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NetSolP: predicting protein solubility in E. coli using language models

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Abstract

Motivation: Solubility and expression levels of proteins can be a limiting factor for large-scale studies and industrial production. By determining the solubility and expression directly from the protein sequence, the success rate of wet-lab experiments can be increased.
Results: In this study, we focus on predicting the solubility and usability for purification of proteins expressed in *Escherichia coli* directly from the sequence. Our model NetSolP is based on deep learning protein language models called transformers and we show that it achieves state-of-the-art performance and improves extrapolation across datasets. As we find current methods are built on biased datasets, we curate existing datasets by using strict sequence-identity partitioning and ensure that there is minimal bias in the sequences.
Availability: The predictor and data are available at https://services.healthtech.dtu.dk/service.php?NetSolP and the open-sourced code is available at https://github.com/tvinet/NetSolP-1.0
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Supplementary information: Supplementary data is attached in submission.

1 Introduction

Successful expression of soluble proteins is desired in research as well as commercial environments. High-throughput purification of proteins enables the production of various products in industries including pharmaceutical, food, and beverage (Chapman *et al.*, 2018). A large-scale protein structure determination effort[1] has shown that low expression and solubility are common issues with about 49% successful expression rate for recombinant proteins and 52% purification rate for expressed proteins. There are several techniques that increase the solubility of wild-type proteins using mutations (Trevino *et al.*, 2008; Miklos *et al.*, 2012; Tan *et al.*, 1998; Dudgeon *et al.*, 2012; Costa *et al.*, 2014). Protein solubility depends on various external physical conditions such as pH and temperature and its interaction with intrinsic factors e.g., the amino-acid composition and structure of proteins. Reducing the search space to only protein sequences that potentially have high solubility and expression is beneficial to reduce the cost and time of wet-lab experiments. Thus, several sequence-based protein solubility prediction tools have been proposed using biophysical and structural features (Smialowski *et al.*, 2012; Sormanni *et al.*, 2015; Hebditch *et al.*, 2017; Bhandari *et al.*, 2020; Hon *et al.*, 2021). Recently, deep learning-based methods have been utilized to learn these features from the amino acid sequence only (Khurana *et al.*, 2018; Raimondi *et al.*, 2020; Wu and Yu, 2021). Evolutionary data in the form of sequence profiles have proven valuable for producing high-quality predictors (Rawi *et al.*, 2017, Khurana *et al.*, 2018). However, computing Multiple Sequence Alignments (MSA) is slow and does not

[1] http://targetdb.rcsb.org/metrics

scale well for large numbers of proteins. All the above methods do not use the same objective. Camsol (Sormanni *et al.*, 2015), Protein-Sol (Hebditch *et al.*, 2017), SWI (Bhandari *et al.*, 2020) predict the solubility, whereas, PROSO (Smialowski *et al.*, 2012), DeepSol (Khurana *et al.*, 2018), SKADE (Raimondi *et al.*, 2020), SoluProt (Hon *et al.*, 2021) and EPSOL (Wu and Yu, 2021) predict soluble expression of proteins.

Language models from Natural Language Processing have successfully been transferred to the protein domain due to the abundance of unlabelled raw sequence data. A protein language model, which is based on the transformer architecture (Vaswani *et al.*, 2017), is trained in a self-supervised fashion on a large corpus, such as the UniRef50 database (Suzek *et al.*, 2014), using the masked language-modelling objective (Devlin *et al.*, 2019). The transformer is a deep learning method to produce a contextual embedding of amino acids in the protein sequence. By using the masked language model objective it is able to build a context around each position and learns to "attend" or "focus" on amino acids and peptides that are relevant in the given context. These language models have been found to encode contact maps, taxonomy, and biophysical characteristics in their distributed representations (Rives *et al.*, 2021; Rao *et al.*, 2021, 2020; Elnaggar *et al.*, 2020; Vig *et al.*, 2020; Brandes *et al.*, 2021; Martiny *et al.*, 2021). In this study, we use a protein language model to predict two objectives, solubility and practical usability for purification of proteins in *E. coli*, and obtain state-of-the-art performance. As we find current datasets are biased by artifacts introduced by the expression vector, we also curate multiple protein datasets for both objectives from publicly available data. Our curation, using strict homology partitioning and ensuring no sequence bias, makes them a better representative of real-world performance than current datasets.

