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Integrating machine learning and protein–ligand interaction profiling for the discovery of METTL3 inhibitors

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RNA modifications are critical in regulating gene expression and cell functions by affecting RNA stability, splicing, translation, and degradation. The catalytic core of N^6-adenosine-methyltransferase catalytic subunit METTL3 has emerged as a key enzyme in tumorigenesis by enhancing the translation efficiency of oncogenic transcripts, which is a promising therapeutic target for cancers, including acute myeloid leukemia. In this study, we presented a novel METTL3 inhibitory bioactivity (pIC_{50}) prediction model (ML3-mix-DPLIFE) by combining machine learning, protein–ligand docking, and protein–ligand interaction analysis, through encoding the conventional physicochemical properties, chemical fingerprint, and the docking-based protein–ligand interaction features (DPLIFE) with leveraging auto-stacking 6 algorithms. A feature selection algorithm further optimized the model (ML3-mix-DPLIFE-FS) and obtained a promising mean squared error (*MSE*) of 0.261 and a Pearson's correlation coefficient (*CC*) of 0.853 on an independent test dataset, and identified 8 residues critical for ligand interactions with METTL3. To further test the model, the pIC_{50}s of recently reported inhibitors were predicted using the ML3-mix-DPLIFE-FS model, and a good *MSE* of 0.418 and *CC* of 0.727 were obtained. This innovative strategy seamlessly integrates machine learning prediction with structural biology insights and reveals a novel way to identify key protein–ligand interactions for further structural rational drug design.

Keywords Human N6-adenosine-methyltransferase catalytic subunit METTL3 , Machine learning, Feature selection, Protein–ligand docking, The docking-based protein–ligand interaction features (DPLIFE)

Abbreviations

UniProtKB	The Universal Protein Knowledgebase
METTL3	The methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit (UniProtKB primary accession number: Q86U44)
METTL14	The methyltransferase 14, N6-adenosine-methyltransferase non-catalytic subunit (UniProtKB primary accession number: Q9HCE5)
AML	Acute myeloid leukemia
MYC	Myc proto-oncogene protein (UniProtKB primary accession number: P01106)
BCL2	B-cell leukemia/lymphoma 2 protein; Apoptosis regulator Bcl-2 (UniProtKB primary accession number: P10415)
DPLIFE	The docking-based protein–ligand interaction features
CC	Pearson's correlation coefficient
IC_{50}	Half maximal inhibition concentration
MSE	Mean squared error
RMSD	Root-mean-square deviation
ECFP	Extended-connectivity fingerprints
mRMR	The minimum redundancy and maximum relevance algorithm
PDB ID	Protein data bank identifier
PCA	Principal component analysis
BA	Bisubstrate analog

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RNA modifications are essential for regulating gene expression and cellular functions, influencing various biological processes, and governing non-coding RNAs[1], including tRNAs, rRNAs, and microRNAs. The most abundant internal modification in eukaryotic mRNA significantly impacts various aspects of RNA metabolism, including stability, splicing, translation, and degradation[2]. To date, over a hundred different types of RNA chemical modifications have been identified in many types of RNAs, including mRNA, rRNA, tRNA, snRNA, microRNA, and long noncoding RNA[3]. Common modifications include 5-methylcytosine (m5C)[4,5], N7-methylguanosine (m7G)[6], N1-methyladenosine (m1A)[7], N4-acetylcytidine (Ac4C)[8], pseudouridine (Ψ)[9], 2′-O-methylation (N$_m$)[10], N6,2′-O-dimethyladenosine (m6A$_m$)[11], and N6-methyladenosine (m6A) [12–14]. Among these modifications, N6-methyladenosine (m6A) stands out as the most prevalent and conserved ribonucleotide modification[15], in which over 25% of human transcripts have been identified with more than 12,000 m6A occurrences[16]. The N6-methyladenosine (m6A) modification, is a complex process involving the crucial participation of multiple proteins that add (writers), recognize (readers), and remove (erasers) the m6A marks[3,17].

N6-adenosine-methyltransferase catalytic subunit METTL3 (METTL3) and its non-catalytic subunit METTL14 form the catalytic core of the m6A RNA methyltransferase complex, a critical player in post-transcriptional RNA modification[18]. These two components were further supported by regulatory elements, including WTAP[17,19], RBM15[20,21], VIRMA[22], ZC3H13[23], YTHDF[23,24], and HAKAI[25]. METTL3's primary function is to catalyze the addition of methyl groups to the N6 position of adenosine residues, enabling the fine-tuning of RNA stability, splicing, and translation. Its critical role in RNA metabolism has positioned METTL3 as a key regulator of cellular processes, with its dysfunction linked to a range of diseases. In oncology, METTL3 has been extensively studied for its role in promoting tumorigenesis. Elevated METTL3 expression has been observed in cancers such as acute myeloid leukemia (AML)[26,27], hepatocellular carcinoma[28], and lung adenocarcinoma[29,30]. In AML, METTL3-mediated m6A methylation promotes the translation of oncogenic transcripts, such as MYC and BCL2, which are crucial for leukemia cell proliferation and survival[31]. Targeting the methyltransferase activity of METTL3 presents a promising strategy to regulate m6A methylation patterns and modulate oncogene expression, offering potential therapeutic benefits in cancer treatment[32].

