Predicting peptide aggregation with protein language model embeddings

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Amyloid fibril formation, i.e., aggregation, is associated with multiple diseases and hinders the development of therapeutics. The experimental characterization of aggregating peptides is resource-intensive, limiting the size of labeled datasets. We present a deep-learning model, PALM (Predicting Aggregation with Language Model embeddings), that uses transfer learning to predict aggregation from pretrained protein language model (pLM) embeddings. PALM is trained on WaltzDB-2.0 to classify peptides and identify aggregation-prone regions within a sequence at single-residue resolution. In comparison to existing models, it exhibits strong performance on held-out experimental datasets. We find that PALM fails to identify single mutations that increase the rate of aggregation of amyloid beta peptide; however, training the PALM architecture on a larger dataset, CANYA NNK1-3, substantially improves performance in this task. These results show that transfer learning with pLM embeddings improves performance when training on small datasets, but highlight that challenging tasks, such as predicting the effect of single mutations, require more experimental data.

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Introduction

Amyloid fibrils are filamentous protein aggregates, characterized by their cross-β structure (1). Fibrils form in a sequence-dependent manner, often irreversibly, and have a wide range of biological effects (2). The formation of amyloid fibrils from native human proteins is associated with Alzheimer's disease, type-2 diabetes, and many other diseases (3). Fibrils pose a challenge to the development of biologic drugs by altering their physical properties and pharmacodynamics (4). Fibrils can also play a functional role, enabling the controlled release of biologically active peptides and displaying unique material properties (5, 6). These challenges and applications motivate the development of models that capture the relationship between amino acid sequence and aggregation.

Many sequence-based methods have been developed to predict aggregation. Most methods use simple descriptors such as hydrophobicity, β-sheet propensity (7, 8), or cross-β pairing energy (9). TANGO uses statistical mechanics to calculate a partition function over secondary structure states (10). While these methods have some predictive ability, they are not machine learning models that can be easily improved with new data, and are more fundamentally limited as they predict values that are proxies of aggregation. More recently, multiple sequence-based methods have been developed that learn from experimental data by estimating a position-specific scoring matrix (11), or directly through supervised learning (12, 13). These models have been trained on WaltzDB-2.0, a database of 1,416 hexapeptides classified as amyloid/non-amyloid by Thioflavin-T fluorescence (ThT) assays and Fourier transform infrared spectroscopy (FTIR) (14). Despite the quality of this dataset, it describes only a very small fraction of the potential sequence space of therapeutic peptides.

One strategy to bridge the gap in aggregation data is to leverage models pretrained on large numbers of protein sequences. Protein language models (pLMs) are transformer architectures trained on a self-supervised masked language modeling (MLM) task (15). The representations learned by pLMs have been used for many tasks, including structure prediction, mutational variant effect prediction, and the design of novel functional proteins (16–19). pLMs have been applied to predict the aggregation of hexapeptides through finetuning (20), but this work has not yet been extended to peptide sequences longer than six amino acids.

We developed the deep-learning model, PALM (Predicting Aggregation with Language Model embeddings), to predict amyloid-forming peptide sequences (Fig.1). PALM is trained on WaltzDB2.0 and uses embeddings from pretrained ESM2 to predict aggregation. The model extracts local sequence patterns with an adapted Light Attention architecture (21), which we refer to as the Aggregation Predictor Module (APM). The key innovation is that the APM infers residue-specific contributions from experimental data with sequence-level annotations. To enable accurate prediction with peptide sequences that are longer than the hexapeptides in WaltzDB, we employ a data augmentation strategy, where non-hydrophobic residues are padded around the original six amino acid WaltzDB sequences prior to training. Relative to existing models, PALM demonstrates strong performance when benchmarked on datasets Serrano158 (10) and AmyPro22 (22), which assess the ability of the model to classify sequences and identify aggregation-promoting regions (APRs), respectively. We further evaluated PALM on additional experimental data, and found that interestingly, the model fails to identify aggregation-inducing single amino acid substitutions in amyloid beta peptide known to cause familial Alzheimer's disease. We demonstrate that training PALM on a larger dataset, NNK1-3, which was released concurrently with this work (23), improves the performance on this task as well as sequence-level classification.