2 Data

2.1 TargetTrack dataset

Rawi *et al.* (2017) curated 69,420 proteins as the training set from a larger collection of 129,643 proteins (Smialowski *et al.*, 2012) and used 2001 protein sequences curated by Chang *et al.* (2013) as an independent test set. All of these sequences were selected from the TargetTrack database (Berman *et al.*, 2017), which was a large-scale project by the Protein Structure Initiative (PSI) from 2000-2017 to greatly increase the number of known protein structures. No explicit solubility label is recorded in the database, although several participating centres registered it separately (Seiler *et al.*, 2014) and thus the binary solubility label for some proteins is available in sources such as the PSI: Biology dataset described below. Proteins from the downloaded version were considered soluble by Smialowski *et al.* (2012) if they reached a set of predetermined soluble experimental states and insoluble if they did not reach those states in the version released 8 months later and also did not already have a structure submitted to the Protein Data Bank (PDB).

2.2 Biases in the TargetTrack dataset

The PaRSnIP (Rawi *et al.*, 2017), DeepSol (Khurana *et al.*, 2018), and SKADE (Raimondi *et al.*, 2020) soluble expression predictors were built using the curated train set and were shown to achieve high scores on the test set. However, it was noticed that these tools generalize poorly (Bhandari *et al.*, 2020, Hon *et al.*, 2021). Raimondi *et al.* (2020) showed that the SKADE model focused mostly on the N- and C- termini and validated that DeepSol did the same using an experiment that involved cropping the starting and ending segments of the sequences. Unfortunately, this behaviour is likely not due to underlying biophysics but a result of unintended bias in both the training and test sets. An example of this is that 11,602 out of 69,420 sequences of the training set and 344 out of 2001 sequences of the test set have the N-terminal His-tag 'MGSDKIHHHHHH' with $\sim$ 99% and $\sim$ 97% of them being insoluble respectively. His-tags are polyhistidine peptides incorporated in the recombinant protein to enable affinity purification (Spriestersbach *et al.*, 2015). Other N-terminal His-tags like 'MGSSHHHH', 'MHHHHHHS', 'MRGSHHHH' with over 100 instances each have 88%, 100%, and 100% mean solubility, respectively. An example of a C-terminal His-tag from the dataset is 'HHHHH'; when sequences have an amino acid other than E preceding this His-tag, they are almost always soluble. Such statistics are not expected naturally and indicate a bias in the selection or the experiments themselves. While it is perfectly possible that His-tags could change solubility properties of a protein, our observations suggest that something else is going on. Such extreme statistics are more likely due to a selection or biased definition of solubility depending on the group that performed the experiments using a particular His-tag.. Moreover, Hon *et al.* (2021) compared the labels of sequences from this dataset with another dataset whose solubility was provided separately (Price *et al.*, 2011) and found that around 18.6% of labels were different, even with 100% identical sequences. The consequence of this is that the trained models focus more on the His-tag instead of the wild-type sequence. Since the SoluProt and EPSOL (Wu and Yu, 2021) training datasets also come from the same source, they face the same issues discussed above. Therefore, the biased selection of proteins in combination with the label noise makes it difficult to train generalizable models using this dataset.

2.3 PSI: Biology dataset

As part of PSI, several centres recorded explicit expression and solubility labels for the target proteins. A subset of this data was extracted by Bhandari *et al.* (2020), which had 12,216 proteins expressed in *E. coli* using two specific expression vectors 'pET21' and 'pET15'. Although newer techniques can change the status of some proteins, explicit labels make this dataset far more reliable. The percentage of soluble proteins in the dataset is $\sim$66%. We use this dataset for 5-fold cross-validation.

