Protein secondary structure prediction using multilayer fully connected neural networks

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Introduction

Proteins serve as the foundational functional units within biological systems, constructed from amino acid chains that intricately fold into simple or highly complex structures. Notably, proteins exhibit four distinct levels of structural organization: primary, secondary, tertiary, and quaternary structures (1,2). These structures are not easy to identify and require multiple experiments, thus extensive efforts have been made to unravel protein structure from their constituent amino acid sequences. This report delves into the realm of secondary protein structures that yield 2D conformations such as α-helices, β-sheets, and coils.

Modern approaches in secondary structure prediction harness advanced computational techniques, prominently including Artificial Neural Networks (ANN). These methods have showcased remarkable prediction accuracies of up to 84%, employing diverse model architectures such as feedforward deep networks, convolutional neural networks (CNNs), and recurrent neural networks (RNNs) (3). Notably, a recent attempt (4) demonstrated that even simple architectures, like their three-layer dense feedforward network, achieved a commendable 78% accuracy. However, a need for further enhancement persists, advocating for exploration of various hyperparameters to attain superior performance. On that note, here we introduced a fully connected neural network model (**Figure 1)** that yields a 76% overall accuracy in the prediction of secondary structures from amino acid sequences.

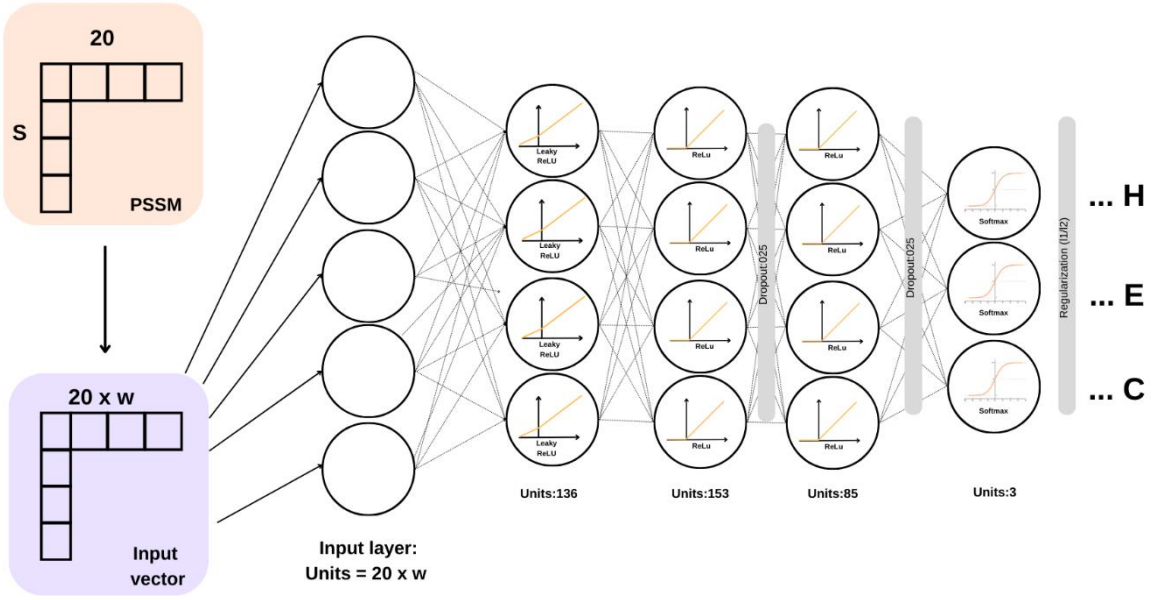

Figure 1. Multilayer feed forward neural network architecture.

Materials/methods

Datasets

Training, validation and blind-test datasets were obtained from (5) Github repository. In short, and as mentioned in her repository; the training set corresponds to proteins, originally taken from a work of (6), that had a resolution < 2.5 A, with residues in the 30-800 range, that display full domains, had DSSP information available, and full-profiles for a total of 1200 proteins with their corresponding Position Specific Scoring Matrix (PSSM) and DSSP assignment. The cross-validation set was generated by partitioning the training set in five equal folds (each one with 240 proteins). Lastly, the blind set comprised 328 sequences with their corresponding PSSM and DSSP.

