylated adenine and 4750 of 33,280 RNA sequences with non-methylated adenine constitute the independent testing dataset. The length of every sample is 21nt.

**H41.** Chen obtained the m6A-containing sequences in *Homo sapiens* from RMBase (Chen et al., 2017). All the m6A sites in these sequences conform to the RRACH motif. The dataset contains 1,130 positive samples and 1,130 negative samples. The length of every sample is 41nt.

**Construction of Input Feature**

Conventional machine learning models require numerical vectors as input features. The feature extraction methods selected have an important impact on the performance of the model. To fully characterize the context of m6A sites, seven sequence-derived features were used. In addition, we build a network based on the whole dataset, by mapping each sample to node and the similarity between samples to edges in the network, and then use graph embedding (neighborhood-based node embedding) methods to extract features in an unsupervised manner. The
The computational framework of our predictor m6AGE is illustrated in Figure 1. In the following, we will introduce the sequence-derived features and the graph embeddings, respectively.

**Sequence-Derived Features**

**CTD Feature**
CTD (Tong and Liu, 2019) is one of the global sequence descriptors. The first descriptor C (nucleotide composition) describes the percentage composition of each nucleotide in the sequence. The second descriptor T (nucleotide transition) describes the frequency of four different nucleotides present in adjacent positions. The third descriptor D (nucleotide distribution) describes five relative positions of each nucleotide along the RNA sequence which are the first one, 25%, 50%, 75%, and the last one.

**PseKNC Feature**
With the successful application of the pseudo component method in peptide sequence processing, its idea has been further extended to the study of DNA and RNA sequences feature representation. The Pseudo k-tuple Composition (PseKNC) combines the local and global sequence information of RNA (Guo et al., 2014) and transforms an RNA sequence into the following vector:

$$\mathbf{D}_{\text{PseKNC}} = [d_1, d_2, \ldots, d_{4^k}, d_{4^k+1}, \ldots, d_{4^k+\lambda}]^T$$  \hspace{1cm} (1)

where,

$$d_u = \begin{cases} \frac{f_u}{\sum_{i=1}^{4^k} f_i + \sum_{j=1}^{\lambda} \theta_j} & (1 \leq u \leq 4^k) \\ \frac{\sum_{i=1}^{4^k} f_i + \sum_{j=1}^{\lambda} \theta_j}{\sum_{i=1}^{4^k+\lambda} f_i} & (4^k < u \leq 4^k + \lambda) \end{cases}$$  \hspace{1cm} (2)

where \(d_u (u = 1, 2, \ldots, 4^k)\) is the occurrence frequency of the \(u\)-th \(k\)-nucleotide in this RNA sequence; the parameter \(w\) is the weight factor; the parameter \(\lambda\) is the number of totals counted tiers of the correlations along an RNA sequence. The \(j\)-tier correlation factor \(\theta_j\) is defined as follows:

$$\theta_j = \frac{1}{L - j - 1} \sum_{i=1}^{L-j-1} \Theta (R_i R_{i+1}, R_{i+j} R_{i+j+1}), \hspace{1cm} (j = 1, 2, \ldots, \lambda; \lambda < L)$$  \hspace{1cm} (3)

The correlation function \(\Theta (, , )\) is calculated by the following formula:

$$\Theta (R_i R_{i+1}, R_{i+j} R_{i+j+1}) = \frac{1}{\mu} \sum_{v=1}^{\mu} \left[ P_v (R_i R_{i+1}) - P_v (R_{i+j} R_{i+j+1}) \right]^2$$  \hspace{1cm} (4)

where \(\mu\) is the number of RNA physicochemical properties used. \(R_i R_{i+1}\) is the dinucleotide at position \(i\) of this RNA.
$P_v (R_t R_{t+1})$ is the standardized numerical value of the v-th RNA physicochemical properties for dinucleotide $R_t R_{t+1}$.

Six RNA physicochemical properties are considered: "Rise", "Roll", "Shift", "Slide", "Tilt", "Twist".