2.4 Price dataset

The North East Structural Consortium (NESG) expressed 9644 proteins in *E. coli* using a unified production pipeline (Price *et al.*, 2011) and provide integer scores (0-5) for both expression (E) and solubility (S). The proteins are part of the TargetTrack database, but the scores were obtained by Hon *et al.* (2021) from the original authors. We remove sequences that have multiple scores and use the remaining 9272 sequences in two ways. First, as an independent test set for solubility consisting of 1323 highly expressed proteins (E = 4 or 5) with high solubility score (S = 4 or 5) as soluble and low score (S = 0) as insoluble. Using this definition, soluble proteins are $\sim$64% of the test set. An alternative objective 'usability', which requires the protein to be successfully purified on a large scale, is used to generate a new dataset. Usability is estimated using the product U=E·S by the authors. Proteins are considered usable if U is greater than 11 and unusable if U is less than 4. The total number of proteins for 5-fold cross-validation is 7,259. We exclude proteins with intermediate scores in both solubility and usability datasets to reduce potential noise.

2.5 Camsol mutation dataset

Sormanni *et al.*, 2015 compiled a set of 19 proteins with 56 total variants from four sources whose change in solubility was experimentally verified. Compared to the wild-type, 53 mutations increased solubility and 3 decreased it. This dataset is used as an independent test set and no partitioning is performed.

3 Methods

3.1 Data Partitioning

To generate high-quality data partitions, we use the four-phase procedure described in Gíslason *et al.*, 2021 to make label-balanced splits for 5-fold cross-validation. This procedure ensures that each pair of train and test fold does not share sequences that have global sequence identity greater than 25% as determined using ggsearch36, which is a part of the FASTA package (Pearson and Lipman, 1988). The datasets after partitioning are as follows, PSI: Biology solubility cross-validation set with 11,226 sequences, the Price usability cross-validation set with 7,259 sequences, and the Price solubility independent test set with 1,323 sequences. The latter dataset is ensured not to share sequences with global identity greater than 25% with the full PSI: Biology dataset using USEARCH v11.0.667, 32-bit (Edgar, 2010).

3.2 NetSolP

Multiple publicly available transformer models are evaluated. We refer to the 12-layer ESM (Evolutionary Scale Modelling, Rives *et al.*, 2021) model with 84M parameters as ESM12, the 12-layer ESM model using multiple sequence alignments (Rao *et al.*, 2021) with 100M parameters as ESM-MSA, the 33-layer ESM model with 650M parameters as ESM1b (Rao *et al.*, 2020) and the 24-layer ProtT5-XL-UniRef50 encoder model (Elnaggar *et al.*, 2020) with 1208M parameters as ProtT5. We follow the guidelines of Rao *et al.* (2021) for generating MSAs. For each protein sequence, we construct an MSA using HHblits, version 3.1.0 (Steinegger *et al.*, 2019) against the UniClust30$_{2020\text{-}6}$ database (Mirdita *et al.*, 2016) with default settings except setting number of iterations to 3 ($-n3$). To reduce the size of MSA and memory requirements, hhfilter is applied (Steinegger *et al.*, 2019) with the -diff 64 parameter. The original MSA transformer uses 256.

The output representations of each amino acid in the sequence are averaged to represent the protein and a linear classification layer is used to predict binary solubility. The trained models have a suffix '-F' and '-P' to indicate whether they are trained end-to-end (fine-tuning) or only the classification layer (pretrained embedding), which is based on the available computational resources. The maximum sequence length used for training is 510, by removing around 3.4% of the training sequences that exceed this length, to speed up the training process. For prediction, amino acids after position 1022 are removed due to the maximum length constraints of the transformer models. Different learning rates for the transformer (3×10^{-6}) and classification layer (2×10^{-5}) are used, and the training is terminated using early stopping. Mixed-precision and model sharding techniques are utilized to efficiently fine-tune the models. The PyTorch-lightning (Falcon *et al.*, 2019) library and hardware provided by Google Colaboratory GPUs[2], and 2 Tesla V100s are used for training and testing. We improve the speed and memory utilization of the final tool using ONNX-runtime[3] and dynamic quantization. The final predictor (NetSolP) is an ensemble of fine-tuned, dynamically quantized ESM1b models. Additionally, we provide a distilled (Hinton *et al.*, 2015) version, NetSolP-D, that preserves most of the performance but runs five times as fast.