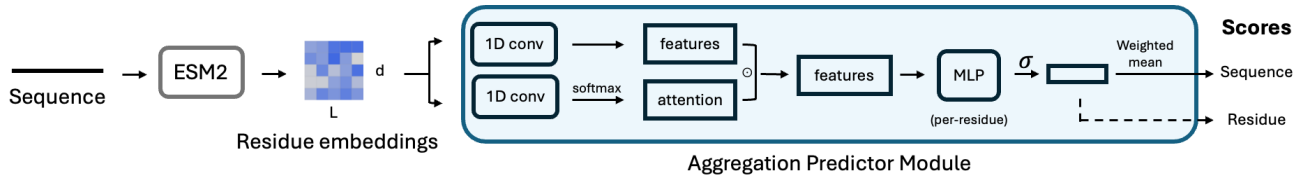

Fig. 1. The architecture of the PALM model. Protein sequences are embedded using ESM2 and then passed to the Aggregation Predictor Module (APM). The module uses one-dimensional convolutions to extract local patterns in the sequence and then predicts the residue aggregation score with an MLP. The weighted mean of the residue aggregation scores is used to compute the overall score for the sequence. Only the sequence-level aggregation score is used to compute the loss during model training.

Methods

Datasets

Training data

The model was trained on WaltzDB-2.0, a database of peptides experimentally labeled as amyloid or non-amyloid (14). The database contains 1,416 six amino acid peptides, 515 of which have been observed to form amyloid fibrils. The labels were experimentally determined through ThT assays and FTIR. Prior to model training, the sequences were randomly assigned into five equally sized folds.

The WaltzDB dataset was augmented to diversify the lengths of the sequences in the training data. We modified each six amino acid sequence by appending padding sequence to the beginning and end of the string. The main assumption of the residue padding strategy is that the addition of residues at either termini does not change experimentally-determined aggregation behavior of the original sequence. More specifically, we assumed that 1) a six-residue aggregation prone region within a larger sequence context was sufficient for aggregation of the sequence overall and 2) that the additional padding residues would not introduce new APRs into the sequence. The first assumption is shared by other methods that search for short windows within a larger sequence context. The second assumption depends on the distribution from which the padding residues are sampled, which influences the rate at which new APRs are introduced into the sequences.

The padding sequences were generated by uniformly sampling L residues from $\{1..L_{max}\}$. Here we considered $5 \leq L_{max} \leq 20$. Three residue type probability distributions were considered: "Random" (uniform), "WaltzDB non-amyloid", and "Non-hydrophobic". Random padding was generated by uniformly sampling from the 20 canonical amino acids. All WaltzDB sequences labeled as non-amyloidogenic were used to generate the WaltzDB non-amyloid residue probability distribution. The non-hydrophobic distribution consisted of residues = (C, D, E, G, H, K, N, P, Q, R, S, T), each sampled with equal probability (Fig. S1). The sequences in WaltzDB-2.0 were oversampled tenfold, with each copy concatenated to a unique padding sequence (Fig. S2). This operation expanded the total size of each augmented dataset to 14,148 sequences. To prevent leakage, the fold assignment of the original six amino acid peptides were preserved with the padded sequences.

Evaluation data

Multiple datasets with sequence or residue level binary amyloid labels were used to evaluate the models, as summarized in Table S1. The first dataset, AmyPro, consists of 162 proteins with experimentally-determined residue-level annotation of amyloid-prone regions (22). To prevent data leakage, we filtered out 130 proteins containing hexapeptides found in WaltzDB-2.0 training samples. