Protein intrinsic disorder prediction using Attention U-Net and ProtTrans protein language model

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Abstract

The prediction of intrinsic disorder regions has significant implications for understanding protein function, structure, and dynamics. It can help to discover novel functions or protein-protein interactions essential to designing new drugs, therapies, or enzymes. Recently, a new generation of predictors based on protein language models is emerging. These algorithms reach state-of-the-art accuracy without calculating time-consuming multiple sequence alignments (MSAs). The article presents a new protein intrinsic disorder predictor DisorderUnetLM based on the Attention U-Net convolutional neural network using features from the protein language model ProtTrans. DisorderUnetLM shows top results in the direct comparison with fIDPnn and IDP-CRF predictors using MSAs and with the SETH predictor using features from the same ProtTrans model. Moreover, among 41 predictors from the latest Critical Assessment of Protein Intrinsic Disorder Prediction (CAID-2) benchmark, it ranks 9th for the Disorder-PDB subset (with ROC-AUC of 0.924) and 1st for the Disorder-NOX subset (with ROC-AUC of 0.844) which confirms its potential to perform well in the upcoming CAID-3 challenge for which DisorderUnetLM was submitted.

Keywords: deep learning, disorder prediction, language models, attention, u-net

Code availability: The inference code and trained models are available on the CodeOcean platform ensuring high reproducibility of the results: <u>doi.org/10.24433/CO.7350682.v1</u>.

1 Introduction

Functional regions in proteins can either be structured or disordered, and these can be considered as two fundamental classes of functional building blocks of proteins (van der Lee *et al.*, 2014). Protein intrinsic disordered regions are segments of proteins that have ambiguous three-dimensional structures in isolated conditions (Dyson and Wright, 2005; Uversky, 2011). They are important in identifying functions of a protein, because, due to their high flexibility, they can engage in numerous different chemical interactions, such as regulation, signalling, transcriptional, and translational processes (Tompa, 2012). Disordered regions can be resolved experimentally, e.g., using nuclear magnetic resonance (NMR) spectroscopy, but it is time-consuming and expensive (Uversky, 2011). Thus, the prediction of disordered regions from their amino acid sequences has become a popular research area in bioinformatics and benchmarks like CAID (Critical Assessment of Intrinsic Protein Disorder) (Necci *et al.*, 2021; Conte *et al.*, 2023) have emerged to assess and compare different predictors. Accurate prediction of disorder regions can help to discover novel functions or protein-protein interactions essential to designing new drugs, therapies, or enzymes.

The simplest predictors are based on the idea that disordered regions usually contain a significantly larger proportion of small and hydrophilic amino acids and proline residues than structured regions (Tompa, 2012). There are also classic approaches based on typical patterns of neighbouring amino acids, i.e., n-grams (or k-mers) (Liu *et al.*, 2008). However, machine learning and deep learning models using evolutionary information, e.g., PSSM (position-specific scoring matrices) (Rost and Sander, 1993) or HHblits (iterative protein sequence search according to the hidden profile) (Remmert *et al.*, 2012) have quickly dominated the benchmarks (Conte *et al.*, 2023; Hu *et al.*, 2021; Hanson *et al.*, 2019; Dass *et al.*, 2020; Akdel *et al.*, 2022; Stapor *et al.*, 2022). These approaches can learn complex patterns from similar sequences and capture subtle features of intrinsic disorder regions. Evolutionary information provides much better features than aminoacid sequences alone (Stapor *et al.*, 2022), but is very computationally expensive to obtain. Recently, pre-trained language models based on the idea of attention and transformers (Vaswani *et al.*, 2017) have been adopted for the protein secondary structure and disorder prediction and they show state-of-the-art results (Ilzhöfer *et al.*, 2022; Elnaggar *et al.*, 2021; Kotowski, Fabian, *et al.*, 2022; Jumper *et al.*, 2021). They are called protein language models and they implicitly embed the evolutionary information in their compact feature space, which allows them to provide better features in a fraction of the time needed for classic multiple sequence alignments.

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The current article follows this trend and presents DisorderUnetLM – a convolutional Attention U-Net (Oktay *et al.*, 2018) architecture using features from the ProtTrans protein language model (Elnaggar *et al.*, 2021). The idea is largely based on our previous ProteinUnetLM (Kotowski, Fabian, *et al.*, 2022) network which showed state-of-the-art results in protein secondary structure prediction. Main novelties are related to (1) the modified output of the network, i.e., binary disorder prediction instead of 8-class secondary structure prediction; (2) additional mechanisms to prevent overfitting for smaller datasets, i.e., fewer convolutional units, weights regularization, stronger dropout, and earlier stopping; (3) ensembling procedure adopted from the first version of ProteinUnet (Kotowski *et al.*, 2021) to boost performance in the CAID-2 benchmark (Conte *et al.*, 2023) and in the latest CAID-3 challenge (caid.idpcentral.org/challenge), for which DisorderUnetLM has been submitted.

2 Materials and Methods

DisorderUnetLM was implemented in the environment containing Python 3.8 with TensorFlow 2.9 accelerated by CUDA 11.7 and cuDNN 8. The inference code and trained models are available on the CodeOcean platform (doi.org/10.24433/CO.7350682.v1) ensuring high reproducibility of the results.

2.1 Datasets

There are 6 datasets used in our study. They are listed in Table 1 with source links and numbers of training, validation, and test sequences as defined by the datasets' authors. There are 3 training sets for flDPnn (Hu *et al.*, 2021), CheZOD (Ilzhöfer *et al.*, 2022), and IDP-CRF (Liu *et al.*, 2018), and they are used to train 3 different versions of the DisorderUnetLM model for a fair and direct comparison with the corresponding predictors (i.e., flDPnn, SETH, and IDP-CRF) in the Results section. The final DisorderUnetLM version submitted to the CAID-3 challenge uses all 8799 sequences for training.

The smallest training set of 445 sequences belongs to the flDPnn dataset. It was introduced together with a predictor of the same name (Hu *et al.*, 2021). It is the only dataset that explicitly defines a validation set (with 100 sequences). The test set of 176 sequences has <25% similarity with the training set. Together, this gives a compact benchmarking dataset with 721 sequences from the DisProt 7.0 database (Piovesan *et al.*, 2017).

The CheZOD (Chemical shift Z-score for quantitative protein Order and Disorder assessment (Nielsen and Mulder, 2016)) dataset with 1174 training and 117 testing sequences is taken from the article about the SETH predictor (Ilzhöfer *et al.*, 2022). Unlike in other datasets, the CheZOD ground truth is not binary. It quantifies the degree of disorder based on the assigned Z-scored nuclear magnetic resonance chemical shifts, ranging between -4 for complete disorder and 16 for complete order with a value of 8 corresponding to an intermediate position in the disorder continuum. For purposes of this study, the CheZOD ground truth was binarized, so all residues with CheZOD scores higher than 8 are marked as ordered and the rest are marked as disordered.

The IDP-CRF is the largest training set in the study with 4590 sequences from MobiDB (Potenza *et al.*, 2015) and 683 sequences from DisProt 7.0 (Piovesan *et al.*, 2017) database. The MxD (Mizianty *et al.*, 2010) dataset with 514 sequences (319 from DisProt 5.0 (Sickmeier *et al.*, 2007) and 205 from Protein Data Bank (PDBe-KB consortium, 2022)) is the oldest in the list and is mainly used to compare the results with the IDP-CRF predictor.

The testing datasets from CAID (Necci *et al.*, 2021) and CAID-2 (Conte *et al.*, 2023) benchmarks contain 652 and 348 sequences from the DisProt database, respectively. Specifically, these numbers concern the Disorder-PDB version of the benchmark which only includes ordered regions if they are observed in the Protein Data Bank database (PDBe-KB consortium, 2022). There are also subsets of sequences where all residues are marked as structured unless they were experimentally annotated as disordered (called Disorder in CAID and Disorder-NOX in CAID-2). Results for both versions are reported in our study.