Recent advances in prediction models related to METTL3 have primarily focused on identifying m6A RNA modification sites. The machine learning model m6Aboost, for instance, tackled the problem of false positives in antibody-based m6A detection by using miCLIP2 datasets[33]. Similarly, the iM6A deep learning model presented high accuracy in predicting m6A deposition sites while identifying critical cis-element sequences that govern site-specific methylation[34]. Recent research has demonstrated that METTL3 has an m6A target-selective role[31,35], with the development of numerous small-molecule inhibitors targeting METTL3 against acute myeloid leukemia, gastric cancer, and neuroblastoma tumor suppression, such as UZH2 and STM2457[18,36,37]. Nevertheless, the prediction model for METTL3 inhibition on drug development has yet to be developed.

To facilitate the drug development for METTL3, this study presented a prediction model using a novel machine learning strategy to conquer the dataset limitation. Due to the small data size of two publicly available datasets of METTL3 inhibitors, the bioactivity distribution of the two datasets derived from METTL3 and METTL3-METTL14 experiments were carefully compared and merged. To overcome the limited number of inhibition bioactivity data for the target protein, a comprehensive approach combining machine learning, protein–ligand docking, and protein–ligand interaction analysis was utilized. This innovative strategy seamlessly integrates computational predictions with structural biology insights, enabling the accurate identification of potent METTL3 inhibitors. Models were developed using AutoGluon, a stacking algorithm leveraging 6 machine learning algorithms for achieving high performance. An optimized METTL3 bioactivity prediction model was developed using the merged datasets with the integration of contact residues of METTL3-ligand docking analysis. Furthermore, key residues with significant impacts on bioactivity were identified using forward feature selection through the minimum redundancy maximum relevance (mRMR) algorithm[38], providing critical guidance for the rational design of future METTL3-targeting inhibitors. The developed model was effective in identifying METTL3 inhibitors and was further validated by external data of recently published inhibitors from the literature.

Methods
The programs were developed on the Ubuntu 20.04 operating system using Python 3.7.11. To achieve various analytical tasks, several Python packages were utilized: numpy and pandas for data manipulation, matplotlib for visualization, scikit-learn for machine learning, rdkit-pypi for cheminformatics, torch and AutoGluon v0.5.2[39] for machine learning.

Datasets
Two datasets (DB1 and DB2) of chemicals and the human METTL3 IC$_{50}$ bioactivities were obtained from ChEMBL[40]. DB1 contains 151 IC$_{50}$ values for METTL3/METTL14 inhibitors (CHEMBL4106140) and DB2 consists of 607 IC$_{50}$ values for METTL3 inhibitors (CHEMBL4739695). A merged dataset of DB1 and DB2 was developed by removing duplicates and was composed of 644 chemicals and their corresponding IC$_{50}$ values. The negative logarithm of the IC$_{50}$ values (pIC$_{50}$) was used as the label for model training.

Protein–ligand docking and docking-based protein–ligand interaction feature encoding (DPLIFE)
For conventional model development, each record of protein–ligand bioactivity was encoded by two commonly used features of 1024 bits of extended-connectivity fingerprints with a diameter of 6 atoms (ECFP)[41] by RDKit[42] and 1444 physicochemical properties by PaDEL-Descriptor[43]. As protein–ligand docking can provide better

structural insights, we propose incorporating the docking-based protein–ligand interaction features (DPLIFE) to further improve the prediction model.

To compute the DPLIFE feature, the reported human METTL3-METTL14 ligand-bound crystal structure was used (PDB ID: 7O2I)[37] for docking experiments with its small inhibitor compound STM2457 removed. The open-source RDKit toolkit 2022.9.4[42] with Python version 3.7.11 was utilized to generate the three-dimensional structures of ligands. The protein structure was prepared and protonated using the AMBER-FB15 force field[44]. Protein–ligand docking was performed to analyze potential binding pockets and protein–ligand interactions using AutoDock Vina 1.1.2 following the protein–ligand docking protocol[45]. The compound STM2457 was employed to validate the docking procedure, ensuring the accuracy of the redocking process with less than 0.8 Å root-mean-square deviation (RMSD). This threshold confirmed that the docking protocol could reliably reproduce the experimentally observed binding pose, thereby validating the precision of the docking methodology for further analyses.

DPLIFE features consisting of one feature of docking score and 185 features of protein–ligand interaction profile were extracted from the docked complex generated from the abovementioned docking process. The protein–ligand interaction features were calculated based on the analysis results of the Protein–Ligand Interaction Profiler (PLIP) tool[46,47]. Target-specific docking analyses were conducted using the crystal structure (PDB ID: 7O2I), which comprises 205 residues. The C-terminal 20 residues without interaction with ligands were removed. The remaining 185 residues-specific interactions were subsequently encoded into numeric features for further model development. The interaction types were coded as follows: 0 for no interaction, 1 for hydrophobic interactions, 2 for π-π stacking, 3 for π-cation interactions, 5 for salt bridges, and 6 for hydrogen bonds.

Principal component analysis (PCA) and t-distributed stochastic neighbor embedding (t-SNE) analysis

To evaluate the chemical space distribution and compatibility of combining two ligand datasets (DS1: CHEMBL4106140 and DS2: CHEMBL4739695) for METTL3 inhibitor modeling, principal component analysis (PCA)[48] and t-distributed stochastic neighbor embedding (t-SNE)[49] were conducted. Prior to dimensionality reduction, non-structural features, *i.e.* molecular identifiers, SMILES strings, and bioactivity labels, were excluded. The PCA was performed to extract 2 principal components, with the explained variance examined to confirm that the primary chemical variance was captured. The t-SNE was applied to the same preprocessed data matrix using a perplexity of 160, 2 dimensions, and 1,000 iterations to preserve local chemical relationships. The results of both PCA and t-SNE analyses were visualized as scatter plots, with samples color-coded by dataset, allowing assessment of chemical space similarity.

