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Protein thermostability prediction using sequence representations from protein language models

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Course work project

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Contents

1 Introduction

This work is a prolongation of the previous work - the model that performed binary classification into thermostability classes. The model, which was a single-layer perceptron (SLP), took protein language model's ESM-1b [1] protein embeddings as input and provided prediction for each protein, how likely it belongs to the thermostable class.

The classes of thermostability were defined the following way: proteins that were considered as stable in lower that 65 degrees of Celsius were labelled with '0' and the remaining proteins were labelled with '1'.

The classifier was trained on the data set [2] that contained proteome identifiers, which were used to collect proteins. The data set also contained information about organism's growth temperature, therefore proteins that belonged to a particular proteome were labelled accordingly.

Nevertheless the classifier showed promising results, yet an important downside of the developed method was emphasized - since ESM-1b embeddings generation was limited by the size of the protein, the model could not provide predictions for proteins that were longer than 1022 amino acids. For this reason, it was decided to try ProtTrans [3] embeddings as an input for the classification model.

Furthermore, it was interesting to exploit not only mean embeddings, but also to check, whether a different connection of per residue embeddings would improve the performance of the classification model.

In addition to these fixed tasks, it was determined to examine whether other variants of model architectures would elaborate the classifier's results.

The outcomes of this collection of tasks will contribute in creation of the final definition of the method for binary protein classification into thermostability classes.

2 Abstract in Lithuanian (Santrauka)

Šis darbas yra ankstesnio darbo - binarinę baltymų klasifikaciją pagal termostabilumą vykdančio modelio vystymo - tęsinys. Vystomas modelis buvo vieno sluoksnio perceptronas, kurio įvestis ESM-1b [1] baltymų kalbos modelio generuojamos skaitinės reprezentacijos, o išvestis - prognozė kiekvienam baltymui, kaip tikėtina, kad jis priklauso termostabilių baltymų klasei.

Termostabilumo klasės buvo atskirtos 65 Celsijaus laipsnių riba: baltymai, kurie yra stabilūs žemesnėje nei nurodyta riba temperatūroje, priskiriami klasei su žyme '0', o stabilūs baltymai 65 laipsnių ir aukštesnėje temperatūroje yra laikomi klasės '1' nariais.

Minėtas klasifikatorius buvo apmokytas naudojant duomenų rinkinį, kuriame saugomos organizmų augimo temperatūros [2]. Į duomenų rinkinį taip pat buvo įtraukti organizmų proteomų identifikatoriai, kurie buvo panaudoti surinkti proteomui priklausančius baltymus ir juos sužymėti pagal organizmo augimo temperatūrą.

Nepaisant to, kad klasifikatorius pateikė neblogus rezultatus, testuojant metodą išryškėjo svarbus trūkumas. Kadangi ESM-1b skaitinių reprezentacijų kūrimas buvo apribotas baltymo ilgiu, nebuvo galima sugeneruoti reprezentacijų baltymams, kurie buvo ilgesni nei 1022 aminorūgštys. Dėl šios priežasties buvo nutarta išmėginti ProtTrans [3] skaitines reprezentacijas kaip klasifikatoriaus įvestį, nes šis baltymų kalbos modelis buvo apmokytas be apribojimo baltymo ilgiui.

Taip pat domino išmėginti ne tik suvidurkintas skaitines reprezentacijas, bet ir išnaudoti galimybę išgauti kitaip apibendrintas kiekvienos aminorūgšties reprezentacijas bei patikrinti, ar gauti kitokie įvesties vektoriai suteikia geresnius rezultatus.

Apart reprezentacijų analizės, taip pat buvo atlikti eksperimentai patikrinimui, ar kitokios modelių architektūros turės ženklios įtakos modelio veikimui.

Gautos analizių išvados suteiks naudingų įžvalgų galutinio metodo baltymų klasifikacijai pagal termostabilumą apibrėžimui.

3 Theory

3.1 ProtTrans embeddings

ProtTrans [3] is a collection of protein language models (LMs) that were trained to learn information about proteins and encode it. ProtTrans embeddings are vector representations taken from the last hidden state of the protein LM.

In particular, ProtT5-XL model was used in this work and exactly this model will be referred to by the name of 'ProtTrans'. Overall, this model has 24 layers. In particular, the size of the hidden layer, from which the embedding is taken, is 1024. This model was trained on BFD-100 data set and fine-tuned on UniRef50. Since ProtT5-XL model was considered by the authors as the best-performing model, it was chosen to be applied in this work. Additionally, this model does not have positional encoding limit, which means that there is no limitation for the protein's size to generate its embedding.

Furthermore, according to the article, in which ProtTrans project was published, ProtT5-XL models (trained on BFD-100 and UniRef50 data sets separately) outperformed ESM-1b [1] model.