Dataset name and ref-	Link to download	Number of sequences			
erence		Training	Validation	Testing	Total
flDPnn	biomine.cs.vcu.edu/servers/flDPnn/	115	100	176	721
(Hu et al., 2021)		445	100	170	/ 2 1
CheZOD (binarized)	github.com/DagmarIlz/SETH	1174		117	1201
(Ilzhöfer et al., 2022)		11/4	-	117	1291
IDP-CRF	mdpi.com/1422-0067/19/9/2483/s1	5273			5273
(Liu et al., 2018)		5275	-	-	5215
MxD	biomine.cs.vcu.edu/servers/MFDp/MxD.txt			514	514
(Mizianty et al., 2010)		-	-	514	514
CAID Disorder	doi.org/10.24433/CO.3610625.v1			652	652
(Necci et al., 2021)		-	=	052	052
CAID-2 Disorder	caid.idpcentral.org/assets/sections/challenge/stati			249	249
(Conte et al., 2023)	c/references/2/disorder_pdb.fasta	-	=	546	540
Total		6892	100	1807	8799

Table 1. List of datasets used in the study with numbers of sequences in training, validation, and testing sets as defined by their authors.

2.2 Attention U-Net for protein intrinsic disorder prediction

U-Net is a state-of-the-art architecture in image segmentation tasks (Isensee *et al.*, 2021; Kotowski, Adamski, *et al.*, 2022; Isensee *et al.*, 2022) and we previously successfully introduced it into the domain of protein secondary structure prediction by creating the ProteinUnet model (Stapor *et al.*, 2022; Kotowski *et al.*, 2021). For the disorder prediction, we base on our latest Attention U-Net architecture of ProteinUnetLM (Kotowski, Fabian, *et al.*, 2022) using features from the ProtTransT5-XL-U50 protein language model (Elnaggar *et al.*, 2021) as input. The detailed architecture is presented in Figure 1, for purposes of this article, it is called DisorderUnetLM. Unlike in ProteinUnetLM, for disorder prediction, we do not use amino acid sequences as additional input because our ablation study in Supplementary Table S1 showed no advantage of such input. Moreover, we decreased the number of convolutional layers at each level or U-Net from 64/128 to 32/64 and increased the dropout rate from 0.1 to 0.25 to avoid overfitting due to smaller training sets and smaller output dimensionality (8-class secondary structure vs binary disorder states). All other hyperparameters are the same as in the ProteinUnetLM. Specifically, we have 2 convolutions with 1D kernels of length 7 and ReLU activations in all blocks. Overall, the model has 628,710 trainable parameters.



Figure 1. The detailed architecture of DisorderUnetLM. Symbols x^{l} and g correspond to the input features and attention coefficients as denoted in Figure 2.

The network learns higher-level features in convolutional contractive paths, concatenates them, and passes them to the additive attention gates (AGs) presented in Figure 2. AGs learn to select and focus (give attention) on the most important features passed by skip connections (Vaswani *et al.*, 2017; Oktay *et al.*, 2018). The output of the AG can be treated as a saliency map which gives high weights to relevant features and low weights to irrelevant ones. Information extracted from lower-scale features is used as a gating signal to disambiguate irrelevant and noisy responses in skip connections. AGs are active both during backward pass (training) and forward pass (prediction), and their role is to filter irrelevant parts of the input features. This should allow for better generalization of the network and improved robustness to noisy data. Finally, the filtered features are passed to the convolutional expanding path that learns to predict the disorder probability as the output layer with softmax activation connected to the last up-block (Figure 1).

DisorderUnetLM takes a sequence of feature vectors $X = (x_1, x_2, x_3, ..., x_N)$ as input, where x_i is the feature vector corresponding to the *i*th residue, and returns a vector $Y = (y_1, y_2, y_3, ..., y_N)$ as output, where y_i is a probability of *i*th residue being in the disordered state. If the probability is greater than 0.5 the residue is marked as disordered. The input sequence length is limited to 7168 which covers all proteins used in this study and nearly all proteins available in the latest DisProt (Aspromonte *et al.*, 2024) database (excluding only Titin with 34350 amino acids). For each amino acid, there are 1024 features from the ProtTransT5-XL-U50 protein language model (Elnaggar *et al.*, 2021). Each feature is standardized across all training residues to ensure a mean of 0 and a standard deviation of 1.