For qualitative analysis, we calculate the contributions of amino acids in the sequence towards predictions, for the ESM12 ensemble, using Integrated Gradients method (Sundararajan *et al.*, 2017) from the Captum[4] library. The baseline is taken to be a <CLS> token followed by <PAD> tokens i.e. an empty protein sequence. Other parameters are set to their default values. The per amino acid contribution for each model in the ensemble is summed and then normalized over the protein sequence using the L1-Norm. The importance is taken to be the absolute value of the contributions. For calculating conserved residue scores the tool provided by Capra and Singh (2007)[5] is used with scaled Shannon entropy and a window-size 0. The protein families are chosen from the PSI: Biology training set using MMseqs2 (Steinegger and Söding, 2017) with a minimum sequence identity 0.2 and the coverage set to 0.5. Three families, FAD/NAD(P)-binding domain (InterPro domain IPR036188), DNA breaking-rejoining enzyme, catalytic core (InterPro domain IPR011010) and Trehalase (Panther domain PTHR31616), are selected such that they have many sequences (48, 71, and 50 respectively) and have average solubility close to 50% (56%, 51% and, 41% respectively).

4 Results & Discussion

We compare PaRSnIP, Camsol, DeepSol-S2, ProteinSol, SWI, SoluProt, and multiple transformer models using threshold-dependent metrics such as accuracy, precision, Matthew's correlation coefficient (MCC), and a threshold-independent metric, area under the Receiver Operating Characteristic curve (AUC). For most models the value recommended by the authors is used as the threshold for predicting soluble proteins. Since Camsol is not built for binary predictions we use a value of 1. The threshold used in cross-validation for each model of the NetSolP ensemble is 0.5 and the threshold for NetSolP is set as the average of optimal thresholds for each of the five validation folds, computed using the Youden Index (Youden, 1950). This value was computed to be 0.69. Since the training dataset is skewed towards the positive class, the value is greater than 0.5. PaRSnIP, DeepSol-S2, and ESM-MSA require sequence profiles as input and thus take a long time to predict a large set of proteins, it takes approximately 5 minutes per sequence on a 2x Intel Xeon Gold 6126 (2.60 GHz) node while NetSolP-D takes about 3 seconds per sequence. Camsol, ProteinSol, and SWI require only the protein sequence and thus scale well. Retrained models of SWI (Bhandari *et al.*, 2020) and SoluProt (Hon *et al.*, 2021) are used only with the cross-validation datasets, with one model trained per fold. SWI was retrained using the scripts shared by the authors. SoluProt was retrained using the downloadable software package as reference, with only the features present in their final model. This is done to better represent the scores with our modified dataset splits since each validation fold might overlap with the original training sets.

NetSolP outperforms existing solubility prediction tools on the PSI: Biology "Solubility" 5-fold cross-validation dataset (Table 1) of 11,226 sequences, with the highest AUC (0.73 ± 0.02), MCC (0.29 ± 0.04) and accuracy (0.70 ± 0.02). Among transformer models the best scores are obtained by ESM-MSA which uses sequence profiles. The independent validation set (Table 2), compared among predictors that do not use sequence profiles, shows that NetSolP also generalizes better with the highest AUC (0.760), MCC (0.402), and accuracy (0.728). NetSolP-D, which is the distilled version of the NetSolP ensemble, performs almost as well with AUC (0.756), MCC (0.391), accuracy (0.723) and precision (0.769).

Interestingly, NetSolP is unable to discriminate between the minute solubility changes produced by mutations compared to other methods (Table 3). However, a high accuracy (94.6%) by ESM12 shows that it may be more suitable for comparing highly similar proteins. Only SODA (Paladin *et al.*, 2017) was trained with the goal of predicting solubility changes upon point mutations, unlike the rest which used the binary solubility objective.

