PRE-TRAINING CO-EVOLUTIONARY PROTEIN REPRESENTATION VIA A PAIRWISE MASKED LANGUAGE MODEL

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ABSTRACT

Understanding protein sequences is vital and urgent for biology, healthcare, and medicine. Labeling approaches are expensive yet time-consuming, while the amount of unlabeled data is increasing quite faster than that of the labeled data due to low-cost, high-throughput sequencing methods. In order to extract knowledge from these unlabeled data, representation learning is of significant value for protein-related tasks and has great potential for helping us learn more about protein functions and structures. The key problem in the protein sequence representation learning is to capture the co-evolutionary information reflected by the inter-residue co-variation in the sequences. Instead of leveraging multiple sequence alignment as is usually done, we propose a novel method to capture this information directly by pre-training via a dedicated language model, i.e., Pairwise Masked Language Model (PMLM). In a conventional masked language model, the masked tokens (i.e. amino acid residues) are modeled by conditioning on the unmasked tokens only, but processed independently to each other. However, our proposed PMLM takes the dependency among masked tokens into consideration, i.e., the probability of a token pair is not equal to the product of the probability of the two tokens. By applying this model, the pre-trained encoder is able to generate a better representation for protein sequences. Our result shows that the proposed method can effectively capture the inter-residue correlations and improves the performance of contact prediction by up to 9% compared to the MLM baseline under the same setting. The proposed model also significantly outperforms the MSA baseline by more than 7% on the TAPE contact prediction benchmark when pre-trained on a subset of the sequence database which the MSA is generated from, revealing the potential of the sequence pre-training method to surpass MSA based methods in general.

1 INTRODUCTION

Life is ruled by biological sequences and molecules, i.e. DNA, RNA, and protein sequences, following the de facto ‘natural’ language of biology. For protein, the structure is determined by the

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sequence, and its function is realized by the structure, according to Anfinsen’s dogma. However, the structure and function label is never easy to obtain (time-consuming and expensive) nor always effective due to the dark regions and structure dynamics. On the other hand, the number of unlabeled protein sequences increases quite faster than that of the labeled ones, due to the large gap between low-cost, high-throughput sequencing methods and expensive yet time-consuming labeling approaches by time- and labor-intensive manual curation process (Consortium, 2019). Understanding the protein sequences is vital to advance in biology, healthcare, and medicine. The protein sequence representation has been exploited in various real applications, including for dark proteome (Perdigão et al., 2015), where the ‘dark’ regions of proteins are never observed by experimental structure determination and inaccessible to homology modeling; therapies, such as cancer diagnosis (Vazquez et al., 2009) and antibody design (Whitehead et al., 2012); function/property prediction for protein engineering, such as fitness prediction (Hsu et al., 2021); phylogenetic inference (Hie et al., 2021b); sequence mutations, such as learning mutational semantics (Hie et al., 2020), virus evolution and escape prediction (Hie et al., 2021a), and cancer diagnosis by mutation prediction (Reva et al., 2011 Martelotto et al., 2014), to name a few.

Co-evolutionary information represented by the inter-residue co-variation is essential for protein sequences in terms of both structure and function due to the evolution pressure from natural selection – sequences with more stable structure and more adequate function are usually remained. A representation that can capture this information from the sequences is beneficial for understanding the molecular machinery of life. This information is previously quantified by analyzing homologous sequences, a.k.a multiple sequence alignment (MSA), for the target sequence, where the homologs are retrieved from public protein sequence databases by applying curated procedure and intensive computation with customized tools and hyper-parameters. For protein function prediction, unsupervised models can be learned from these homologous sequences, such as mutational effect prediction from sequence co-variation (Hopf et al., 2017) and mutational landscape inference (Figliuzzi et al., 2016). It is demonstrated that incorporating inter-residue dependencies using a pairwise model that power the predictions to agree more with the mutational experiment observations (Mann et al., 2014, Boucher et al., 2016, Riesselman et al., 2017). The reason is that, the function of the sequence is the combination effect of the residues other than the sum of the properties of each residue (Hopf et al., 2017). As the cost to quantify the co-effect of multiple residues is combinatorial while modeling their interactions is critical, pairwise co-variation analysis becomes the best choice, meanwhile, Figliuzzi et al. (2018) demonstrates that pairwise models are able to capture collective residue variability. For protein structure prediction, the key step is to predict inter-residue contacts/distances, while the shared cornerstone of prediction is performing evolutionary coupling analysis, i.e. residue co-evolution analysis, on the constructed MSA for a target protein (Ju et al., 2021). The underlying rational is that two residues which are spatially close in the three-dimensional structure tend to co-evolve, which in turns can be exploited to estimate contacts/distances between residues (Seemayer et al., 2014, Jones & Kandathil, 2018). Although the contacts are usually rare in the residue pairs of the sequence, they are critical to rebuilding the 3D structure of the protein due to their roles of constraints on the sketch.