4 Methods

4.1 Objective of this work

The main objective of this work is to analyse which numerical representation of proteins is the most suitable to use as input for the neural network model that solves our thermostability prediction problem. Additionally, it was decided to try model architectures with one or two hidden layers and evaluate, whether the different architecture improves the performance.

4.2 Data set

In this work two types of data sets were used: for the analysis of principal components' the data set was inherited from the previous work, although the analysis of other representations was carried out using the data set that was filtered from identically matching sequences to get more accurate evaluations.

Table 1: Number of sequences with embeddings before and after filtering the data set

Subset	Original	After filtering
Training	284309	283360
Validation	65156	63158
Testing	73662	73308

Table 2: Number of sequences with embeddings in each class before and after filtering the data set

Class	Original	After filtering
0	216595	212129
1	212729	207697

4.3 Analysed representations

As a consequence of the former work, this thesis includes analysis of ProtTrans protein language model's mean embeddings usage in protein classification.

Besides the original mean embeddings, principal components of ESM-1b and ProtTrans mean embeddings were retrieved and taken as input for the model. In particular, principal components that explained 95 percent and 100 percent of the data variance were picked.

Table 3: Sizes of the analysed principal components vectors

Explained variance	ESM-1b	ProtTrans
95%	540	453
100%	1280	1024

Furthermore, both protein language models - ESM-1b and ProtTrans - provide per token or per residue representations - each amino acid of the protein gets a 1280 or 1024-dimensional vector from ESM-1b or ProtTrans model respectively. Therefore, each protein is originally represented by the $m \times n$ matrix, where m is the number of dimensions of the chosen type of embedding and n is the number of amino acids that compose the protein. These representations are processed to get vectors with the same dimension for each protein in the data set. The representations included in the analysis were:

1. Mean ESM-1b and ProtTrans
2. Joined mean ESM-1b and ProtTrans
3. Normalised mean ESM-1b and ProtTrans
4. Joined normalised mean ESM-1b and ProtTrans
5. Median ESM-1b and ProtTrans
6. Minimum, median, and maximum ESM-1b and ProtTrans
7. Quantiles (including minimum and maximum) ESM-1b and ProtTrans
8. Quantiles (including minimum and maximum) and mean ESM-1b and ProtTrans
9. Octiles (including minimum and maximum) ESM-1b and ProtTrans

Table 4: Sizes of the analysed representations' vectors

Representation	ESM-1b	ProtTrans
Mean	1280	1024
Joined mean	2304	
Median	1280	1024
Minimum, median, maximum	3840	3072
Quantiles	6400	5120
Quantiles and mean	7680	6144
Octiles	11520	9216

4.4 Analysed architectures

All representations that were described in the previous section were taken as input for the baseline single-layer perceptron models, although another important part of this work was to explore several different model architectures.

Architectures that were chosen to run experiments with had one or two hidden layers. The sizes of hidden layers were chosen to be original embeddings size divided by several multiples of 2 (Tables 5 and 6). That is, since the size of the ESM-1b embedding is 1280, there were models defined with one hidden layer of size 640, 320, or 160 that take ESM-1b embeddings as input. For models with two hidden layers, sizes of hidden layers were assigned by combining two sequential sizes received from division. Analogously, the same operation was done for models adjusted for ProtTrans.

Table 5: Models that were tested with ESM-1b embeddings input

Model	Number of hidden layers	Size of hidden layers
C2H2_h640-320	2	640, 320
C2H2_h320-160	2	320, 160
C2H1_h640	1	640
C2H1_h320	1	320
C2H1_h160	1	160
SLP_ESM-1b	0	-

Table 6: Models that were tested with ProtTrans embeddings input

Model	Number of hidden layers	Size of hidden layers
C2H2_h512-256	2	512, 256
C2H2_h256-128	2	256, 128
C2H1_h512	1	512
C2H1_h256	1	256
C2H1_h128	1	128
SLP_ProtTrans	0	-

5 Results

5.1 Correlation analysis of embeddings' components

One of the tasks of this work was to try joined ESM-1b and ProtTrans representations and pass them as input to the thermostability classification model. Additionally, the correlation coefficients between the components of these embeddings were analysed.

The results of the analysis showed that there are five ESM-1b embeddings' components that have absolute correlation coefficients higher than 0.5 with more than 10 ProtTrans embeddings' components (Figure 1). However, overall the majority of components' pairs had correlation coefficients close to zero (Figure 2).

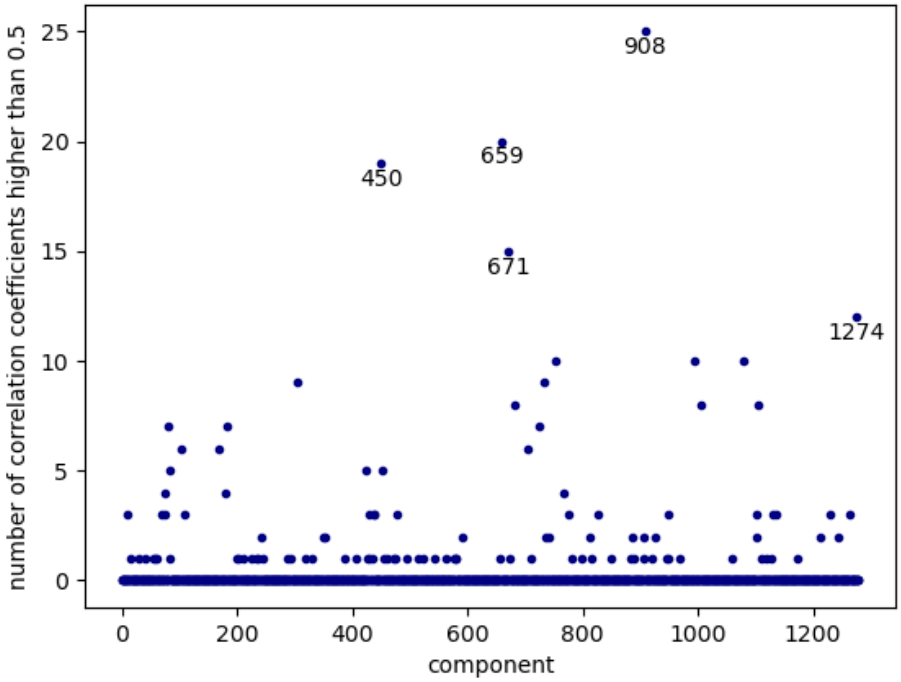


Figure 1: Plot of ESM-1b components that have got high absolute correlation coefficients with ProtTrans components

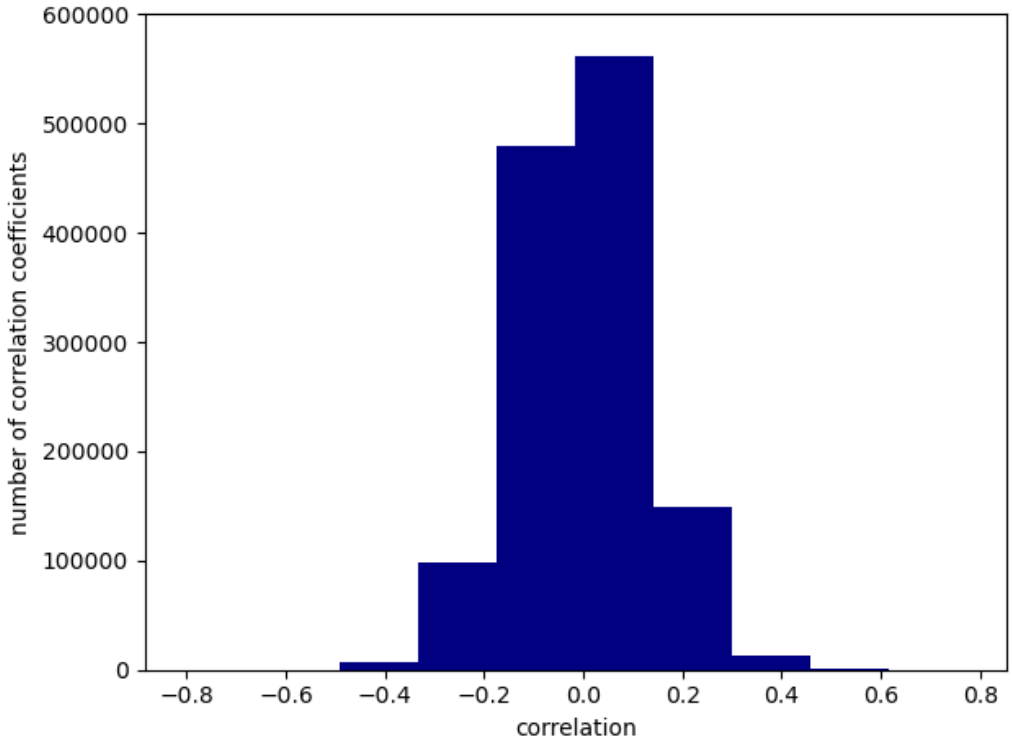
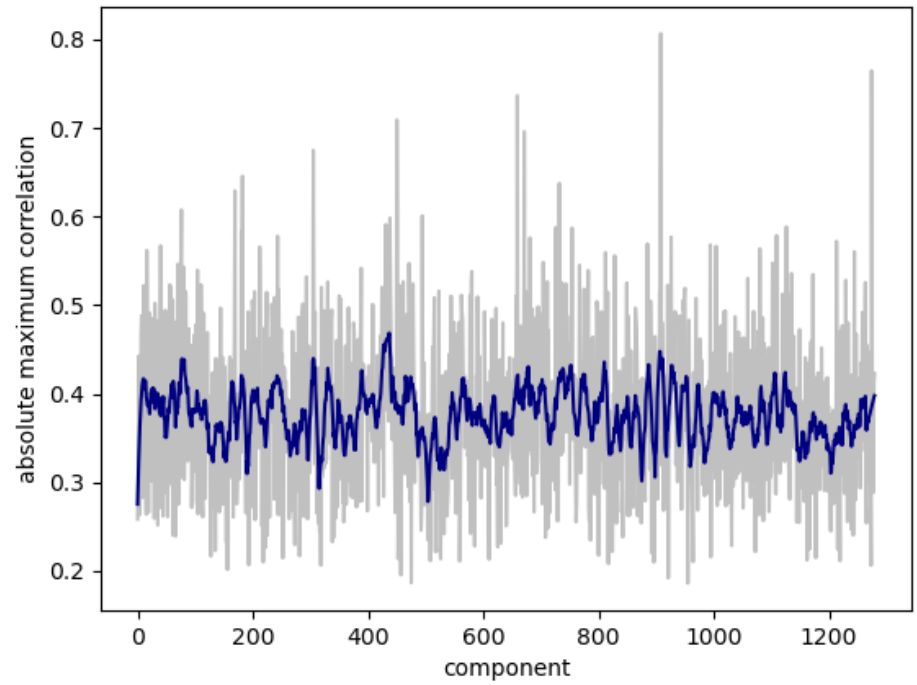


Figure 2: Histogram of correlation coefficients between ESM-1b and ProtTrans components

The plot that visualises absolute maximum correlation coefficients between pairs of components has two curves (Figure 3). The grey curve provides raw coefficient correlation values - there are peaks at ESM-1b positions that correspond with positions that have the biggest number of high (absolute value above 0.5) correlation coefficients (Figure 1). The blue curve shows the trend of absolute maximum values of correlation coefficients along the ESM-1b components. None of the peaks of the trend curve overstep 0.5. Therefore, it can be concluded that there are no intervals of ESM-1b components that have a considerably high correlation with ProtTrans components.

The observations that can be made from the plot of averaged correlation coefficients (Figure 3) do not change the overview of the correlation between ESM-1b and ProtTrans components - the trend of mean correlation coefficients fluctuates around 0.1.



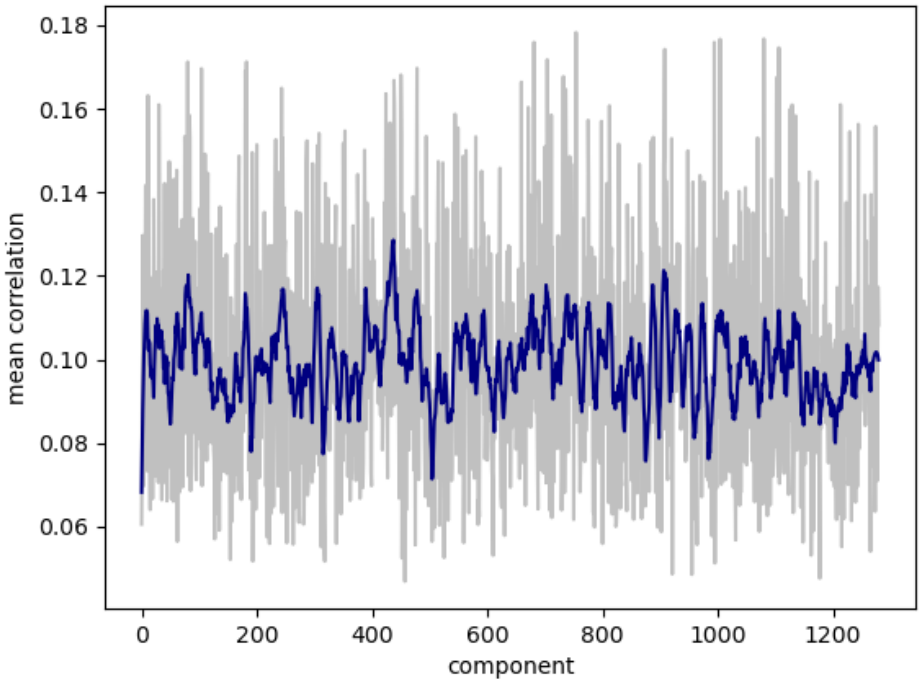


Figure 3: Plots of ESM-1b components' maximum and mean absolute correlation coefficients with ProtTrans components

Additionally, it was attempted to analyse correlation coefficients between embeddings' principal components that explain 95 percent of data variation.