Model development

To ensure a rigorous process for model development, the original dataset was uniformly divided into 64% training, 16% validation, and 20% test sets ratio for model training, validation, and independent test, respectively. To construct a prediction model, the AutoGluon-Tabular[39] was used for developing an auto-staking[50] model based on 6 different algorithms of neural networks[51], LightGBM boosted trees[52], XGBoost[53], CatBoost[54], random forests[55], and extremely randomized trees[56]. The maximum training time was limited to 2 h. To assess model performance, both the mean squared error (*MSE*) and Pearson's correlation coefficient (*CC*) were employed. The mean squared error (*MSE*) was utilized as the objective function for model training, as detailed in *Eq.* 1 below.

$$MSE = \frac{\sum_{i=1}^{n}(Y_i - y_i)^2}{n} \tag{1}$$

In this equation, Y_i and y_i represent the experimental and predicted values for each instance, and i and n denote the specific instance and the total number of instances, respectively. The formula for calculating CC was provided in *Eq.* 2 below.

$$CC = \frac{\sum \left(Y_i - \overline{Y}\right)(y_i - \overline{y})}{\sqrt{\sum \left(Y_i - \overline{Y}\right)^2 \cdot \sum (y_i - \overline{y})^2}} \tag{2}$$

In this equation, $\overline{Y}$ represents the average of the experimental values for the variable being predicted while $\overline{y}$ denoting the average of the predicted values.

Feature selection for identifying key residues

The minimum redundancy maximum relevance (mRMR) algorithm[38] was used to rank the features based on the training dataset in order to determine which features were most pertinent for forecasting METTL3 bioactivities. Subsequently, a sequential forward feature selection algorithm[57] was applied to select the optimal feature subset giving the highest validation *MSE* performance on the validation dataset. In brief, the top-ranked *n* features were selected for model development and validation using the training and validation datasets, respectively, where the feature number of *n* was incrementally increased until reaching a convergence of validation *MSE* values. Since the chemical fingerprint was usually considered as a whole, the feature selection applies only to the physicochemical properties and DPLIFE features while keeping the ECFP information unselected for model development. The incremental selection stops when less than 5% improvement in validation *MSE* value was obtained by including an additional feature.
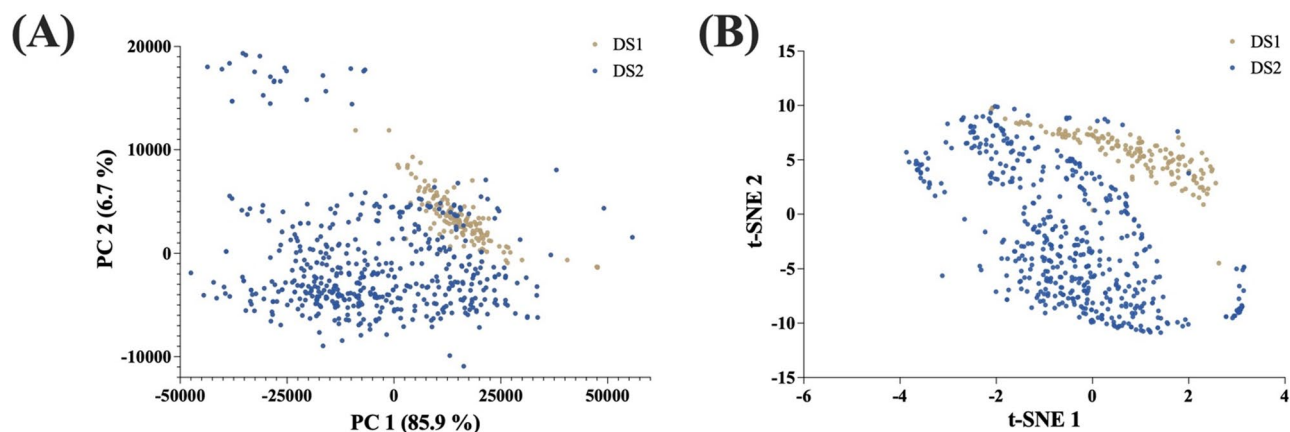
Fig. 1. Chemical space visualization of DS1 and DS2 datasets using (**A**) the first two principal components (PC1 and PC2) obtained from a principal component analysis (PCA), with explained variations shown in parentheses; (**B**) t-SNE.

Models	Training dataset	# features	MSE (validation)	DS1 test set		DS2 test set		Overall test set*	
				MSE	CC	MSE	CC	MSE	CC
ML3-DS1	DS1	2478 (ECFP & PP)	0.176	0.057	0.971	1.261	0.138	0.972	0.370
ML3-DS2	DS2	2478 (ECFP & PP)	0.335	2.839	0.644	0.130	0.935	0.781	0.620
ML3-mix	DS1 + DS2	2478 (ECFP & PP)	0.303	0.231	**0.829**	0.296	**0.830**	0.280	**0.829**
ML3-mix-DPLIFE	DS1 + DS2	2664 (ECFP, PP & DPLIFE)	0.307	0.168	0.874	0.299	0.840	0.264	**0.849**
ML3-mix-DPLIFE-FS	DS1 + DS2	1636 (ECFP, PP-FS & DPLIFE-FS)	0.309	0.168	0.874	0.294	0.845	0.261	**0.853**

Table 1. The validation and test performance of developed models. *CC*, Pearson's correlation coefficient; *MSE*, mean squared error; ECFP, extended-connectivity fingerprints; PP, physicochemical properties, DPLIFE, docking-based protein–ligand interaction features; PP-FS, selected PP features; DPLIFE-FS, selected DPLIFE features; *: the combined test set of DS1 and DS2 test sets. The baseline performances were indicated with underscores, while the improved performances were highlighted in bold font.