A natural question arises that, as both derived from unlabeled sequences, can we directly capture this co-evolutionary information via sequence pre-training instead of extracting from MSA? To answer this question, we first discuss the key ingredient missed by conventional language models, then we propose a pairwise masked language model for the protein sequence representation learning. The key information to extract from the MSA (denoted as $X$) is the statistics for $Q(x_i, x_j | X)$, typically the co-variation for each pair, where $i, j$ are two indexes of the tokens/residues in the sequences. The intuition is that, if the two residues are independent, their co-variance will be close to zero, otherwise, it indicates the co-evolutionary relation between these two positions. While for the conventional masked language model, although they scan through all the same sequences as MSA-
based methods and the losses for all the residues in the masked tokens will be back-propagated, they are processed in an accumulative way within the batches, i.e. independently updating the weights. This means that \( P(x_i | X_{/M}) \) is modeled by the LMs. Table 1 presents an example for this kind of method, originating from NLP but sub-optimal for protein sequences due to the critical and much frequent co-evolution relationship among the residues. Here, we argue that for the protein sequences \( P(x_i, x_j | X_{/M}) \neq P(x_i | X_{/M}) \cdot P(x_j | X_{/M}) \). Auto-regressive methods, i.e. generative pre-training (GPT), where the latter tokens are predicted by conditioning on the previous ones from the same direction, still having the similar issue. Inspired by this, we design a pairwise masked language model and pre-train the model with a pairwise loss calculated from the protein sequences directly. Following the masked language model where the tokens of each sequence are picked at a probability and then be masked, replaced, or kept, our proposed model takes the masked sequences as input and predicts these original tokens, especially, the label for each masked token pair.

To examine the capability of the model to capture the co-evolutionary information, we select protein contact prediction as our main downstream task due to its well-established relationship to the co-evolutionary information and the plentiful data that are publicly available and well measured by empirical experiment. Here, protein contact prediction is a binary classification task for amino acid residue pairs, a residue pair is called a contact if their distance is less than or equal to a distance threshold, typically 8 Å (i.e., \( 8 \times 10^{-10} \) m). The result shows that this method can significantly improve the learned representation on the contact prediction task, it even surpasses the MSA baseline when pre-trained on another sequence subset UR50 with the sequence-only input on the TAPE benchmark. Meanwhile, an additional experimental evaluation for secondary structure prediction illustrates that the proposed model does not hurt performance, while significant improvements are observed in some other settings for remote homology prediction.

## 2 RELATED WORK

### Evolutionary Coupling Analysis

Co-evolution information is closely correlated to the contacts, due to the rational that two residues are likely co-evolving when they are spatially close. To this end, techniques such as mutual information (MI) (Gloor et al., 2005) are exploited to quantify this feature. Later than MI, direct coupling analysis (DCA) proves to be more accurate and be widely adopted, for example, EVfold (Sheridan et al., 2015), PSICOV (Jones et al., 2012), GREMLIN (Kamisetty et al., 2013), plmDCA (Ekeberg et al., 2013), CCMpred (Seemayer et al., 2014) are all built on DCA. For protein sequences, the common idea of these methods is to use statistical modeling to quantify the strength of the direct relationship between two positions of a protein sequence, excluding effects from other positions. A variety of DCA methods are further proposed to generate the direct couplings of residues by fitting Potts models (Ekeberg et al., 2013) or precision matrix (Jones et al., 2012) to MSAs, e.g. mean-field DCA (Morcos et al., 2011), sparse inverse covariance (Jones et al., 2012) and pseudo-likelihood maximization (Ekeberg et al., 2013; Balakrishnan et al., 2011; Seemayer et al., 2014).