**NPS Feature**
The nucleotide pair spectrum (NPS) (Zhou et al., 2016) encoding method describes the RNA sequence context of the site by calculating the occurrence frequency of all k-spaced nucleotide pairs in the sequence. The k-spaced nucleotide pair $n_1[k]n_2$ means that there are k arbitrary nucleotides between $n_1$ and $n_2$, and its occurrence frequency is calculated as follows:

$$d_{n_1[k]n_2} = \frac{C(\{n_1[k]n_2\})}{L-k-1}$$

where $C(\{n_1[k]n_2\})$ is the count of $n_1[k]n_2$ in this RNA sequence, and $L$ is the sequence length. The parameter $k$ ranges from 1 to $d_{max}$. The parameter $d_{max}$ is set to 3, so this encoding method transforms an RNA sequence into a matrix $D_{NPS}$ with a dimension of $4 \times 4 \times 3 = 48$.

**NPPS Feature**
The nucleotide pair position specificity (NPPS) (Xing et al., 2017) encoding method extracts statistical information by calculating the frequency of single nucleotide and k-spaced nucleotide pairs at specific locations. Based on the positive training dataset, we can get the frequency matrix

$$F^+_{\pm} = \begin{bmatrix} f^+_{s(A,1)} & \cdots & f^+_{s(A,L)} \\ \vdots & \cdots & \vdots \\ f^+_{s(G,1)} & \cdots & f^+_{s(G,L)} \end{bmatrix}$$

$$F^+_{d} = \begin{bmatrix} f^+_{d(AA,1)} & \cdots & f^+_{d(AA,L-k-1)} \\ \vdots & \cdots & \vdots \\ f^+_{d(GG,1)} & \cdots & f^+_{d(GG,L-k-1)} \end{bmatrix}$$

where the element of $F^+_{\pm}$ is the frequency of single nucleotide appearing at each location in the positive training dataset; the element of $F^+_{d}$ is the frequency of k-spaced nucleotide pair appearing at each location in the positive training dataset; and $L$ is the sequence length. The frequency matrix $F^+_{\pm}$ and $F^+_{d}$ are calculated similarly on the negative training dataset.

Assuming that the $i$-th nucleotide is "A" and the $(i+k)$-th nucleotide is "C", $p_i^+$ is calculated through conditional probability formula and frequency matrix:

$$p^+_i = \frac{f^+_i}{f^+_s(A,C,i+k)}$$

NPPS encoding method transforms a sequence into a vector $\mathbf{D}_{NPPS} = [p^+_1, \ldots, p^+_L]$ with a dimension of $L - k - 1$, where $p_t = p^+_t - p^-_t$.

**NCP-ND Feature**
Different nucleotides have different chemical properties. According to the difference of ring structure (purine or pyrimidine), hydrogen bond (strong or weak), and functional group (amino or keto), nucleotide A, U, C, and G can be represented by (1, 1, 1), (0, 1, 0), (0, 0, 1), and (1, 0, 0), respectively (Golam Bari et al., 2013).

The nucleotide density (ND) is used to measure the relevance between the frequency and position of the $i$-th nucleotide $n_i$ in the sequence:

$$d_{ni} = \frac{1}{L} \sum_{j=1}^{L} t(n_j), \quad t(q) = \begin{cases} 1, & \text{if } n_i = q \\ 0, & \text{otherwise} \end{cases}$$

where $L$ is the sequence length. Combined with the chemical properties of nucleotides, each sequence is transformed into a vector $\mathbf{D}_{NCP-ND}$ with a dimension of $L \times 4$.

**EIIP Feature**
This encoding method uses the electron-ion interaction pseudopotentials (EIIP) values (Nair and Sreenadh, 2006) to represent the nucleotide in the sequence. The EIIP values of nucleotides A, T (we replace T with U), C, G are 0.1260, 0.1340, 0.0806, and 0.1335, respectively. Thus the dimension of the vector $\mathbf{D}_{EIIP}$ is equal to the sequence length.

**BPB Feature**
The Bi-profile Bayes (BPB) encoding method was first proposed by (Shao et al., 2009), and then has been successfully applied in other fields of bioinformatics. This method uses the occurrence frequency $f_{i,n}$ of the $i$-th nucleotide $n$ to estimate the posterior probability $p_i, n$, and transforms a sequence into the following vector:

$$\mathbf{D}_{BPB} = [f^+_1, f^+_n, f^+_2, f^+_m, f^+_3, f^+_{2,2}, \ldots, f^+_{L, n}, f^+_{L, m}]$$

where $n$ is the $i$-th nucleotide of the sequence; $f^+_i$, $n$ denotes the frequency of nucleotide $n$ appearing at the $i$-th position of the sequence in the positive training dataset, while $f^-_i$, $n$ denotes the frequency of nucleotide $n$ appearing at the $i$-th position of sequence in the negative training dataset. $L$ is the sequence length. The dimension of the vector $\mathbf{D}_{BPB}$ is $2 \times L$.