Results and discussion
Ligand-based model development for METTL3 bioactivity

To develop prediction models of METTL3 inhibitory activities, two datasets of DS1 and DS2 sourced from CHEMBL4106140 and CHEMBL4739695 were collected. To investigate the potential for combining the two datasets to enlarge the training set, the bioactivity distributions of the two datasets were compared and found to be similar (Fig. S1). Principal component analysis (PCA)[48] and t-distributed stochastic neighbor embedding (t-SNE) analysis[49], based on ECFP and physicochemical features, were then utilized to visualize the chemical spaces of the two datasets. As shown in Fig. 1, both datasets exhibit comparable bioactivity distributions. PCA analysis revealed that the first two principal components explain over 92% of the total variance and that the chemical spaces of DS1 and DS2 substantial overlap, indicating structural similarity between the datasets. Additionally, t-SNE visualization demonstrated that the two datasets occupy partially overlapping regions in chemical space, with clear areas of intersection. These analyses indicated that the two datasets possessed substantial structural similarity and overlap, suggesting enhancement of the chemical space coverage of the developed model by the combination of two datasets.

Two single-dataset models (ML3-DS1 and ML3-DS2) were trained separately on DS1 and DS2 using features of ECFP and physicochemical properties employing AutoGluon-Tabular. The mixed-dataset model (ML3-mix) was trained on the concatenated DS1 and DS2 dataset, which pooled samples from both DS1 and DS2, removed redundant entries, and used their ECFP and physicochemical features. Table 1 shows the validation and test results of the developed models. The model training for ML3-DS1, ML3-DS2, and ML3-mix takes 29, 53, and 60 min. While good validation *MSE* of 0.176 and test *MSE* and *CC* of 0.057 and 0.971, respectively, were obtained for the ML3-DS1 model on the DS1 dataset, the generalization ability of ML3-DS1 was poor on the DS2 dataset. Similarly, the ML3-DS2 model with a good validation *MSE* of 0.335 and good test *MSE* and *CC* of 0.130 and 0.935, respectively, using the DS2 dataset performed poorly on the DS1 dataset. The overall test performance on

both DS1 and DS2 datasets of ML3-DS1 and ML3-DS2 was poor performance with an *MSE* greater than 0.78 and a CC less than 0.62. It shows that a small dataset was not sufficient to train a robust model even with good validation and test results. It was therefore important to investigate the utilization of a combined dataset for developing a robust model.

The performance of the ML3-mix model utilizing both DS1 and DS2 datasets was reasonably good with a validation *MSE* of 0.303 and test *MSE* and *CC* of 0.280 and 0.829, respectively using the combined dataset. The test *MSE* and *CC* on individual datasets were satisfying with 0.231 and 0.829 for DS1 and 0.296 and 0.830 for DS2. These results indicate that dataset integration was beneficial for model development.

Incorporation of protein–ligand interaction features (DPLIFE) for model improvement

While the ligand-based model provides good performance on the prediction of pIC_{50} values of METTL3 ligands, no structural insights into the protein–ligand interactions can be obtained from ligand-based approaches making further validation and investigation difficult. To overcome the limitation, this study incorporated the protein–ligand interaction information to facilitate the identification of METTL3 inhibitors. In short, the protein–ligand docking process was conducted to identify potential ligand binding poses based on the human METTL3-METTL14 heterodimer crystal structure (PDB ID 7O2I)[37] with the inhibitor STM2457 removed (Fig. 2). The docking-based protein–ligand interaction information (DPLIFE) was then represented as numeric feature vectors including one feature of the docking score and 185 protein–ligand interaction features.

A ML3-mix-DPLIFE model was developed using the mixed dataset. As shown in Table 1, a slightly increased validation *MSE* of 0.307 was obtained, while a significant improvement on the DS1 test set and a slight improvement on the DS2 test set was found. The test *MSE* and *CC* were 0.168 and 0.874 for the DS1 test set, and 0.299 and 0.840 for the DS2 test set, respectively. The overall test performance of *MSE* and *CC* for ML3-mix-DPLIFE were 0.264 and 0.849, which was better than the ML3-mix model with 0.280 and 0.829, respectively. The results showed that the DPLIFE features could facilitate learning from small datasets.