Feature extraction

Extensive documentation within the literature mention the utilization of Position-Specific Scoring Matrices (PSSM) for protein structure prediction. A PSSM comprises a matrix with 20 columns representing amino acids and S rows corresponding to each residue in the sequence, where values denote the frequency of residues at each position providing evolutionary insights (7–10). To obtain positional information from the PSSM, a sliding window technique was employed to analyze the residue of interest alongside a window of neighboring residues. This entailed the creation of a new vector of dimensions 20 x W, where 'W' signifies the window size. An array containing all such vectors serves as input for the initial input layer of our neural network.

Network architecture

We started with a simple network: a multilayer feed forward network with one input layer with an equal number of units corresponding to the width of the represented PSSM matrix . A Bayesian Optimization and Hyperband algorithms from Keras tuner were (11) to obtain a first skeleton of an optimal architecture for the model using all the training and test data. Selecting for number of layers (maximum of five layers), number of units per layer (min:17, max:240), dropout layers and activation functions.

Performance optimization

Following the work of Drozdetskiy et., al (4) we focused on improving the initial network by tuning the window size for feature selection, number of epochs for training, activation functions (‘*leaky ReLU*’, *‘tanh’* and *‘ReLU’),* adding regularization techniques such as L1-L2 for weight penalization and dropout to add some randomness to the model to avoid overfitting. Batch normalization was also tested. To see all the process see: GITHUB

Results

Network architecture

The models chosen by Hyperband and the Bayesian algorithms were similar. The best model from the Bayesian algorithm was selected for optimization **(Table 1).** Utilizing the cross-validation sets, we scrutinized different window sizes for feature extraction from the Position-Specific Scoring Matrix (PSSM). After evaluation, a window size of 17 emerged as optimal, yielding superior accuracy results across both training and validation sets **(Supplementary Figure 1a and 1b**). Furthermore, the number of epochs revealed that employing 10 epochs led to rapid overfitting, evidenced by pronounced accuracy deviations in both training and validation sets **(Supplementary Figure 1c).**

Table 1. Best model architecture based on Keras Tuning utils

Tuner	Bayesian	Hyperband
Best model	activation: ReLU dropout: False num_layers: 4 Input_layer: 340 units_0: 136 units_1: 153 units_2: 85 Output_layer: 3 Score: 0.7494206428527832	num_layers: 4 activation: ReLU dropout: False Input_layer: 340 units_0: 136 units_1: 102 units_2: 204 Output_layer: 3 Score: 0.7489377856254578

Thus, 4 epochs were selected as the ideal training duration. An activityRegularization layer incorporating L1 and L2 regularization was strategically introduced after the final layer, with experimentation revealing that a weight value of 1e-5 improved accuracy on the validation set (**Supplementary Figure 2**). While ReLU activation was employed across all layers, the implementation of *'leaky ReLU'* in the second layer notably improved the model's learning capacity and overall accuracy. Lastly, the incorporation of dropout layers within hidden layers further augmented model accuracy to a mean of 0,7592 (±0,0043) **(Figure 2).**