**Graph Embeddings**

**Network Construction**
To extract the graph embedding feature of each sample, we construct a network based on the whole dataset. Each sample in the dataset is taken as a node, and the relationships between samples are taken as edges. Generally, edges exist two similar sample nodes. The fast linear neighbor similarity approach (FLNSA) (Zhang et al., 2017, 2019) is a method to extract “sample-sample” similarity, which has been successfully applied to many bioinformatics classification tasks. In this study, FLNSA is utilized to calculate the similarity between samples.

First, we extract sequence-derived features and use the feature fusion strategy to transform all the samples in the dataset into $n$-dimensional vector $\{x_1, x_2, \ldots, x_m\}$, where $x_i (0 < i \leq m)$ is the vector of the $i$-th sample. Then these vectors are concentrated into a matrix $X \in \mathbb{R}^{m \times n}$, each row of which represents a sample
vector. FLNSA tries to minimize the objective function:

$$\min_{\mathbf{W}} \frac{1}{2} \| \mathbf{X} - (\mathbf{C} \odot \mathbf{W}) \mathbf{X} \|_F^2 + \frac{\mu}{2} \sum_{i=1}^{m} \left\| (\mathbf{C} \odot \mathbf{W}) \mathbf{e} \right\|_F^2$$  \hspace{1cm} (11)

subject to

$$\left( \mathbf{C} \odot \mathbf{W} \right) \mathbf{e} = \mathbf{e}, \mathbf{W} \geq 0$$

where $\odot$ is the Hadamard product operator, $\| \cdot \|_F$ represents the Frobenius norm and $\mu$ is the regularization coefficient. $\mathbf{e}$ is an $m$-dimensional column vector with all elements equal to $1$. The element $w_{ij}$ of matrix $\mathbf{W}$ in $\mathbb{R}^{m \times m}$ represents the reconstruction contribution weight of the sample $x_j$ to the sample $x_i$, and is used to quantify the similarity between two samples. The element of indicator $\mathbf{C} \in \mathbb{R}^{n \times m}$ is

$$c_{i,j} = \begin{cases} 1 & x_j \in N(x_i) \\ 0 & x_j \notin N(x_i) \end{cases}$$  \hspace{1cm} (12)

where $N(x_i)$ denotes the set of all neighbors of $x_i$. The Euclidean distances between $x_i$ and other samples are calculated and the nearest $c(0 < c < m)$ samples are selected to form $N(x_i)$. FLNSA uses the Lagrange method to get matrix $\mathbf{W}$. After mathematical derivation, the Equation (13) is obtained.

$$w_{ij} = \begin{cases} \frac{w_{ij}(x_i^T + \mu \mathbf{e} \mathbf{e}^T)}{((\mathbf{C} \odot \mathbf{W}) x_i^T + \mu (\mathbf{C} \odot \mathbf{W}) \mathbf{e} \mathbf{e}^T)_{ij}} & x_j \in N(x_i) \\ 0 & x_j \notin N(x_i) \end{cases}$$  \hspace{1cm} (13)

Randomly generated matrix $\mathbf{W}$ was updated according to Equation (13) until convergence. Taking $\mathbf{W}$ as the adjacency matrix, an undirected weighted graph $\mathbf{G}$ is obtained. The graph embedding methods require a connected graph as input. Note that if $\mathbf{G}$ is not connected, we can increase $c$ (the number of neighborhoods of a sample). Under the condition of ensuring the connectivity of the graph, the edges whose weights are lower than the threshold $t$ are removed and the weights of the remaining edges are set to 1. Finally, an undirected unweighted graph is constructed based on the dataset.