Permutation feature importance assay

A permutation feature importance assay was performed to thoroughly evaluate the individual and combined contributions of each feature set (Table S1). The model integrating all features, ECFP, PP, and DPLIFE, yielded the highest performance (*MSE* 0.264, *CC* 0.849). When evaluated individually, the model using only PP demonstrated superior performance (*CC* 0.833) compared to models using only ECFP (*CC* 0.788) or only DPLIFE (*CC* 0.500) (Table S1). Combinations of DPLIFE with either ECFP or PP resulted in only marginal improvements over the respective single-feature models, with less than a 1% increase in *CC*. The resulting order of feature importance for predictive accuracy was determined as PP > ECFP > DPLIFE. While DPLIFE features alone had limited predictive power, their inclusion with molecular fingerprints and physicochemical properties improved model robustness and accuracy, resulting in more than a 2.4% improvement in *CC*. This analysis
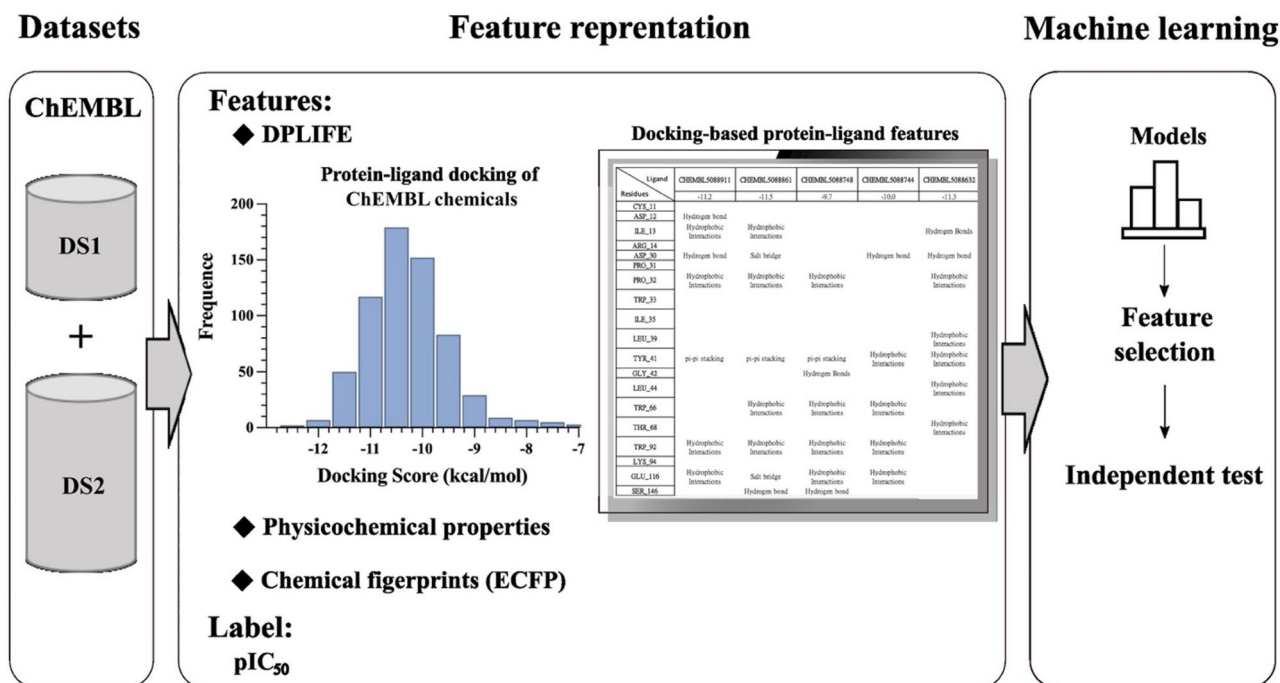

Fig. 2. Flowchart of the stacked ensemble model development for the METTL3 bioactivities using 186 docking-based protein–ligand interaction features (DPLIFE), 1024-bit chemical fingerprint (ECFP), and 1444 physicochemical features.

suggested the necessity of feature complementarity for developing effective and generalizable predictive models for METTL3 bioactivity.

Identification and analysis of key residues for METTL3 bioactivities

Since the incorporation of DPLIFE features was useful for improving the model performance, it was therefore interesting to know which were the key residues and interactions by using machine learning algorithms. To achieve this goal, an mRMR-based forward feature selection algorithm was used to identify the most relevant DPLIFE features and physicochemical property features.

As shown in Fig. 3A, the *MSE* performance was converged in the feature number of 1,636 with a validation *MSE* of 0.309 which was similar to the model using the full feature set. The feature selection removed redundant and irrelevant features with reduced training time while keeping the performance unaffected. The final feature set includes 1,024 ECFP, 603 physicochemical properties, and 9 DPLIFE (*i.e.* 8 key contact features and one docking score feature) (Fig. 3B). The selected DPLIFE features comprised 8 key protein–ligand contact residues and one docking score. The model ML3-mix-DPLIFE-FS based on the selected features yields a slightly reduced test *MSE* of 0.261 and an improved test *CC* of 0.853 (Table 1) for the overall test set. Excluding a significant number of DPLIFE features was reasonable by the observation that only a few key residues were responsible for the bioactivity of proteins, consistent with the fact that fewer than 2% of protein residues were involved in binding and activity[58].

The 8 key residues influencing METTL3 bioactivities ranked by the mRMR algorithm were Arg 379, Pro 396, Ile 400, Trp 431, Trp 457, His 512, Lys 513, and Gln 550, in a sequential order. The identified residues were mapped onto the METTL3 structure (PDB ID: 7O2I) (Fig. 3C) for visualization. The mean pIC_{50} values of chemicals interacting with 0 and at least 1, 2, and 3 key residues out of the 8 key residues are 7.357, 7.494, 7.569, and 7.600, respectively, indicating a potential influence of the number of interacted key residues on the pIC_{50}.