Figure 2. Schematic of the additive attention gate (AG). Input features (x^l) are scaled with attention coefficients (α) computed in AG. Spatial regions are selected by analysing both the activations and contextual information provided by the gating signal (g) which is collected from a coarser scale. Grid resampling of attention coefficients is done using trilinear interpolation. Source: (Oktay et al., 2018).

2.3 Training procedures and loss function

Following the ProteinUnetLM (Kotowski, Fabian, *et al.*, 2022) procedures, DisorderUnetLM was trained to simultaneously minimize the binary cross-entropy (BCE, Equation 1) and maximize the Matthews correlation coefficient (MCC, Equation 2) by defining a loss function as a difference between average BCE and average MCC across the training batch (Equation 3).

$$BCE = \mathbf{y} \log(\hat{\mathbf{y}}) + (1 - \mathbf{y})\log(\hat{\mathbf{y}}), \quad \text{where } \mathbf{y} \text{ is a target vector and } \hat{\mathbf{y}} \text{ is a model output,}$$
(1)

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN) + e}}$$
(2)

where $TP = \mathbf{y} \cdot \hat{\mathbf{y}}$, $TN = (\mathbf{1} - \mathbf{y}) \cdot (\mathbf{1} - \hat{\mathbf{y}})$, $FP = (\mathbf{1} - \mathbf{y}) \cdot \hat{\mathbf{y}}$, $FN = \mathbf{y} \cdot (\mathbf{1} - \hat{\mathbf{y}})$, and e is a very small number preventing division by zero,

$$Loss = BCE - MCC \tag{3}$$

(2)

Adam optimizer (Kingma and Ba, 2015) is used with a batch size of 8 and an initial learning rate of 0.001. The learning rate is reduced by a factor of 10 when there is no improvement in the validation loss in consecutive epochs. The training is stopped when the validation loss is not improving for 4 epochs and the checkpoint with the lowest validation loss among all epochs is selected as the final model.

Ensembling for the CAID-2 benchmark and the CAID-3 challenge

To train the final DisorderUnetLM model for purposes of the CAID-3 challenge, we use the ensembling procedure introduced in ProteinUnet (Kotowski *et al.*, 2021). All collected datasets (including test sets) are merged into a single training set of 8799 sequences and a 10-fold stratified cross-validation is performed. The folds are stratified based on the sequence lengths and ratios of disordered residues. The 10 resulting models (each trained on 9 folds and validated on the remaining one) are ensembled by taking the average of their output probabilities for each residue. If the average probability is greater than 0.5 the residue is marked as disordered. Note that this final ensemble is not tested in the current article as all available data are used for training to maximize the result in the upcoming CAID-3 challenge. However, the described ensembling is used to evaluate the model on the CAID-2 test set (excluding this test set from the training data).

2.4 Metrics and evaluation procedures

MCC has been evaluated as one of the most reliable, universal, and informative metrics in machine learning and bioinformatics problems in the literature (Chicco and Jurman, 2020; Chicco *et al.*, 2021; Abhishek and Hamarneh, 2021). MCC is also commonly used as the primary metric in the domain of intrinsic disorder prediction (Hu *et al.*, 2021; Liu *et al.*, 2018; Hanson *et al.*, 2018) and addresses the imbalance problem of disorder prediction (there are only 14.6% disordered residues in all collected datasets). For these reasons, MCC is used both in the loss function and as the primary metric in our study. The area under receiver operating characteristic (ROC-AUC) and F1-score are also calculated to directly compare the results of DisorderUnetLM with the results of other predictors as reported in the literature.

3 Results

In this section, DisorderUnetLM is benchmarked against evaluation procedures proposed by authors of flDPnn (Hu *et al.*, 2021), IDP-CRF (Liu *et al.*, 2018), and SETH (Ilzhöfer *et al.*, 2022) predictors, and against 41 predictors from the latest CAID-2 (Conte *et al.*, 2023) competition.