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[2] https://colab.research.google.com/

[3] https://github.com/microsoft/onnxruntime

[4] https://captum.ai/

[5] https://compbio.cs.princeton.edu/conservation/score.html

Table 1. PSI:Biology Solubility. 5-Fold CV

Models	ACC	PRE	MCC	AUC
SoluProt	0.59 ± 0.03	0.70 ± 0.02	0.10 ± 0.03	0.59 ± 0.02
Parsnip*	0.61 ± 0.10	0.71 ± 0.03	0.16 ± 0.11	0.64 ± 0.08
Camsol	0.59 ± 0.08	0.77 ± 0.03	0.21 ± 0.09	0.65 ± 0.06
DeepSol S2*	0.54 ± 0.02	$\mathbf{0.81 \pm 0.05}$	0.22 ± 0.05	0.67 ± 0.04
ProteinSol	0.70 ± 0.03	0.70 ± 0.03	0.23 ± 0.08	0.68 ± 0.05
SWI	0.64 ± 0.04	0.80 ± 0.02	0.29 ± 0.05	0.69 ± 0.04
SWI†	0.63 ± 0.04	0.79 ± 0.03	0.28 ± 0.05	0.69 ± 0.03
ESM12-F	$\mathbf{0.71 \pm 0.03}$	0.75 ± 0.03	0.32 ± 0.04	0.73 ± 0.04
ESM1b-F	0.70 ± 0.02	0.74 ± 0.03	0.29 ± 0.03	0.73 ± 0.03
ProtT5-P	0.70 ± 0.03	0.77 ± 0.02	$\mathbf{0.33 \pm 0.04}$	0.73 ± 0.02
ESM-MSA-P*	$\mathbf{0.71 \pm 0.03}$	0.76 ± 0.02	$\mathbf{0.33 \pm 0.04}$	$\mathbf{0.75 \pm 0.03}$
NetSolP	0.70 ± 0.02	0.74 ± 0.03	0.29 ± 0.04	0.73 ± 0.02

† = Retrained on this dataset
* = Method requires sequence profiles

On the Price *et al.* (2011) dataset (Table 4) with the "Usability" objective the highest AUC (0.71 ± 0.01), MCC (0.30 ± 0.03), precision (0.64 ± 0.03) and accuracy (0.65 ± 0.01) is obtained by NetSolP. Quantization proves to be very effective as NetSolP is able to retain most of the performance of the constituent ESM1b models while reducing its data storage by a factor of four.

Fig 1 shows that the solubility of shorter sequences is predicted slightly better than that of longer sequences by our method as well as by SWI which could be due to the abundance of shorter sequences in the datasets. The trend seems to reverse for much longer sequences for our method, but note that due to the small number of very long test sequences, the confidence interval of the score is much larger for those. Raimondi *et al.* (2020) observe that the ends of the protein sequence are more important for predicting the solubility. However, in our case (Fig 2) the magnitude of the effect is insignificant, with the exception of the initial amino acid. The initial 1% of the amino acid sequence has 3% of the total importance indicating that it is only a small bias. The signed contributions averaged over all the positions for an amino acid (Fig 3) show an interesting relationship between NetSolP and SWI. The spearman rank correlation for these two sets of amino acid solubility scores is 0.66 (p-value= 1.48×10^{-3}) suggesting that the average statistics learned are similar but the performance improvement for NetSolP over SWI could be due to the context-dependent contribution of amino acids towards solubility. From the figure we can see that E,D,K are the most positively correlated with solubility and C,W,R, the most negatively. Fig 4 shows that NetSolP is the most effective at prioritizing proteins. The percentage of soluble proteins in the selected proteins is the most when considering sequences with the highest NetSolP scores. The conservation versus importance plots (Fig 5) show that highly conserved regions tend to be more important but not vice versa. The full importance plots for each of the families are provided in the Supplementary Material (Supplementary Fig 1). It would be interesting to try to synthesize proteins with modifications in positions of high importance and observe the experimental solubility values. We leave that for future work.