The analogous scatter plot of the number of absolute correlation coefficients higher than 0.5 was drawn (Figure 4). The plot demonstrated that there are few high correlation coefficients between components' pairs overall and this observation is supported by the histogram of correlation coefficients (Figure 5). Note that the number of correlation coefficients for principal components' correlation analysis was around five times smaller than in the raw embeddings' components analysis (Table 3).

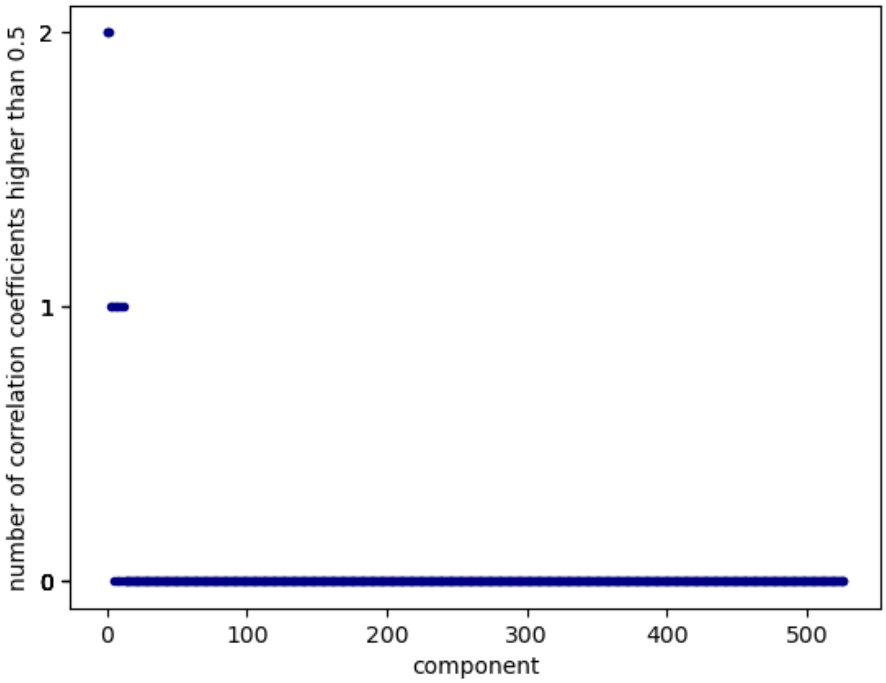


Figure 4: Plot of ESM-1b principal components (explaining 95% of data variation) that have got high correlation coefficients with ProtTrans principal components (95%)

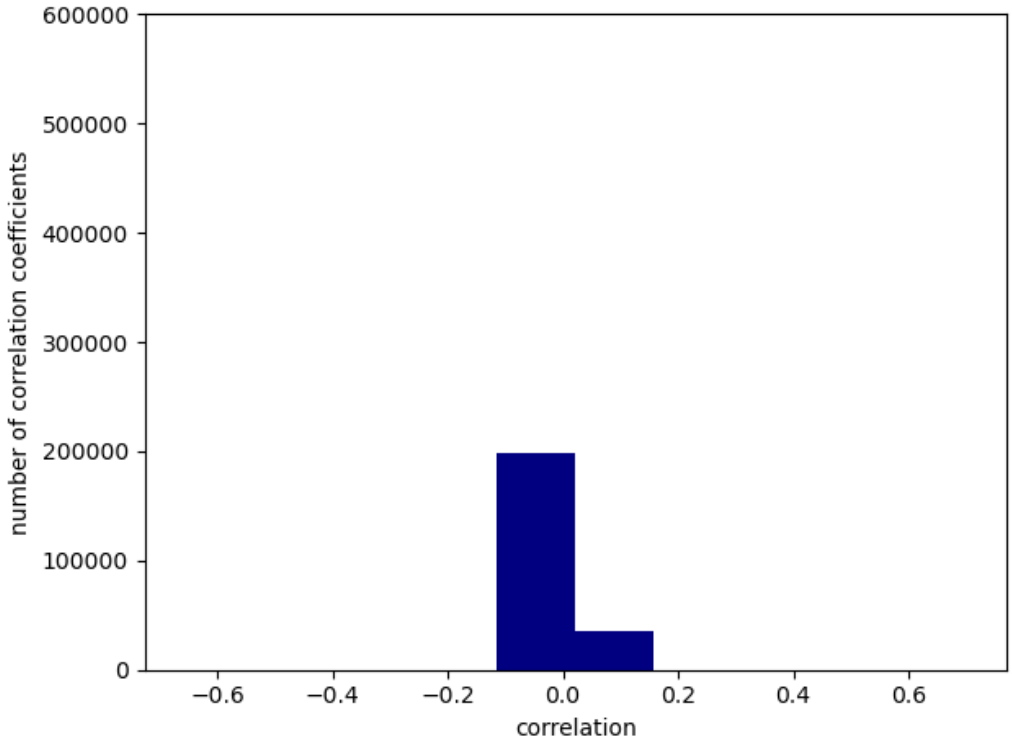


Figure 5: Histogram of correlation coefficients between ESM-1b and ProtTrans principal components (95%)