By taking DCA-derived scores as features, deep neural networks based supervised methods significantly outperform the unsupervised methods (Senior et al., 2020; Jones & Kandathil, 2018; Wang et al., 2017; Xu, 2019; Yang et al., 2020). Most neural network models, including AlphaFold (AlQuraishi, 2019) and RaptorX (Xu, 2019), rely on this feature. However, due to the considerable information loss after transforming MSAs into hand-crafted features, supervised models, such as CopulaNet (Ju et al., 2021) and AlphaFold2 (Jumper et al., 2021), are proposed to directly build on the raw MSA. The superior performance over the baselines demonstrates that residue co-evolution information can be mined from the raw sequences by the model. A noticeable drawback of the MSA based methods is that, when the MSA quality (i.e. the number of the homologous sequences) is low for a target sequence, the model performance drops a lot.

### Pre-Training Methods

Following the pre-train and fine-tune paradigm (Peters et al., 2018; Kenyon & Toutanova, 2019; Liu et al., 2019; Radford et al., 2018), various pre-training methods are proposed recently to learn better representations for protein sequences. TAPE (Rao et al., 2019) is built as a benchmark to evaluate the protein sequence pre-training method. It demonstrates the performance can be improved by pre-training compared to one-hot representation, however, also indicates that the performance of the pre-training based models on pure sequences still lags behind the alignment-based method in the downstream tasks.
Among pre-training methods, RNNs are exploited as the pre-training model. Bepler & Berger (2019) use two layers of Bi-LSTM as an extraction part of the original protein sequences by applying next token prediction for pre-training language modeling. UniRep (Alley et al., 2019) uses a similar training scheme as Bepler & Berger (2019), then use evo-turning (evolutionary fine-tuning) to address the importance of evolutionary information for the protein embedding. UDSMProt (Strodthoff et al., 2020) relies on an AWD-LSTM language model, which is a three-layer LSTM regularized by different kinds of dropouts.

A variety of techniques based on Transformer (Vaswani et al., 2017) are applied to build dedicated models for proteins. ESM (Rives et al., 2021) demonstrates that Transformer models can outperform RNN based models, it also illustrates that the protein sequence diversity and model size have significant impacts on the pre-trained encoder performance. PRoBERTa (Nambiar et al., 2020) follows RoBERTa to pre-train the model with Byte Pair Encoding and other optimization techniques. Lu et al. (2020) applies contrastive learning by mutual information maximization to the pre-training procedure, MSA Transformer (Sturmfels et al., 2020) learns the representation on the MSAs for a protein sequence, however, it relies on expensive database searches to generate the required MSAs for each sequence.

Large-scale models are explored due to the fact that protein sequence datasets are large in size and complex in interaction. ProtTrans (Elnaggar et al., 2020) trains the protein LMs (pLMs) on the Summit supercomputer using more than $4 \times 10^3$ GPUs and TPU Pod up to $10^4$ cores, the most informative embeddings even outperform state-of-the-art models without multiple sequence alignments (MSAs) for the secondary structure prediction. Xiao et al. (2021) also demonstrate that sequence evolution information can be accurately captured by a large-scale model from pre-training, up to $3 \times 10^9$ parameters pre-trained on a 480 GPUs cluster.

Additional supervised labels are exploited for protein sequence pre-training. PLUS (Min et al., 2019) tries to model the protein sequence with masked language model together with an auxiliary task, i.e. same family prediction, in their work. Sturmfels et al. (2020) add a pre-training task named profile prediction for pre-training.

Generative pre-training is also exploited for protein engineering, for example, ProGen (Madani et al., 2020) is a generative model conditioned on taxonomic information as an unsupervised sequence generation problem in order to leverage the exponentially growing set of proteins that lack costly, structural annotations.