Following the procedures from the article about the flDPnn predictor, a single DisorderUnetLM trained on the flDPnn training set is compared with 5 predictors (flDPnn (Hu *et al.*, 2021), ESpritz-D (Walsh *et al.*, 2012), SPOT-Disorder-Single (Hanson *et al.*, 2018), IUPred2A-long (Mészáros *et al.*, 2018), and IUPred-2A-short (Mészáros *et al.*, 2018)) on the flDPnn test set (Figure 3) and with 10 predictors (flDPnn (Hu *et al.*, 2021), flDPlr (Hu *et al.*, 2021), RawMSA (Mirabello and Wallner, 2019), ESpritz-D (Walsh *et al.*, 2012), DisoMine (Orlando *et al.*, 2022), SPOT-Disorder2 (Hanson *et al.*, 2019), AUCpreD (Wang *et al.*, 2016), SPOT-Disorder-Single (Hanson *et al.*, 2018), AUCpreD-np (Wang *et al.*, 2016), PreDisorder (Deng *et al.*, 2009)) on the well-established CAID Disorder-PDB test set (Figure 4). DisorderUnetLM results on the flDPnn test set are comparable to the results of the flDPnn predictor. Our model is slightly better in terms of F1-score (0.629 vs 0.626), but slightly worse in ROC-AUC (0.835 vs 0.839) and MCC (0.478 vs 0.491) metrics. However, DisorderUnetLM shows a clear advantage over the flDPnn predictor on the larger CAID test set in every metric, F1-score (0.516 vs 0.483), ROC-AUC (0.826 vs 0.814), and MCC (0.414 vs 0.370). Both DisorderUnetLM and flDPnn overcome other predictors by a large margin in all metrics.



Figure 3. Comparison of DisorderUnetLM (marked in red) with 5 other predictors on the flDPnn test set. The visualization is adapted from the article about the flDPnn predictor (Hu et al., 2021).



Figure 4. Comparison of DisorderUnetLM (marked in red) with 10 other predictors on the CAID test set. The visualization is adapted from the article about the fIDPnn predictor (Hu et al., 2021).

To confront DisorderUnetLM with the IDP-CRF (Liu *et al.*, 2018) predictor, it was trained on the IDP-CRF training set using a random 10% of sequences as a validation set and tested on the MxD dataset. The results of 6 other predictors (MFDp (Mizianty *et al.*, 2010), MD (Schlessinger *et al.*, 2009), PONDR-FIT (Xue *et al.*, 2010), DISOPRED2 (Ward *et al.*, 2004), IUPred-long (Dosztányi *et al.*, 2005), and PONDR VSL2B (Peng *et al.*, 2006)) are also given for comparison in Figure 5. Our network dominated the competition by a large margin in the MCC metric (0.583 vs 0.460 for the second-best IDP-CRF). However, the competing predictors are relatively old and do not use advanced evolutionary information or protein language models.



Figure 5. Comparison of DisorderUnetLM (marked in red) with 7 other predictors on the MxD test set.

Following the procedures from the article about the SETH predictor, the binarized CheZOD training set was used to train a single DisorderUnetLM model using a random 10% of sequences as a validation set. In Figure 6, ROC-AUC scores on the binarized CheZOD test set were compared with 15 selected predictors described in the SETH article. Besides DisorderUnetLM, two predictors use features from protein language models – SETH and ADOPT-Esm1b. They show a clear advantage over the 9 predictors using evolutionary information from multiple sequence alignments (ODiNPred (Dass *et al.*, 2020), SPOT-Disorder (Hanson *et al.*, 2017), AlphaFold2-rsa-25 (Akdel *et al.*, 2022), AUCpreD (Wang *et al.*, 2016). MetaDisorder (Kozlowski and Bujnicki, 2012), MFDp2 (Mizianty *et al.*, 2013), PrDOS (Ishida and Kinoshita, 2007), DISOPRED3(Jones and Cozzetto, 2015), flDPnn(Hu *et al.*, 2021)) and remaining 4 using only amino acids sequences (AUCpreD-noEvo (Wang *et al.*, 2016), IUPred (Dosztányi *et al.*, 2005), DISPROT VSL2b (Vucetic *et al.*, 2005)). DisorderUnetLM achieves the same ROC-AUC score (0.910) as SETH. However, SETH was trained using continuous CheZOD scores which should give some advantage on the CheZOD test set, thanks to more detailed information beyond the binarized disorder status. Thus, DisorderUnetLM proved its effectiveness.