The curve of absolute maximum correlation coefficients shows that there is a trend of correlation coefficients to decrease as the index of ESM-1b component is increasing (Figure 6). The following plot of mean correlation coefficients (Figure 6) supports the statement that high maximum values of absolute correlation coefficients are not dominating because the mean correlation is very small between the pairs.

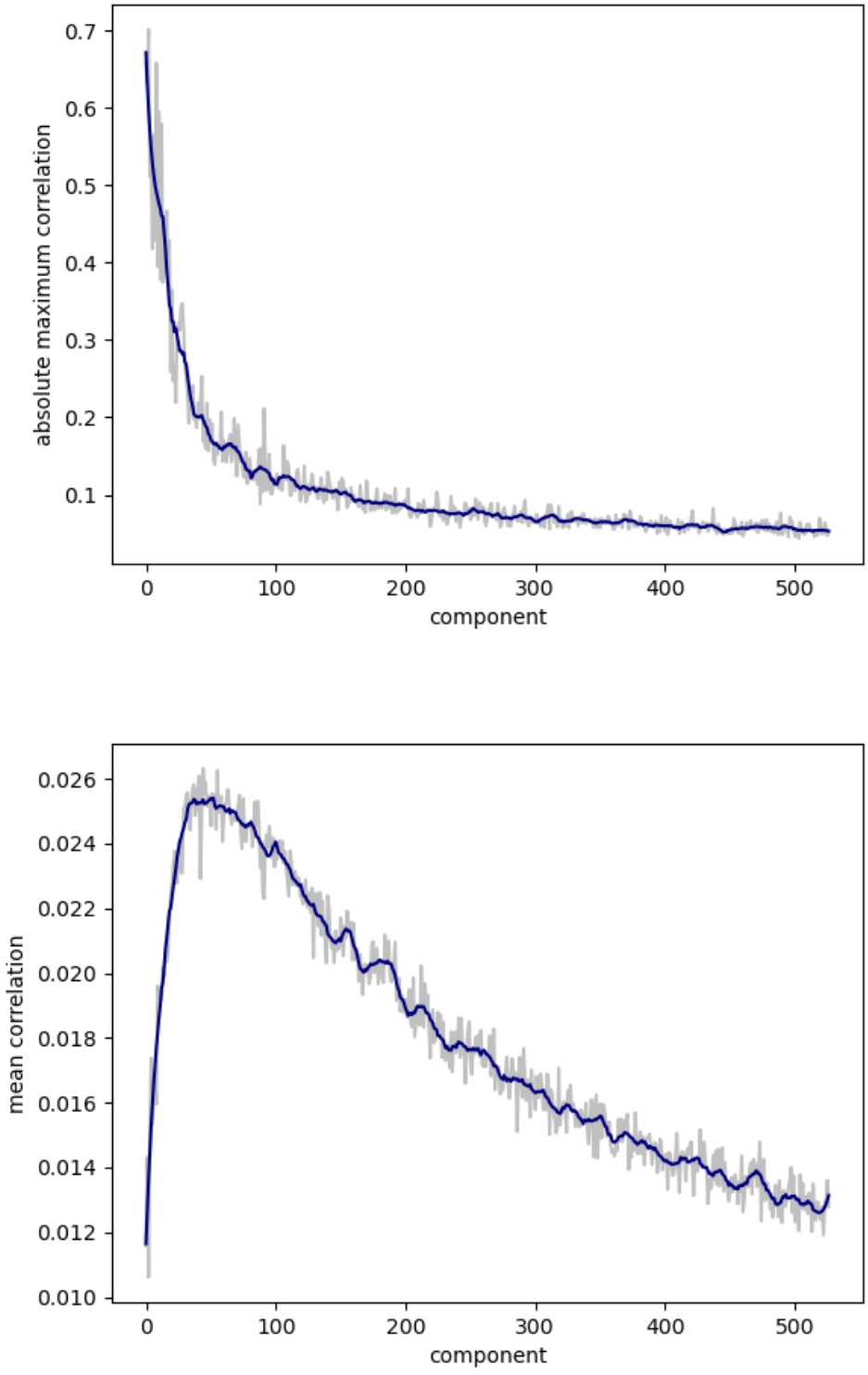


Figure 6: Plot of ESM-1b principal components' (95%) mean and maximum correlation coefficients with ProtTrans principal components (95%)

5.2 Representation analysis

The first step of the analysis was to try ProtTrans embeddings as input for the SLP model and compare its results with the testing metrics of model trained with ESM-1b. For the comparison, the primary model with ESM-1b was retrained using the filtered data set. The comparison disclosed that the model that uses ProtTrans embeddings as input performs better (Table 8).