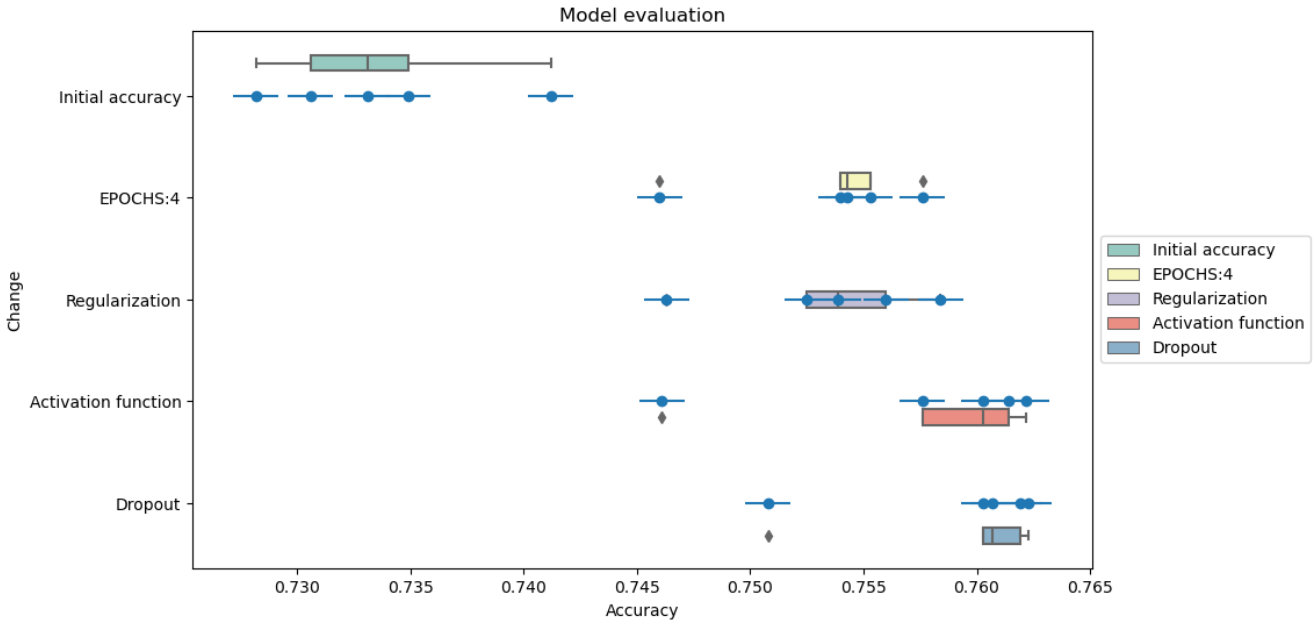

Figure 2. Model accuracy during the hyperparameter search across cross-validation sets (seen in blue) for the different changes implemented: change in the number of epochs, regularization techniques for the loss function in the last layer, addition of 'leaky ReLU' as an activation function and lastly the addition of dropout layers within hidden layers.

Network performance.

All cross-validation models were tested against a blind-set yielding an average accuracy of 76% (± 0.005) **(Supplementary table 2)**. A confusion matrix was calculated **(Figure 3 and supplementary figure 4**) from which scores were assessed for all predicted classes (H, E, C) **(Table 2)**. Helices as the class with better scores overall.

Class	Precision	SD	Recall	SD	F1	SD
H	0.82110142	0.0117517	0.80378451	0.01053579	0.81224197	0.00593778
E	0.72351835	0.01214003	0.60701534	0.00902925	0.66007415	0.00683902
C	0.72952938	0.00630561	0.80352486	0.008741	0.76467704	0.00257725

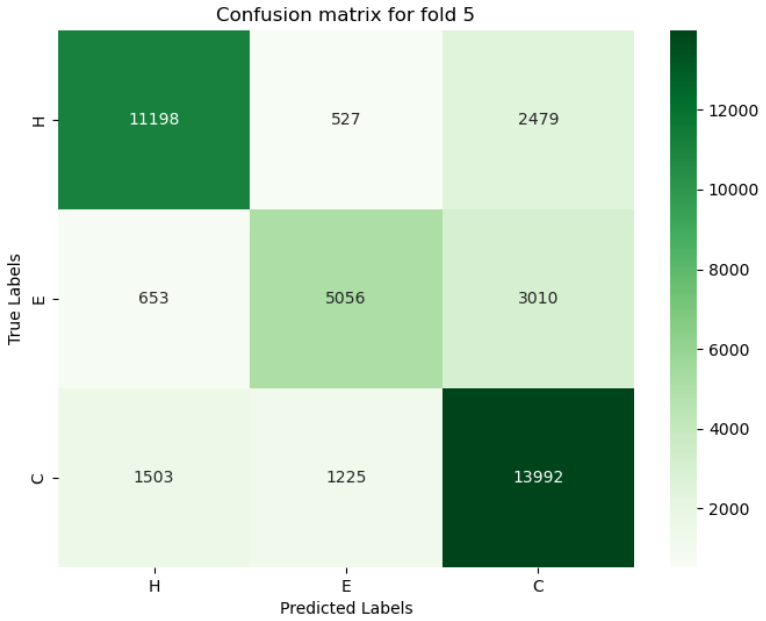

Figure 3. Confusion matrix for blind test using model of cross-validation train set number five.

Conclusion/discussion

In conclusion, this project underscores the efficacy of regularization techniques, notably regularization with L1 and L2 and dropout, in mitigating overfitting during training, thereby enhancing model generalization (going from 74% to 76% accuracy). The careful selection of activation functions also played a pivotal role in improving model performance. Likewise, the consistent performance observed across cross-validation sets underscores the reliability of our model. However, exploring alternative architectures and incorporating specific features to provide the network with additional structural and geometrical information may yield further improvements in accuracy.

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