Figure 6. Comparison of DisorderUnetLM (marked in red) with 15 selected disorder predictors on the binarized CheZOD test set. 2 predictors use features from protein language models (marked in light red), 9 predictors explicitly use evolutionary information (marked in blue) and 4 predictors use only classic features from amino acid sequences (marked in green).

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Finally. CAID DisorderUnetLM compared with 41 predictors available the Prediction Portal was at (https://caid.idpcentral.org/challenge#Benchmarking). To maximize the performance of the model, all the collected datasets (excluding the CAID-2 test set) were used to train an ensemble of 10 DisorderUnetLM models as described in Methods. As presented in Figure 7 and Figure 8, the ensembled DisorderUnetLM achieved the 9th best ROC-AUC for CAID-2 Disorder-PDB (0.924 vs 0.949 for the best SPOT-Disorder2 (Hanson et al., 2019)) and is the best algorithm for smaller CAID-2 Disorder-NOX test set (0.844 vs 0.838 for the second best Dispredict3 (Kabir and Hoque, 2024)). It shows the potential of DisorderUnetLM to achieve top results in the upcoming CAID-3 as well.



Figure 7. The comparison of methods on CAID-2 Disorder-PDB dataset. Our proposed DisorderUnetLM is marked in red.



CAID-2 Disorder-NOX

Figure 8. The comparison of methods on CAID-2 Disorder-NOX dataset. Our proposed DisorderUnetLM is marked in red.

4 Conclusion

The Attention U-Net using features from the ProtTrans protein language model proved their high utility in the task of protein intrinsic disorder prediction, just like it recently did in the domain of protein secondary structure prediction (Kotowski, Fabian, *et al.*, 2022). In this study, DisorderUnetLM is compared with more than 50 predictors in 6 different evaluation scenarios. It shows top results in direct comparisons with flDPnn (Hu *et al.*, 2021) and IDP-CRF (Liu *et al.*, 2018) predictors using classic and evolutionary features, and with the SETH (Ilzhöfer *et al.*, 2022) predictor using features from the same ProtTrans model. Moreover, it is ranked in the top 10 best-performing methods among 41 predictors in the CAID-2 benchmark (9th place in Disorder-PDB with ROC-AUC of 0.924 and 1st place in Disorder-NOX test sets with ROC-AUC of 0.844) and has potential to perform well in the upcoming CAID-3 challenge for which it was submitted.

The convolutional Attention U-Net architecture is characterized by relatively fast training and inference as compared to recurrent neural networks for protein structure prediction (Kotowski, Fabian, *et al.*, 2022). Additionally, DisorderUnetLM does not use computationally expensive evolutionary features but the output of the ProtTrans model - calculated in a fraction of a second per sequence. It is useful in large-scale predictions and low-grade devices. We share the complete code and models on the CodeOcean platform to support the reproducibility of our work and to encourage the community of protein scientists to use our method in their research, e.g., to study functions of proteins (Babu *et al.*, 2012), protein-protein interactions (Roterman *et al.*, 2023), or cellular signalling and regulation (Wright and Dyson, 2015). In the future, the Attention U-Net architecture can be easily adapted to many other use cases like a prediction of continuous CheZOD scores (Ilzhöfer *et al.*, 2022), binding sites, or linkers.

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Supplementary Material

The exact version of the ProtTransT5-XL-U50 model used is the study can be downloaded from <u>https://huggingface.co/Rostlab/prot_t5_xl_uniref50/blob/main/pytorch_model.bin</u> and has been run_using Prot-TransT5XLU50Embedder class from bio_embeddings 0.2.2 Python library (<u>https://github.com/sacdallago/bio_embeddings/releases/tag/v0.2.2</u>).

Supplementary Table S1. Ablation study of DisorderUnetLM trained on the flDPnn training set and tested on the CAID dataset.

Model	MCC	F1	ROC-AUC
DisorderUnetLM	0.411	0.516	0.825
With AA on input	0.385	0.493	0.817
With AA on input and 64 lay- ers (like in ProteinUnetLM)	-0.002	0.0	0.797