The outputs of the pre-trained language models can be used in different ways. For example, Vig et al. (2020), Rao et al. (2020), and Bhattacharya et al. (2020) demonstrate that the attention weights from the pre-trained models have a strong correlation with the residue contacts. Hie et al. (2021b) analyze the correlation between the pseudo likelihood of the mutated sequences predicted by the language model and the evolution of the mutations, the results suggests that pre-trained language models on protein sequences are able to predict evolutionary order at different timescales. Hie et al. (2021a, 2020) apply pre-trained protein language models to predict mutational effects and virus escape.

3 Method

Given the sequence data $D = \{X\}$, where $X = (x_1, x_2, \ldots, x_N)$, language models can be built on the sequences for pre-training.

3.1 Pairwise Masked Language Model

The loss function of vanilla Masked Language Model (MLM) can be formulated as follows:

$$L_{mlm} = \mathbb{E}_{X \sim D} \left( \mathbb{E}_M \sum_{i \in M} \left( - \log P_{\theta}(x_i | X_{/M}) \right) \right)$$

where $D$ is the sequence set, $X$ is a sequence in $D$, $X_{/M}$ represents the masked sequence of $X$ where the masked token indices are in $M$, $x_i$ stands for the $i$-th token in the sequence $X$, and $\theta$ denotes the encoder parameters.
In this paper, we propose a novel Pairwise Masked Language Model (PMLM) whose loss function can be written as:

\[ \mathcal{L}_{pmlm} = \mathbb{E}_{X \sim \mathcal{D}} \left( \mathbb{E}_M \sum_{i,j \in M, i \neq j} (- \log P_\theta(x_i, x_j | X_M)) \right) \]

The combined loss for both MLM and PMLM can be used for pre-training as:

\[ \mathcal{L} = \mathcal{L}_{mlm} + \lambda \cdot \mathcal{L}_{pmlm} \]

where \( \lambda \) is a weight coefficient to balance two losses. In this paper, \( \lambda \) is set to 1 during pre-training the PMLM models. As we can see, all the MLM and PMLM labels for pre-training are from the sequences themselves, thus still self-supervised. For multiple rounds of updates, if \( i \)-th and \( j \)-th positions are independent, \( P_\theta(x_i, x_j | X_M) \) degenerates to \( P_\theta(x_i | X_M) \cdot P_\theta(x_j | X_M) \). When they are co-evolutionary, the model learns a different distribution from the independent case.

### 3.2 Model Architecture

The goal of pre-training is to build a good protein sequence encoder that generates better representation. The pre-training model mainly consists of a protein sequence encoder and two prediction heads, i.e., a token prediction head and a pair prediction head. The sequence encoder is built on stacked Transformer encoder layers. The overview of our model is illustrated in Figure 1. Transformer is believed to be a powerful tool for modeling sequence data and has been applied to various tasks, including natural language understanding, question answering, computer vision, and so on. Thus we exploit a Transformer encoder as the sequence encoder of our model. The sequence encoder takes raw protein sequences as input and converts them into their vector representations. The model was trained on protein sequences using both masked token prediction (MLM) and masked pair prediction (PMLM) for protein language modeling. Each prediction head is an MLP, i.e. two-layer fully-connected (FC) neural network, where the output is mapped into the vocabulary via softmax, i.e. 20 amino acid residues (denoted as \( V_{res} \)) for the token prediction head and 400 amino acid pairs (denoted as \( V_{pair} \)) for the pair prediction head.

### 3.3 Masked Pair Prediction

The masked language model lets the model to reconstruct the masked tokens conditional on the other tokens in the sequences. Predicting masked pairs follows the same idea of the masked token prediction, however, with losses for the pairs. A two-layer FC neural network is exploited for the prediction of masked pairs, whereas another one for that of masked tokens. During pre-training, the dot and difference of the vectors for the residue pair are concatenated before feeding into the pair prediction head. Then, the output is mapped into a vector of dimension \( |V_{pair}| \) by softmax. This prediction is finally compared with the pair label from the sequence itself.