Numerous studies have explored the significance of key residues in METTL3 through mutagenesis experiments, providing critical insights into its catalytic mechanism and structural dynamics. Among the identified key residues, Lys 513, ranked second in this study, has been extensively studied using the Lys513Ala
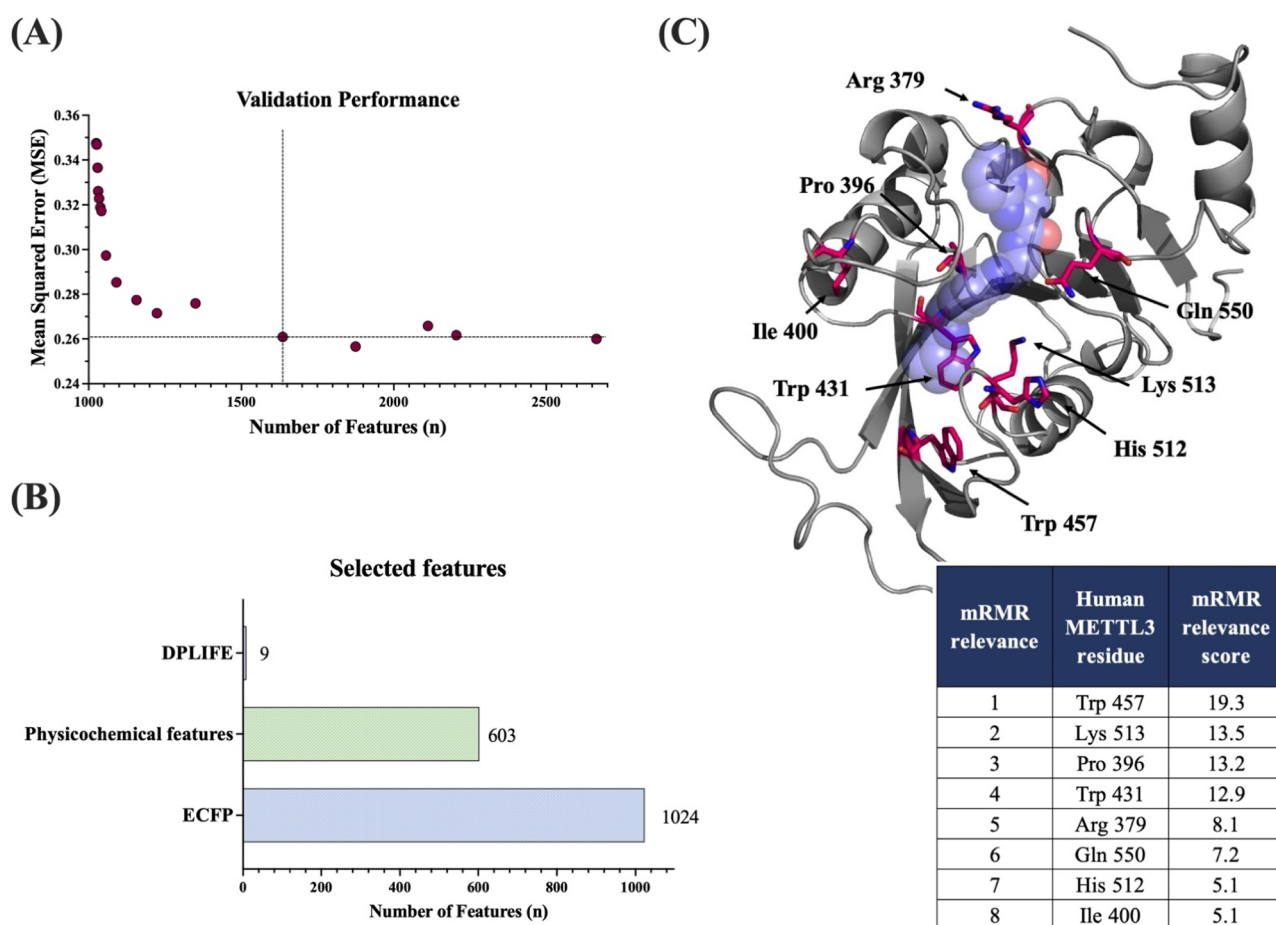

mRMR relevance	Human METTL3 residue	mRMR relevance score
1	Trp 457	19.3
2	Lys 513	13.5
3	Pro 396	13.2
4	Trp 431	12.9
5	Arg 379	8.1
6	Gln 550	7.2
7	His 512	5.1
8	Ile 400	5.1

Fig. 3. The mRMR-based forward feature selection. (**A**) The validation performance of top-ranked features with the highlighted final feature set. (**B**) Composition of the final feature set; the 603 selected physicochemical features were listed in Table S2. (**C**) The identified 8 key residues (red sticks) critical for METTL3 bioactivities (PDB ID: 7O2I). The inset table ranks these residues by mRMR relevance score.

mutant. This mutation abolished the recruitment of the ligand's adenosine and eliminated METTL3 catalytic activity, confirming its essential role in RNA methyltransferase function[59]. Similarly, His 512, ranked 7th in this study, exhibits flexibility in the apo state and was stabilized upon adenosine binding making the catalytic site accessible. Furthermore, Trp 457 and Trp 431, ranked 1st and 4th in this study, respectively, have been highlighted as essential aromatic residues forming the ligand-binding aromatic cages. These residues play pivotal roles in stabilizing the ligand within the METTL3 active site, a process vital for enzymatic activity[35]. Additionally, Pro 396, ranked third in this study, contributes significantly to ligand binding through its backbone carbonyl, which interacts with reactive groups and nearby ion pairs. This interaction was critical for the stability of the enzyme-ligand complex and efficient catalysis[59]. Likewise, Gln 550, ranked 6th in this study, was involved in stabilizing the hydroxyl groups of ribose in the ligand. Mutagenesis of this residue to alanine (Gln550Ala) was shown to moderately reduce enzymatic activity, indicating its supporting role in METTL3 function[60]. Furthermore, Pro 396 and Ile 400, ranked 3rd and 8th in this study, have been implicated as a key residue within gate loop 1 (Pro 396 - Gly 407)[61], a flexible region critical for regulating cofactor and substrate binding, as well as product release[62].