**GraRep**

GraRep (Cao et al., 2015) proposes a graph embedding model that can be learned from weighted graphs and integrate global structure information of the graph. GraRep forms $k$ different vectors by separating $k$ kinds of relationships. For a specific $k$, GraRep samples a set of $k$-step paths from the graph. The $k$-step path which starts with node $v_w$ and ends with node $v_c$ is denoted as $(v_w, v_c)$. For all pairs, it increases the probability of the pairs come from the graph and decreases the probability of the pairs do not come from the graph. Based on the normalized adjacency matrix, GraRep obtains $W^k$ for different values of $k$, and each column vector of $W^k$ represents an embedding of the node. Finally, this method concatenates all the $k$-step representations $W^1, W^2, ..., W^k$.

**CatBoost Classifier**

CatBoost (Dorogush et al., 2018; Prokhorenkova et al., 2018) is an improved implementation of gradient enhanced decision trees (GDBT) algorithm developed by Yandex. It has demonstrated excellent performance on many classification and regression tasks. Compared with other advanced gradient boosting algorithms such as XGBoost (Chen and Guestrin, 2016) and lightBGM (Ke et al., 2017), CatBoost has the following advantages: (1) It can better process categorical features. (2) To solve the problem of gradient bias and prediction shift, ordered boosting is proposed instead of the classic GDBT gradient estimation algorithm. (3) The requirement of super parameter tuning is reduced.

CatBoost uses oblivious decision trees (Langley and Sage, 1994) as base predictors. As oblivious decision trees are balanced, they can prevent overfitting. Moreover, it optimizes the traditional boosting algorithm which transforms the category features into numerical features, and the algorithm of calculating the leaf value to improve the generalization ability of the model. Since the CatBoost algorithm is running on GPU, the model is trained efficiently and parallelly.

**Evaluation Metrics**

Our predictor predicts whether the adenosine at the center of an RNA sequence segment is an $m^6$A site. We used the following metrics to evaluate the performance of binary classification predictors: accuracy (ACC), Matthew’s correlation coefficient (MCC), sensitivity (SEN), specificity (SPE), and F1. These metrics...
are calculated as follows:

$$\text{ACC} = \frac{TP + TN}{TP + FN + TN + FP} \times 100\%$$ (15)

$$\text{MCC} = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FN)(TN + FP)(TP + FP)(TN + FN)}}$$ (16)

$$\text{SEN} = \frac{TP}{TP + FN} \times 100\%$$ (17)

$$\text{SPE} = \frac{TN}{TN + FP} \times 100\%$$ (18)

$$F_1 = \frac{2TP}{2TP + FP + FN}$$ (19)

where $TP$ is the number of true positive samples; $TN$ is the number of true negative samples; $FP$ is the number of false positive samples; $FN$ is the number of false negative samples.

Additionally, the receiver operating characteristic (ROC) curve is also an important measurement to evaluate the performance of classifiers, and the area under receiver operating characteristic curve (AUC) is the quantitative indicator. High values of AUC indicate better performance of predictors.

**RESULTS**

We redivided the four datasets introduced in section “Datasets” into the training sets and test sets with the ratio of 4:1, respectively. The training datasets were used to train models and the test datasets were utilized to evaluate model performance.

**TABLE 1 | The performance of m6AGE against other existing predictors.**

| Datasets | Predictors | ACC (%) | MCC | SEN (%) | SPE (%) | AUC   |
|----------|------------|---------|-----|---------|---------|-------|
| A101     | m6AGE      | 89.11   | 0.7822 | 90.49   | 87.68   | 0.9500 |
|          | M6A-HPCS   | 86.43   | 0.7286 | 86.64   | 86.22   | 0.9284 |
|          | Targetm6A  | 87.36   | 0.7471 | 87.65   | 87.06   | 0.9358 |
|          | RAM-NPPS   | 83.88   | 0.6777 | 86.44   | 81.21   | 0.9077 |
|          | M6APred-EL | 86.02   | 0.7205 | 85.63   | 86.43   | 0.9055 |
|          | DeepM6ASeq | 87.77   | 0.7595 | 93.32   | 82.05   | 0.9419 |
| A25      | m6AGE      | 87.97   | 0.7708 | 74.65   | 98.85   | 0.8867 |
|          | M6A-HPCS   | 68.35   | 0.3577 | 61.97   | 73.56   | 0.7238 |
|          | Targetm6A  | 82.91   | 0.6542 | 76.06   | 88.51   | 0.8570 |
|          | RAM-NPPS   | 82.91   | 0.6538 | 77.46   | 87.36   | 0.8621 |
|          | M6APred-EL | 87.34   | 0.7642 | 71.83   | 100.00  | 0.8464 |
|          | DeepM6ASeq | 77.85   | 0.5515 | 67.61   | 86.21   | 0.8054 |
| H41      | m6AGE      | 90.93   | 0.8325 | 81.94   | 100.00  | 0.9181 |
|          | M6A-HPCS   | 71.46   | 0.4336 | 64.76   | 78.22   | 0.7765 |
|          | Targetm6A  | 90.49   | 0.8249 | 81.06   | 100.00  | 0.9205 |
|          | RAM-NPPS   | 90.49   | 0.8249 | 81.06   | 100.00  | 0.9051 |
|          | M6APred-EL | 89.82   | 0.8136 | 79.74   | 100.00  | 0.9132 |
|          | DeepM6ASeq | 86.50   | 0.7566 | 73.57   | 99.56   | 0.9051 |