It is not trivial to generate pair labels from multiple masked tokens. The pairwise label construction process is demonstrated in Figure 2. For each pair \( x_i, x_j \) for the masked token where \( i \neq j \), a pairwise label is generated as \( w_{ij} = (x_i, x_j) \), where \( x_i, x_j \in V_{res} \) and \( w_{ij} \in V_{pair} \). Obviously, we have \( |V_{pair}| = |V_{res}|^2 \) for \( V_{res} \) and \( V_{pair} \). The pairs for \( x_i, x_j \) whose \( i = j \) are ignored since they are already a part of the MLM loss. Notably, \( i \neq j \) does not necessarily mean \( x_i \neq x_j \) since two different positions may be the same residue. For each valid pair, the pair prediction head generates the probability over \( V_{pair} \). For a masked sequence \( X_M \), the size of the pairwise labels is \(|M|^2 - |M|\) while each label is a one-hot encoding for \( V_{pair} \).

Intuitively, the masked pair loss is consistent with the masked token loss. For example, the loss can be treated as a combined loss for the two masked tokens if the two positions within the pair are independent, otherwise this masked pair loss provides extra information of the sequence besides each position.
3.4 FINE-TUNING FOR CONTACT PREDICTION

The generated encoder is further fine-tuned on a given supervised dataset for contact prediction. As the prevalent approach does, different neural networks can be built on top of the pre-trained model for further fine-tuning, whereas, a simple FC layer followed by a softmax operator can be applied to evaluate the representation. For the residue pairs task, e.g. contact prediction, the fine-tuning network is built on top of the first FC layer of the pair prediction head, other tasks are fine-tuned on the encoder outputs.

4 EXPERIMENT EVALUATION

4.1 EXPERIMENT SETUP

To evaluate the performance of the pairwise masked language model, several models with different settings are pre-trained on two prevalent datasets, i.e. Pfam (El-Gebali et al., 2019) and UR50 (release 2018_03). Following the RoBERTa-base setting, the hidden size, feed forward dimension, number of encoder layers, and attention heads of the base models are set as 768, 3072, 12, 12 respectively (denoted as MLM-base for MLM and PMLM-base for PMLM). A larger model named PMLM-large is pre-trained with the same setting except using 34 as the number of encoder layers. Moreover, the largest model with a hidden size of 1280 and a number of encoder layers of 36 is also pre-trained on UR50, denoted as PMLM-xl. The implementation is optimized to speed up the training procedure, e.g., the pairwise loss is applied alone by enabling the diagonal elements and disabling the token prediction head. MLM-base and PMLM-base are pre-trained with maximum length 512, while PMLM-large is pre-trained with a maximum length of 1024. The positional encoding of both models are non-learnable. MLM-base and PMLM-base are pre-trained using the Adam optimizer (0.9, 0.98) with peak learning rate 0.0003 and clip norm 1.0, the learning rate scheduler is a polynomial decay scheduler where the learning rate is decreased linearly after warming up by 20,000 steps to the peak. The hyper-parameters are almost the same for pre-training PMLM-large.
Figure 2: Pairwise Label Construction from the Masked Sequence

except that the peak learning rate is set to 0.0001. MLM-base, PMLM-base, and PMLM-large are pre-trained on 24 Tesla V100 GPU cards, about three weeks for MLM-base/PMLM-base and about seven weeks for PMLM-large. PMLM-xl is pre-trained on 16 Tesla V100 GPU cards for more than two weeks.

Two groups of experiments are conducted to examine the generated model on the TAPE contact prediction test set (denoted as TAPE-Contact) and the RaptorX contact prediction test set (denoted as RaptorX-Contact). The models are all evaluated on or fine-tuned from the checkpoint of the sixtieth epoch, except PMLM-base on UR50, which is fine-tuned from that of the ninetieth epoch. For TAPE-Contact, we use a shallow decoder as TAPE does, i.e., simply use an outer-dot and outer-difference of each pair followed by a linear projection with softmax, for the sequence representation to evaluate the representation. To see the potential of the representations, RaptorX-Contact is evaluated on a more expressive decoder, i.e., a ResNet with stacked convolution blocks, on top of the pre-trained encoder as ESM does, however, the convolution blocks are not dilated. The fine-tuning process for each model on each dataset is finished on one Tesla V100 GPU card.