5 Conclusion

We propose NetSolP, a predictor based on protein language models and deep learning, that outperforms existing tools for *in silico* solubility and usability prediction. We curate new datasets with an emphasis on strict partitioning based on sequence identity and ensuring that there are no spurious correlations between the sequences and target labels. Our experiments find that larger transformer models are better and fine-tuning the models is more effective than the pretrained embeddings. Qualitative analysis reveals an interesting correlation with a previous method SWI and finds no significantly important regions for solubility in contrast to the study done by Raimondi *et al.* (2020), which can be explained by the bias present in the dataset they use.

The predictor is available at https://services.healthtech.dtu.dk/service.php?NetSolP and the open-sourced code is available at https://github.com/tvinet/NetSolP-1.0.

Table 2. Price Solubility. Independent Validation

Method	ACC	PRE	MCC	AUC
SoluProt	0.624	0.704	0.187	0.634
Parsnip	0.558	**0.786**	0.221	0.663
Camsol	0.570	0.751	0.199	0.646
DeepSol-S2	0.450	0.764	0.117	0.595
Protein-Sol	0.641	0.694	0.190	0.679
SWI	0.680	0.712	0.269	0.690
ESM12-F	0.698	0.743	0.328	0.732
ESM1b-F	0.723	0.764	0.386	**0.761**
ProtT5-P	0.702	0.714	0.314	0.733
ESM-MSA-P	0.712	0.715	0.340	0.745
NetSolP	**0.728**	0.773	**0.402**	0.760
NetSolP-D	0.723	0.769	0.391	0.756

Table 3. Camsol Solubility Mutation. Independent Validation

Method	Trevino	Miklos	Tan	Dudgeon	Total	Accuracy
SoluProt	17 / 22	3 / 3	1 / 1	11 / 30	32 / 56	57.1
PROSO II	16 / 22	3 / 3	1 / 1	12 / 30	32 / 56	57.1
SolPro	15 / 22	3 / 3	1 / 1	21 / 30	40 / 56	71.4
Parnsip	8 / 22	3 / 3	1 / 1	11 / 21	54 / 56	41.1
Camsol	22 / 22	3 / 3	1 / 1	28 / 30	54 / 56	96.4
DeepSol-S2	8 / 22	3 / 3	1 / 1	23 / 30	35 / 56	62.5
ProteinSol	14 / 22	3 / 3	1 / 1	1 / 30	19 / 56	33.9
SWI	21 / 22	3 / 3	1 / 1	30 / 30	55 / 56	98.2
SODA	22 / 22	3 / 3	1 / 1	30 / 30	56 / 56	100.0
ESM12-F	19 / 22	3 / 3	1 / 1	30 / 30	53 / 56	94.6
ESM1b-F	14 / 22	3 / 3	0 / 1	14 / 30	31 / 56	55.3
NetSolP	16 / 22	3 / 3	0 / 1	18 / 30	37 / 56	66.1

Table 4. Price Usability. 5-Fold CV

Method	ACC	PRE	MCC	AUC
SoluProt†	0.63 ± 0.02	0.63 ± 0.03	0.26 ± 0.03	0.63 ± 0.02
SoluProt	0.62 ± 0.01	0.60 ± 0.02	0.24 ± 0.02	0.67 ± 0.02
Parsnip	0.61 ± 0.01	0.66 ± 0.02	0.22 ± 0.01	0.66 ± 0.01
Camsol	0.55 ± 0.02	0.63 ± 0.12	0.13 ± 0.07	0.62 ± 0.05
DeepSol-S2	0.54 ± 0.02	0.59 ± 0.06	0.09 ± 0.05	0.57 ± 0.02
Protein-Sol	0.55 ± 0.02	0.53 ± 0.02	0.13 ± 0.04	0.60 ± 0.02
SWI	0.58 ± 0.01	0.54 ± 0.01	0.20 ± 0.02	0.64 ± 0.02
ESM12-F	0.64 ± 0.01	0.63 ± 0.01	0.29 ± 0.03	$\mathbf{0.71 \pm 0.01}$
ESM1b-F	$\mathbf{0.65 \pm 0.01}$	$\mathbf{0.64 \pm 0.03}$	$\mathbf{0.30 \pm 0.03}$	$\mathbf{0.71 \pm 0.01}$
NetSolP	0.65 ± 0.02	0.65 ± 0.04	$\mathbf{0.30 \pm 0.04}$	0.70 ± 0.01