The optimal value of each evaluation metric is marked in bold.

**FIGURE 2 | The ROC curves of m6AGE and comparing predictors on three datasets.** (A) The ROC curves on the A101 dataset. (B) The ROC curves on the A25 dataset. (C) The ROC curves on the H41 dataset.
Due to the difference between datasets, we selected suitable sequence-derived features for each dataset. For A101, the PseKNC, CTD, and NPS features were selected; For A25, the EIIP, NPPS, NPS, PseKNC, and NCP-ND were selected; For S21, the NPPS and NCP-ND features were selected; For H41, the NCP-ND, PseKNC, and NPPS features were selected.

**Comparison With Existing Predictors**

In this section, we compared the performance of our predictor m6AGE with several other state-of-the-art predictors, including M6A-HPCS (Zhang et al., 2016), Targetm6A (Li et al., 2016), RAM-NPPS (Xing et al., 2017), M6APred-EL (Wei et al., 2018), and DeepM6ASeq (Zhang and Hamada, 2018). M6A-HPCS uses PseDNC and DACC features and a support vector machine (SVM) classifier to identify m6A sites. Targetm6A utilizes position-specific kmer propensities (PSKP) feature and SVM classifier. RAM-NPPS uses the NPPS feature and SVM classifier to identify m6A sites. M6APred-EL creates an ensemble model with PseKNC, PSKP, and NCP-ND features. DeepM6ASeq develops a deep learning framework and uses one-hot encoding for the identification of m6A sites. The predictor M6A-HPCS, M6APred-EL, Targetm6A, and RAM-NPPS were reproduced faithfully, and their parameters were optimized by grid search. All predictors were trained and evaluated on the same dataset for fairness of comparison.

The evaluation results were summarized in Table 1. We employed ACC, MCC, SEN, SPE, and AUC as evaluation metrics, and compared the evaluation metrics of m6AGE with five other predictors on three datasets: A101, A25, and H41. As shown in Table 1, our predictor m6AGE achieved all optimal values on three datasets, except for SEN and SPE on the A25 dataset, and AUC on the H41 dataset.

### Table 2: The performance of different predictors on S21 dataset.

| Predictors   | SEN (%) | SPE (%) | F1    | MCC   | AUC   |
|--------------|---------|---------|-------|-------|-------|
| m6AGE        | 68.68   | 83.02   | 0.5723| 0.4593| 0.8103|
| HPCS         | 71.70   | 46.63   | 0.3622| 0.1459| 0.6330|
| Targetm6A    | 70.57   | 76.73   | 0.5260| 0.3984| 0.7818|
| RAM-NPPS     | 66.42   | 81.49   | 0.5440| 0.4218| 0.7778|
| M6APred-EL   | 78.59   | 75.20   | 0.5554| 0.4433| 0.7899|
| DeepM6ASeq   | 63.77   | 83.38   | 0.5480| 0.4253| 0.8056|

*The optimal value of each evaluation metric is marked in bold.*

On the A101 dataset, m6AGE obtained the optimal ACC, MCC, SEN, and SPE with 90.93%, 80.325, 81.94%, and 100%, which is 0.44%, 0.0076, 0.88%, and 0 higher than the predictor Targetm6A and RAM-NPPS, respectively.