4.2 Pre-training Validation

The two heads of the PMLM model output \( P(x_i, x_j) \) and \( P(x_i), P(x_j) \) separately, this raises another question that if they have already learned the difference between the independent and dependent positions of the sequence. To answer this question, we aggregate the validation losses and accuracy scores of pre-training on the Pfam and UR50 sequence datasets, as shown in Table 2. As we can see, even the fine-tuned performance of PMLM-base is better than that of MLM-base, the loss \( \mathcal{L}_{mlm} \) of PMLM-base is slightly higher than that of MLM-base. We further use \( \Delta \text{Acc} = \text{Acc}_{pmlm} - \text{Acc}_{mlm}^2 \) to quantify the difference. For PMLM-base and PMLM-large on Pfam and UR50, \( \Delta \text{Acc} \) is not negligible as an expectation, which indicates the difference of the correct probability for the most likely residue on each position. The joint probability is quite dif-
ferent to the multiplication of the individual probability, illustrating that PMLM is able to capture the co-evolutionary information via pre-training on pure sequences. This observation can be further validated by the histograms of $KL(P(x_i) \cdot P(x_j) \| P(x_i, x_j))$ of the PMLM-large model on the Pfam and UR50 validation sequences, which is shown in Figure 3, each $(x_i, x_j)$ pair of the sequence is masked when predicting the probabilities.

Table 2: Validation Losses and Accuracy Scores of Pre-training

| MODEL      | DATA   | $\mathcal{L}_{mlm}$ | $\mathcal{L}_{pmlm}$ | $\text{Acc}_{mlm}$ | $\text{Acc}_{pmlm}$ | $\Delta\text{Acc}$ | $\Delta\text{Acc} / \text{Acc}_{pmlm}$ |
|------------|--------|---------------------|---------------------|---------------------|---------------------|-------------------|-------------------------------------|
| MLM-base   | Pfam   | 1.6475              | -                   | 48.0%               | -                   | -                 | -                                   |
| PMLM-base  | Pfam   | 1.6728              | 3.3446              | 47.1%               | 22.4%               | 0.22%             | 1.0%                                |
| MLM-base   | UR50   | 2.2725              | -                   | 32.0%               | -                   | -                 | -                                   |
| PMLM-base  | UR50   | 2.2784              | 4.5557              | 31.8%               | 10.9%               | 0.79%             | 7.2%                                |
| PMLM-large | UR50   | 2.1710              | 4.3419              | 35.1%               | 13.2%               | 0.88%             | 6.7%                                |
| PMLM-xl    | UR50   | -                   | 3.9746              | 37.3%               | 16.9%               | 2.95%             | 17.5%                               |

Figure 3: KL Divergence of PMLM-large on Pfam Valid (Left) and UR50 Valid (Right)

4.3 COMPARISON ON TAPE-CONTACT

For a fair comparison, the models are evaluated on the TAPE-Contact with the same contact prediction head, which is an FC layer with a 0.5 dropout followed by a softmax operator. Medium and long range precision at $L/5$ is used for evaluation, where medium and long range means the there are at least 12 residues between the pair to test. With precision at $L/5$, the top $L/5$ contact predictions are compared to the ground-truth, where $L$ is the length of the input sequence.

To better control the setting, a Transformer encoder for MLM (denoted as MLM-base) as well as a Transformer encoder (denoted as PMLM-base) are pre-trained on the Pfam and UR50 datasets. The hyper-parameters for these models resemble that of the RoBERTa-base for NLP, i.e., hidden size as 768, feed forward dimension as 3072, and number of layers as 12. To further evaluate the effect of PMLM, we also pre-train a deeper Transformer encoder with the number of layers increased to 34, denoted as PMLM-large.

The fine-tuned performance of the models are compared in Table 3. The performance of TAPE transformer and MSA baseline are reported in the paper. As we can see, the performance is increased from 36.0 for TAPE to 37.3 for MLM-base. Comparing the two models which is close in model size pre-trained on Pfam, i.e. MLM-base and PMLM-base, a huge performance gain over 9% is witnessed, demonstrating the positive effect of the PMLM for pre-training.