Out of the scope of this representation and model architecture analysis for binary classification, there were several attempts made to overtrain the classification model to predict three thermostability classes (one more class added after dividing the zero-labelled class at the temperature threshold of 40 degrees Celsius). The purpose of overtraining was to check whether the selected architecture has a potential to be trained for the multiclass classification problem. The usage of principal components of protein embeddings showed that overfitting can be done successfully.

Therefore, it was decided to check whether a vector of principal components could be a suitable input for the binary classification task. The testing stage metrics showed worse model's performance than using the original representations (Tables 7).

Table 7: The comparison of scores between models trained with ESM-1b and ProtTrans mean representations and their principal components that account for 95% and 100% of the unfiltered data set variance

	Mean ESM-1b	Mean ProtTrans	ESM-1b (95%)	ProtTrans (95%)	ESM-1b (100%)	ProtTrans (100%)
MCC	0.843	0.902	0.699	0.767	0.698	0.766
Accuracy	0.922	0.951	0.845	0.880	0.845	0.879
Loss	0.208	0.128	0.383	0.442	0.382	0.443
Precision	0.919	0.949	0.910	0.940	0.909	0.939
Recall	0.921	0.951	0.768	0.813	0.767	0.812
ROC AUC	0.979	0.990	0.901	0.945	0.901	0.943

Before joining the embeddings, the normalisation of ESM-1b and ProtTrans vectors was done. Normalised representations were taken as input to the model with the same SLP architecture. For both types of embeddings the results were improved (Table 8).

After joining ESM-1b and ProtTrans mean embeddings, an SLP was trained using these joined representations. The results of this model were similar to the scores of the model that was trained using only ProtTrans embeddings, though the results did not improve (Table 8).

Table 8: The comparison of testing stage scores between models trained with mean, normalised mean, joined mean, and normalised joined ESM-1b and ProtTrans representations

	ESM-1b	Normalised ESM-1b	ProtTrans	Normalised ProtTrans	Joined	Normalised joined
MCC	0.843	0.858	0.901	0.915	0.899	0.920
Accuracy	0.921	0.929	0.951	0.957	0.949	0.960
Loss	0.208	0.248	0.128	0.143	0.131	0.139
Precision	0.921	0.923	0.949	0.951	0.945	0.954
Recall	0.917	0.931	0.949	0.962	0.951	0.964
ROC AUC	0.979	0.982	0.990	0.991	0.991	0.992

However, joining the normalised ESM-1b and ProtTrans mean representations showed the best results. Since joined representations require generation of ESM-1b embeddings, this type of representation does not solve the length limitation problem that was noticed in the previous work. Nevertheless slightly improved results can be observed when normalised representations are used, the process of normalisation depends on the data set, which is not convenient in the process of development until the final data set is established. Therefore, the optimal choice for this stage of development was mean ProtTrans embeddings.

Nonetheless, ProtTrans already demonstrated the impact for the model's improvement on the performance, it was decided to finish up the different representation and architecture analysis using embeddings of both protein language models for completeness. The results of the consequent analysis did not change the conclusion regarding ProtTrans influence for the results using any variation of analysed representations (listed in the section 4.3) - in all cases model that took ProtTrans embeddings as input performed significantly better (Figures 7 and 8).

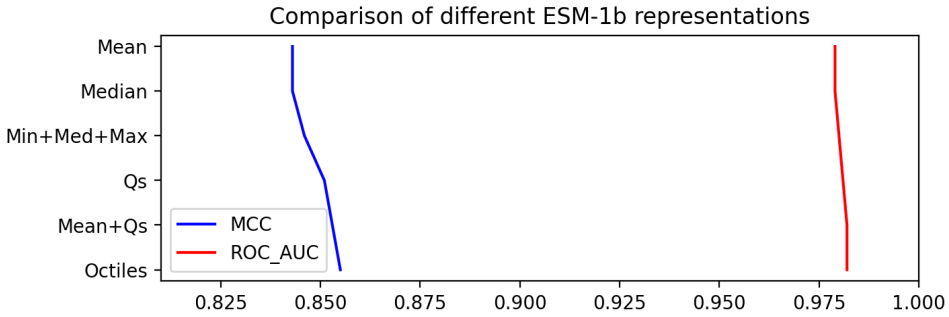


Figure 7: Comparison of SLP models', which were trained with different ESM-1b representations, MCC and ROC AUC scores