The ROC curves of these predictors on three datasets were plotted in Figure 2. As shown in Figure 2, our predictor outperformed other predictors on the A101 and A25 datasets. Although the AUC of m6AGE on dataset H41 is lower than other predictors, m6AGE achieved the optimal value of ACC, MCC, SEN, and SPE. These evaluation results demonstrate that our predictor m6AGE is superior to other predictors in terms of these three datasets.

### Performance on Imbalanced Dataset

The non-m6A sites on mRNA are much more than m6A sites, so testing the performance of our predictor on imbalanced datasets is of great importance. The imbalance ratio of the S21 dataset is about 1:4. We redivided the S21 dataset, and randomly selected 80% samples as the training set, and the remaining 20% samples as the test set.

CatBoost solves the imbalance data issues by setting weights for each class or sample. The weight of each class is generally inversely proportional to the number of its samples. The metrics F1 and MCC are usually used as the evaluation criteria for imbalanced datasets (Zhao et al., 2018; Wang et al., 2019; Dou et al., 2020). We compared m6AGE with five other predictors on the S21 dataset.

The evaluation results were summarized in Table 2. The optimal value of each evaluation metric is marked in bold. As shown in Table 2, our predictor m6AGE got the optimal values of F1, MCC, and AUC with 0.5723, 0.4593, and 0.8103.

The ROC curves of these predictors on the S21 dataset were plotted in Figure 3. As shown in Figure 3, our predictor outperformed other predictors on the S21 dataset.
Comparison With Different Classifiers

To further demonstrate the effectiveness of CatBoost, we compared it with other popular classifiers, including Random Forest, Logistic Regression, and Decision Tree, which are commonly and widely used in bioinformatics classification tasks. All classifiers were trained and assessed under the same conditions for a fair comparison.

The prediction results were summarized in Table 3. We compared the prediction results with three other classifiers on the A101, A25, and H41 dataset. The evaluation metrics used are

| Datasets | Classifiers | Metrics | ACC (%) | MCC    | SEN (%) | SPE (%) | AUC    |
|----------|-------------|---------|---------|--------|---------|---------|--------|
| A101     | CatBoost    |         | 89.11   | 0.7822 | 90.49   | 87.68   | 0.9500 |
|          | Random forest|        | 87.67   | 0.7534 | 87.04   | 88.31   | 0.9377 |
|          | Logistic regression | | 89.00   | 0.7800 | 89.07   | 88.94   | 0.9489 |
|          | Decision tree |      | 80.99   | 0.6197 | 82.39   | 79.54   | 0.8096 |
| A25      | CatBoost    |         | 87.97   | 0.7708 | 74.65   | 98.85   | 0.8867 |
|          | Random forest|        | 87.34   | 0.7642 | 71.83   | 100.00  | 0.8729 |
|          | Logistic regression | | 79.11   | 0.5767 | 74.65   | 82.76   | 0.8562 |
|          | Decision tree |      | 81.65   | 0.6349 | 84.51   | 79.31   | 0.8191 |
| H41      | CatBoost    |         | 90.93   | 0.8325 | 81.94   | 100.00  | 0.9181 |
|          | random forest |      | 89.38   | 0.8031 | 79.74   | 99.11   | 0.9098 |
|          | Logistic regression | | 86.95   | 0.7422 | 82.38   | 91.56   | 0.9125 |
|          | Decision tree |      | 86.28   | 0.7258 | 85.46   | 87.11   | 0.8629 |

The optimal value of each evaluation metric is marked in bold.

FIGURE 4 | The feature importance scores on the four datasets. (A) The feature importance scores on the A101 datasets. (B) The feature importance scores on the A25 datasets. (C) The feature importance scores on the H41 datasets. (D) The feature importance scores on the S21 datasets.
ACC, MCC, SEN, SPE, and AUC. As shown in Table 3, CatBoost achieved all optimal metrics on three datasets, except for SPE on the A101 dataset and SEN on the A25 and H41 dataset.

**Feature Importance Analysis**

CatBoost can output the scores of feature importance, which reflect the contributions of the features in specific feature space for identifying m^6A sites. The first 20 important features and their scores on the four datasets were plotted in Figure 4.