† = Retrained on this dataset

Competing interests

The authors have declared no competing interests.

Fig. 1. Top: The length distribution of the test set. Bottom: Change in accuracy based on the length of the protein sequences computed on the Price Solubility independent validation set of 1323 sequences. A dip in accuracy with longer sequences can be seen for both NetSolP and the best existing tool SWI

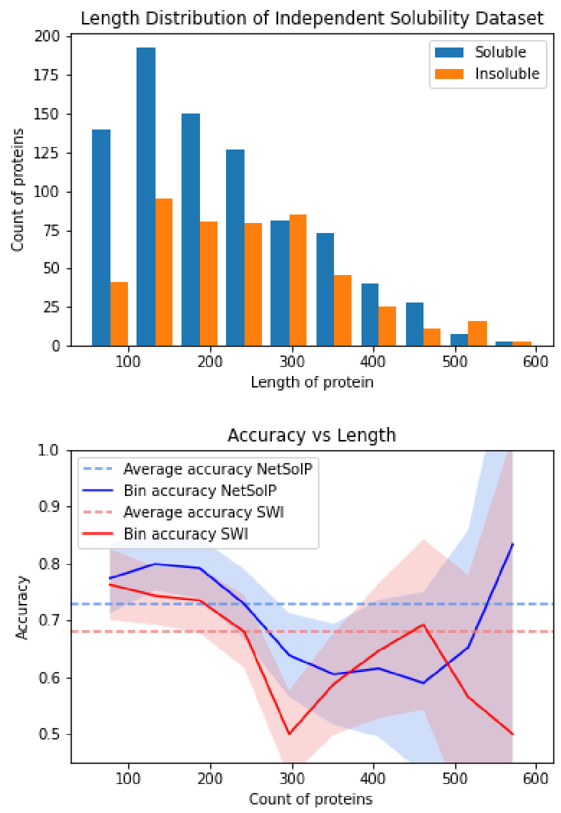

Fig. 2. Qualitative analysis of ESM12 model using Integrated Gradients computed on the independent solubility dataset. The importance of position in solubility prediction is shown.

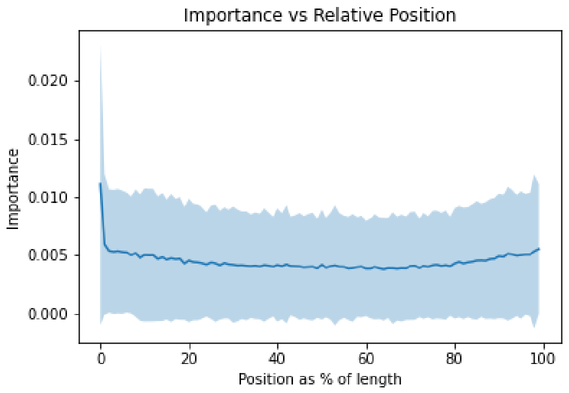

Fig. 3. Qualitative analysis of ESM12 model using Integrated Gradients computed on the independent solubility dataset. Solubility scores per amino acid shows strong correlation between NetSolP and SWI.