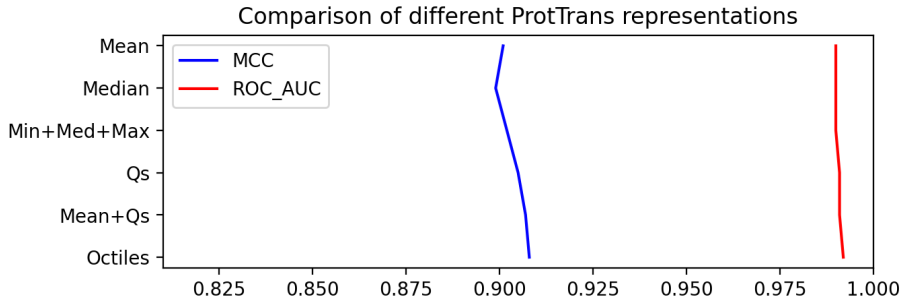


Figure 8: Comparison of SLP models', which were trained with different ProtTrans representations, MCC and ROC AUC scores

5.3 Architecture analysis

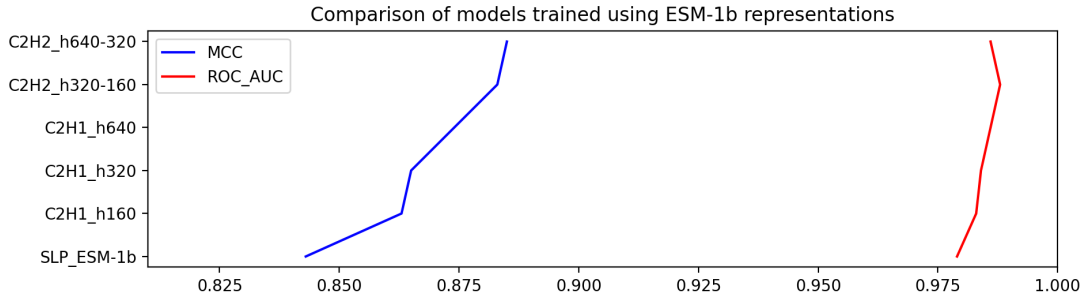


Figure 9: Comparison of models', which were trained using ESM-1b embeddings, MCC and ROC AUC scores

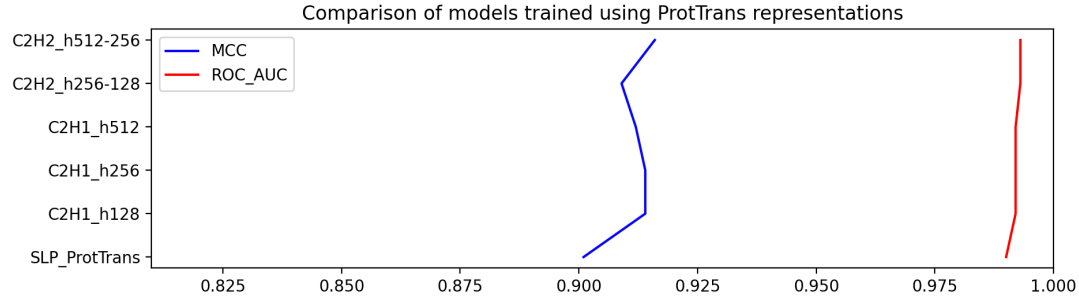


Figure 10: Comparison of models', which were trained using ProtTrans embeddings, MCC and ROC AUC scores

6 Conclusions

The results of this work provided following conclusions: for further development of the final method ProtTrans mean and octiles embeddings will be used. Additionally, this work showed that it is worth to use the model's architecture with two hidden layers with sizes 512 and 256.

7 Availability

The code that was used to receive the results of this work can be found in the designated Github repository: https://github.com/ievapudz/Course_Work_Project.

References

[1] A. Rives, J. Meier, T. Sercu, S. Goyal, Z. Lin, J. Liu, D. Guo, M. Ott, C. L. Zitnick, J. Ma, *et al.*, "Biological structure and function emerge from scaling unsupervised learning to 250 million protein sequences," *Proceedings of the National Academy of Sciences*, vol. 118, no. 15, 2021.

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[3] A. Elnaggar, M. Heinzinger, C. Dallago, G. Rihawi, Y. Wang, L. Jones, T. Gibbs, T. Feher, C. Angerer, M. Steinegger, *et al.*, "Prottrans: towards cracking the language of life's code through self-supervised deep learning and high performance computing," *arXiv preprint arXiv:2007.06225*, 2020.