On the A101 dataset, the first three important sequence-derived features are “PseKNC_44”, “PseKNC_59”, and “PseKNC_40”, which correspond to the occurrence frequency of “GUA”, “UGU”, and PseKNC_40 respectively. On the A25 dataset, the first three important sequence-derived features are “NCP Nd_58”, “NPPS Xi2_14”, and “NPPS Xi1_14,” which correspond to the position +1 (Assuming that the position of m^6A site is 0), +2 and +4, +2 and +3, respectively; On the H41 dataset, the first three important sequence-derived features are “NPPS Xi2_20”, “NPPS Xi2_22”, and “NCP Nd_72”, which correspond to the position 0 and +1, +2 and +3, respectively; On the S21 dataset, the first three important sequence-derived features are “NPPS Xi2_17”, “NPPS Xi2_17”, and “NPPS Xi2_18”, which correspond to the position +6 and +7, +6 and +8, +7 and +9, respectively.

In addition, graph embeddings account for 20%, 25%, 35%, and 50% of the top 20 important features in the four datasets, respectively, which indicates that graph embeddings could supplement the information of the sequence-derived features.

**DISCUSSION**

The methods for extracting sequence features are indispensable for building a reliable predictor. Contributing sequence features, such as the physical and chemical properties of nucleotides, the frequency of k-nucleotides, and the frequency of specific positions, can fully reflect the information related to the m^6A site recognition. In this study, we integrated and selected suitable sequence-derived features for each dataset. However, most of the feature encoding methods are based on the primary sequence, and only a few of them calculate the frequency of nucleotides in the training dataset, so it is difficult to obtain more helpful information from the whole dataset. This paper innovatively introduces a feature extraction method based on the graph embedding methods as a supplement to sequence-derived features. First of all, a network is constructed based on the whole dataset and sequence-derived features. Samples are abstracted as nodes of the network, and the similarity relationships between samples are abstracted as edges. This network reflects global information of the whole dataset. Then, graph embedding (neighborhood-based node embedding) methods are used to learn the feature representation of each node in an unsupervised manner. The graph embedding features of samples contain the related information with other samples. Finally, we integrate sequence-derived features and graph embeddings based with the feature fusion strategy. Therefore, the final input features can reflect the information of samples more comprehensively.

It is also significant to choose an appropriate classifier. CatBoost is a GBDT algorithm, which shows excellent performance in many classification tasks. Because of its good effect of restraining overfitting and fast running, the CatBoost algorithm is selected to train our predictor m6AGE.

To further prove the effectiveness of our predictor, we compare the evaluation results with that of other existing m^6A site predictors. The results show that our predictor m6AGE outperforms other existing methods. In the future, we will apply m6AGE to more m^6A site datasets and seek more suitable graph embedding methods. It is worth mentioning that the computational framework proposed in this study is possible to extend to other bioinformatics site identification tasks.

The source code of m6AGE is available at https://github.com/bokunoBike/m6AGE. Users can download and run it on the local machines. The data is imported through the file paths of the positive training set, negative training set, and test set. Then m6AGE is trained and generates prediction results. Note that the corresponding python packages need to be installed first (see GitHub page for details). For a new dataset, our predictor will automatically select the appropriate sequence-derived features (or specified by the users in the corresponding configuration file) according to the feature importance scores.

**CONCLUSION**

The identification of N^6-methyladenosine (m^6A) modification sites on RNA is of biological significance. In this study, a novel computational framework called “m6AGE” is proposed to predict and identify the m^6A sites on mRNA. Our predictor combines sequence-derived features with the features extracted by graph embedding methods. The context information of sites is directly extracted from primary sequences by the sequence-derived features, and the global information is extracted by the graph embeddings. Experiments showed that the proposed m6AGE achieved successful prediction performance on four datasets across three species. It could be expected that m6AGE would be a powerful computational tool for predicting and identifying the m^6A modification sites on mRNA.

**DATA AVAILABILITY STATEMENT**

Publicly available datasets were analyzed in this study. Codes and data are available here: https://github.com/bokunoBike/m6AGE which contains detailed steps to run m6AGE.

**AUTHOR CONTRIBUTIONS**

YW and RG conceived the algorithm and developed the program. RG, YW, and SY wrote the manuscript and prepared the datasets. YW and SY helped with manuscript editing, design. XMH, LH, and KH helped to revise the manuscript. All authors contributed to the article and approved the submitted version.
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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