Notably, PMLM-xl pre-trained on UR50 can even outperform the MSA baseline (about 8%), although UR50 is a small subset of UniParc + Metagenome, which shields the light for the single sequence pre-training methods to exceed the MSA based methods.
Table 3: Medium-/Long-Range Precision@L/5 on TAPE Contact Prediction Test Set. The predictions are generated from a linear projection of the encoder outputs during fine-tuning. UR50 is a small subset of UniParc + Metagenome.

| MODEL          | #PARAMS | DATA   | PRE-TRAIN TASK | RESULT |
|----------------|---------|--------|----------------|--------|
| TAPE Transformer | 38M     | Pfam   | MLM            | 36.0   |
| MLM-base       | 85M     | Pfam   | MLM + PMLM     | 37.3   |
| PMLM-base (ours) | 87M   | Pfam   | MLM + PMLM     | 47.2   |
| PMLM-base (ours) | 87M   | UR50   | MLM + PMLM     | 57.7   |
| PMLM-large (ours) | 250M  | UR50   | MLM + PMLM     | 66.7   |
| PMLM-xl (ours) | 713M    | UR50   | MLM + PMLM     | 71.7   |
| MSA baseline   | -       | UniParc + Metagenome | -      | 64.0   |

4.4 COMPARISON ON RAPTORX-CONTACT

The models are evaluated on the RaptorX-Contact with the same contact prediction module, however, with different contact prediction modules, customized networks may be optimal for different pre-trained encoder.

As shown in Table 4, PMLM-base outperforms ESM Transformer-12 by about 4\% given that their model sizes are close, demonstrating the effect of PMLM for pre-training again. When comparing the larger models of a close model size pre-trained, PMLM-xl significantly outperforms ESM models (i.e., more than 9\% for ESM Transformer-34 and 3\% for ESM-1b) even that a systematic hyper-parameter searching on a 100M model is conducted to generate the hyper-parameters for ESM-1b, which is not performed for PMLM-xl, showing the superior performance of the PMLM encoder.

Table 4: Long-Range Precision@L on RaptorX Contact Prediction Test Set. A ResNet with stacked convolution blocks is built on top of each pre-trained encoder during fine-tuning. The performance numbers of the models except PMLM are reported by Rives et al. (2021). *The PMLM-xl checkpoint fine-tuned from is pre-trained by a half of GPU time as that of the ESM-1b model does.

| MODEL          | #PARAMS | DATA   | PRE-TRAIN TASK | RESULT |
|----------------|---------|--------|----------------|--------|
| TAPE Transformer | 38M     | Pfam   | MLM            | 23.2   |
| UniRep         | 18M     | UR50   | Autoregressive | 21.9   |
| SeqVec         | 93M     | UR50   | Autoregressive | 29.0   |
| ESM Transformer-12 | 85M   | UR50   | MLM            | 37.7   |
| PMLM-base (ours) | 87M   | UR50   | MLM + PMLM     | 41.6   |
| ESM Transformer-34 | 669M  | UR50   | MLM            | 50.2   |
| ESM-1b         | 650M    | UR50   | MLM            | 56.9   |
| PMLM-xl (ours) | 715M    | UR50   | MLM + PMLM     | 59.9*  |

4.5 ABLATION STUDY

To study the factors which influence the model performance, a systemic ablation study is conducted.

PRE-TRAINING DATA  Pfam is a dataset with protein sequences from a few thousand families, while UR50 consists of much more diverse sequences, i.e., sequences from UniRef with 50\% sequence identity. In other words, UR50 is more diverse than Pfam and representative for more sequences. As shown in Table 5, when PMLM-base is pre-trained on UR50, which consists of sequences with higher diversity, instead of Pfam, the performance gain for the task is more than 10\%, increased from 47.2 to 57.7. As shown in Table 5.

MODEL SIZE  Model under-fitting is observed in both ESM model pre-training and ours. A straightforward way to tackle this is by increasing the model size. To examine this factor, we compare two models with different sizes pre-trained on the same data, i.e. PMLM-base and PMLM-large. As shown in Table 5, when PMLM-base and PMLM-large are both pre-trained on UR50, the performance gap is about 14\% between 57.7 (PMLM-base) and 71.7 (PMLM-xl), illustrating that
Table 5: Ablation Study on RaptorX Contact Prediction Test Set (Long-Range Precision@L)