Independent test on external data of novel METTL3 inhibitors

Recent studies have introduced a series of novel bisubstrate analogs (BAs) as METTL3 inhibitors, with experimentally determined pIC_{50} values for 5 novel BAs (BA1, BA2, BA3, BA4, BA6) reported[59]. While the independent test on the test dataset has been conducted, an additional test on the unseen BAs structures can provide a better understanding of the model performance. The predicted pIC_{50} values using the ML3-mix-DPLIFE-FS model and experimentally determined pIC_{50} values of the 5 BAs were shown in Table 2. The BA2 and BA4, the two most effective inhibitors reported in the study, were predicted to be the best inhibitor with pIC_{50} values of 7.061 and 7.129, respectively, which closely aligned with the experimentally reported pIC_{50} of 8.046 and 7.745[59]. The CC between the predicted and experimental pIC_{50} values for all BAs was 0.727, with a mean squared error (MSE) of 0.418. BA6 exhibited the lowest predicted (6.021) and experimental (≤ 6.301) values, showing a promising tool for prioritizing inhibitors. Since experimental conditions can lead to a large variation of pIC_{50} values[63], some deviation was expected when comparing the predicted and experimental values.

For external benchmarking, the predictive performance of the ML3-mix-DPLIFE-FS model was evaluated against the recently reported deep learning-based bioactivity predictor HAC-Net[64] and alternative approaches, including NNscore 2.0[65], and GNINA 1.3[66] (Table S4). The ML3-mix-DPLIFE-FS model showed strong predictive capability, with predicted pIC_{50} values for BA1, BA2, BA3, BA4, and BA6 closely matching experimental measurements, achieving a Spearman correlation coefficient (ρ) of 0.90. In comparison, conventional molecular docking with AutoDock Vina yielded a lower correlation (CC 0.62, ρ 0.80). Nevertheless, HAC-Net predictions for pKa showed weak association with experimental data ($CC - 0.49$, $\rho - 0.30$) (Table 2 and S4). In addition, NNscore 2.0 yielded negligible correlations ($CC - 0.01$, $\rho - 0.30$), and GNINA predictions were similarly weak correlations ($CC - 0.43$, $\rho - 0.40$). These results indicated that, for this test set, the ML3-mix-DPLIFE-FS model exhibited higher correlation with METTL3 inhibitor bioactivity data.

Since the cocrystal structures of these BAs have been reported[59], the analysis of their binding poses and interactions with the identified 8 key residues can further validate the developed model. This study therefore highlighted the 8 key residues and the key residues within 4 Å of the ligand in the protein–ligand complex on the newly available crystal structures (Fig. 4). Results indicated that in every analyzed complex, at least 2 key residues were located in close proximity to the ligand, further substantiating their functional importance in inhibitor bioactivities. During the course of this study, three new METTL3-inhibitor cocrystal structures were published, allowing us to incorporate additional PDB entries (PDB IDs: 9G4S, 9G4U, and 9G4W) into our analyses. As shown in Fig. S2, the 8 key residues identified as essential for METTL3 bioactivity were consistently detected across all analyzed protein–ligand crystal structures. Analysis of these cocrystal structures further supports the elongated ligand binding pocket with 8 key residues in defining the binding pocket architecture required for METTL3 inhibition (Fig. S2).

Newly discovered compounds from reference[59]	Key residue interactions from reported structure*	Experimental pIC_{50}[59]	Predicted pIC_{50}	Docking score by AutoDock Vina[45] (kcal/mol)
BA1	Arg 379, Gln 550	6.461	6.965	− 8.0
BA2	Arg 379, Pro 396, Ile 400, His 512, Lys 513, Gln 550	8.046	7.061	− 7.3
BA3	Not available	7.678	7.039	− 8.4
BA4	Arg 379, His 512, Lys 513, Gln 550	7.745	7.129	− 6.9
BA6	Arg 379, His 512, Lys 513, Gln 550	≤ 6.301	6.021	− 8.5