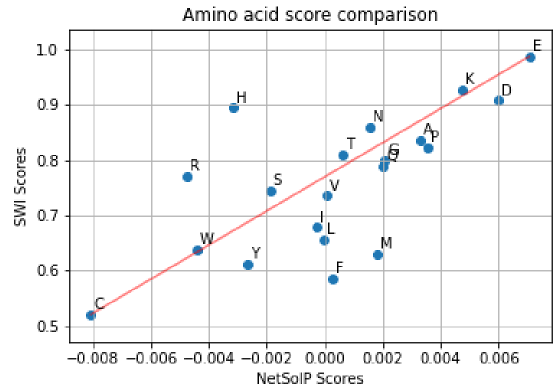

Fig. 4. Percentage of proteins that are soluble by selecting a variable fraction of the proteins sorted by the predicted scores of various methods on the Price independent test set with 1323 proteins.

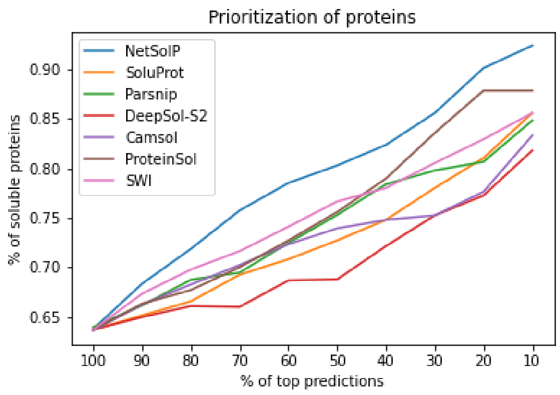

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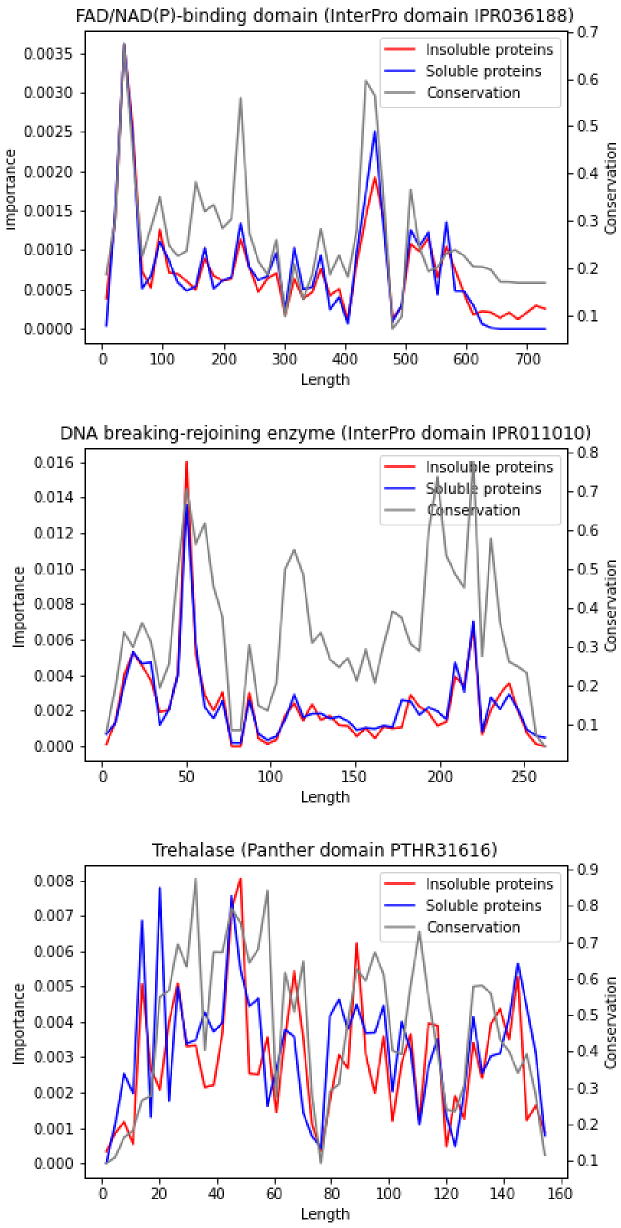
Fig. 5. Plotting the residue conservation score and importance for each position in the aligned protein families shows that highly conserved regions are important but not vice versa. Three protein families from the training set were chosen.



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