| MODEL | #PARAMS | DATA | PRE-TRAIN TASK | RESULT |
|-------|---------|------|----------------|--------|
| MLM-base (+ Linear) | 85M     | Pfam | MLM           | 19.8   |
| PMLM-base (+ Linear) | 85M     | Pfam | MLM + PMLM    | 25.2   |
| MLM-base (+ Linear) | 85M     | UR50 | MLM           | 36.9   |
| PMLM-base (+ Linear) | 87M     | UR50 | MLM + PMLM    | 39.2   |
| PMLM-base (+ ResNet) | 88M     | UR50 | MLM + PMLM    | 41.6   |
| PMLM-large (+ ResNet) | 252M | UR50 | MLM + PMLM    | 54.1   |
| PMLM-xl (+ ResNet) | 715M | UR50 | MLM + PMLM    | 59.9   |

Increasing model size indeed benefits the performance for contact prediction. Performance increases by more than 18% as shown in Table 5 when comparing PMLM-xl (+ ResNet) with PMLM-base (+ ResNet). Both are pre-trained on UR50.

**Downstream Task Module** On top of the pre-trained encoder, there are various methods to fine-tune the model for downstream tasks. To study the impact of this factor, we also compare the pre-trained encoder PMLM-base with two different downstream modules, namely, a simple linear layer (+ Linear) and an eight-layer ResNet with convolution blocks (+ ResNet). As shown in Table 4, by comparing the performance of PMLM-base (+ Linear) and PMLM-base (+ ResNet), we can see that the precision score is increased from 39.2 to 41.6, indicating that a more expressive model can be trained to improve the performance on the ever pre-trained encoder.

Table 6: Secondary Structure Prediction on the TAPE Benchmark

| MODEL | CB513 | CASP12 | TS115 |
|-------|-------|--------|-------|
| TAPE Transformer | 0.730 | 0.710 | 0.770 |
| PMLM-base (pre-trained on Pfam, ours) | 0.728 | 0.706 | 0.771 |

Table 7: Remote Homology Prediction on the TAPE Benchmark

| MODEL | FOLD | SUPERFAMILY | FAMILY |
|-------|------|-------------|--------|
| TAPE Transformer | **0.210** | 0.340 | 0.880 |
| PMLM-base (pre-trained on Pfam, ours) | 0.199 | **0.446** | **0.946** |

**Other Downstream Tasks** To evaluate our pre-trained encoder on other tasks, we also compared the performance of PMLM-base and the TAPE Transformer for Secondary Structure Prediction and Remote Homology Prediction on the TAPE benchmark, the results in terms of accuracy scores are listed in Tables 6 and 7. The performance numbers of PMLM-base for secondary structure prediction are quite close to that of the TAPE baseline Transformer, illustrating that the proposed model does not hurt the performance with the additional loss. For Remote Homology Prediction, the performance gap varies on the fold-level, superfamily-level, and family-level holdout test sets. The performance of PMLM-base is slightly worse than that of the TAPE Transformer on the fold-level test set, however, much better on the superfamily-level and family-level test sets up to 10%, showing the potential improvement from the extracted co-evolutionary information on other tasks.

5 Discussion

In this paper, we propose a novel model named pairwise masked language model for the protein sequence encoder to capture co-evolutionary information during pre-training. The pre-trained model generates a better representation for global structure by applying this model. Our result shows that the proposed method significantly improves the performance of contact prediction compared to the baselines. Meanwhile, the proposed model surpasses the MSA baseline on the TAPE contact benchmark. Although the performance is encouraging, the potential capability of the proposed model, i.e. PMLM, is not fully developed, neither it is the only way to extract the co-evolutionary
information from the sequences. For example, an observation is that increasing the mask probability for the tokens might improve the representation for PMLM.

Although it sheds light on the single sequence based methods for representation learning for proteins, more endeavors are needed to push forward the pre-training methods for protein sequences. Following the idea of PMLM, supervision from multiple residues, e.g. a Triple Masked Language Model, might also be helpful for pre-training the representation. The metaphor is that three points in Euclidean space follows the triangle inequations inspired by AlphaFold2. However, the storage and computation cost will be cubic to the count of the masked tokens, thus how to efficiently pre-train the model might be an important issue to tackle, which will be left to future work.

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