Table 2. Comparison of predicted and experimentally reported bioactivities of recently reported METTL3 inhibitors. Columns included experimentally measured pIC_{50} values[59], the pIC_{50} values predicted by the ML3-mix-DPLIFE-FS model, and docking score predicted by the AutoDock Vina[45]. *Key-residue interactions from reported crystal structures were defined as those within 4 Å of the inhibitor.
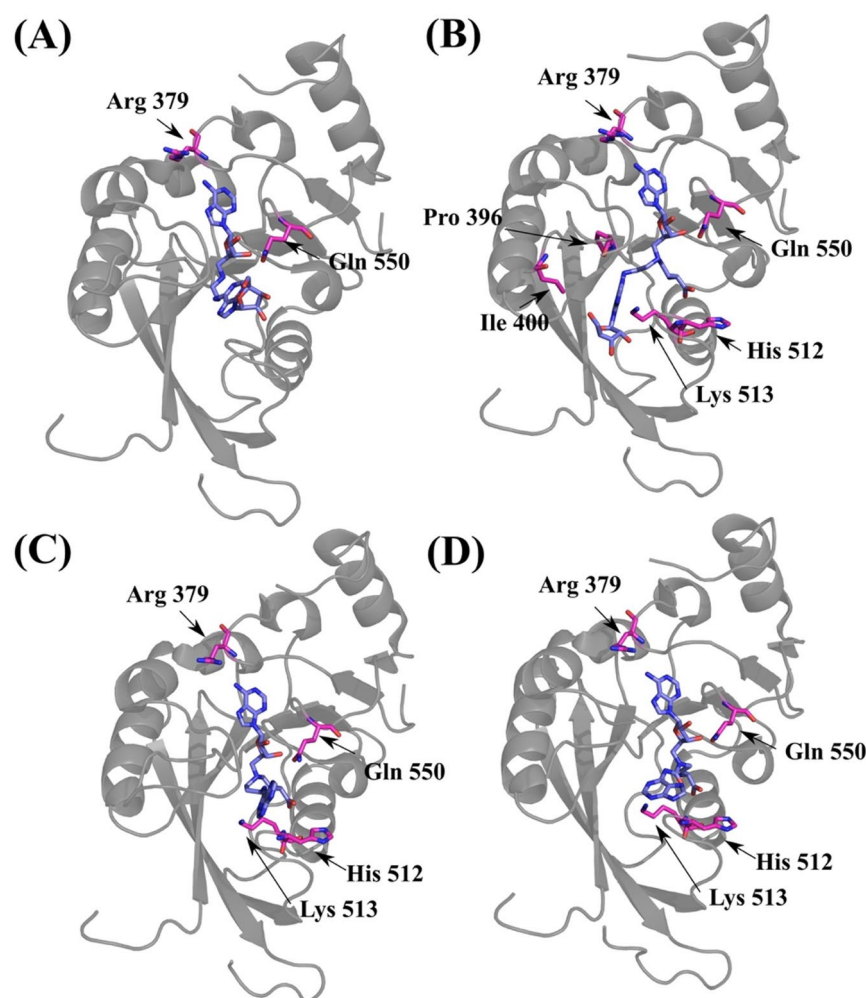
Fig. 4. Key residues relevant to METTL3 bioactivities in recently reported protein-inhibitor structures. (**A**) The METTL3-BA1 protein–ligand crystal structure (PDB ID: 8PW9). (**B**) The METTL3-BA2 protein–ligand crystal structure (PDB ID: 8PW8). (**C**) The METTL3-BA4 protein–ligand crystal structure (PDB ID: 8PWA). (**D**) The METTL3-BA6 protein–ligand crystal structure (PDB ID: 8PWB). The key residues within 4 Å of the ligand were highlighted in magenta stick representations.

Detailed analysis of METTL3–inhibitor cocrystal structures demonstrates that the ligand binding site is defined by the spatial arrangement of the 8 key residues: Arg 379, Pro 396, Ile 400, Trp 431, Trp 457, His 512, Lys 513, and Gln 550. These residues collectively shape an elongated binding pocket capable of accommodating a variety of inhibitors, as illustrated in Fig. 4 and Fig. S2. Notably, Trp 457 was consistently located within 4 Å of the bound ligands in the newly reported METTL3–inhibitor cocrystal structures (Fig. S2), indicating its frequent involvement as a lower pocket interaction residue. In contrast, this proximity was not observed in the BA-bound cocrystal structures (Fig. 4), suggesting potential differences in binding mode and pocket engagement between the newly reported inhibitors and the bisubstrate analogs. Further quantification of interaction contributions revealed that Trp 457 and Trp 431 are characterized by a high frequency of hydrophobic interactions with ligands (22.6% and 22.7%, respectively) (Table S3). Trp 431 also occasionally participates in π−π stacking interactions, highlighting the importance of both residues in providing aromatic and hydrophobic stabilization of the ligand. Hydrogen bonding is predominantly contributed by Arg 379, Pro 396, Gln 550, and Lys 513, with Gln 550 and Pro 396 exhibiting hydrogen bonding in 11.7% and 5.6% of cases, respectively. Additionally, Arg 379 forms salt bridges (0.2%) alongside hydrogen bonds, further anchoring the ligand within the binding pocket (Table S3). Notably, inhibitors exhibiting interactions with a greater number of these key interaction residues were observed to demonstrate enhanced effectiveness, highlighting the importance of these residues in the design of potent METTL3 inhibitors.

Conclusion

The discovery of METTL3 inhibitors has garnered significant interest in recent years due to their potential applications in cancer treatment[67] and their potential as antiviral therapies[68]. METTL3, a key catalytic subunit of the m[6]A RNA methyltransferase complex, plays a critical role in modulating oncogenic and antiviral pathways

by regulating RNA methylation. Targeting METTL3 provides a promising avenue for developing therapeutic interventions, particularly for cancers such as acute myeloid leukemia (AML), where it promotes the translation of oncogenic transcripts MYC and BCL2. This study introduces a novel method for METTL3 inhibitor discovery. In addition to conventional features of chemical fingerprint and physicochemical properties, the newly introduced DPLIFE features representing ligand–protein interaction information were found to be useful for improving model predictivity and interpretability. Eight key protein–ligand interaction residues were identified via feature selection, and the optimized ML3-mix-DPLIFE-FS model achieved high predictive accuracy, with predicted pIC_{50} values closely matching experimental IC_{50} data for benchmark inhibitors. Validation with additional BAs confirmed both the model performance and the relevance of these residues. Considering the importance of interaction type and its contribution to the final prediction, which was not fully addressed in this work, future studies could incorporate interaction type–wise features derived from advanced protein–ligand complex modeling methods, such as NeuralPLexer[69] and UMol[70], to further improve accuracy and generalizability. This approach not only provided a powerful METTL3-focused drug discovery tool for addressing unmet therapeutic needs in oncology and virology, but also demonstrated a combinational methodology that could be applied to rational drug design for other targets.

Data availability

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Author contributions

W.C.H. and C.W.T. designed and conducted the experiments, wrote the main manuscript, and prepared all figures and tables. C.W.T. and H.P.H. contributed to project management and participated in manuscript editing and review. All authors reviewed and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

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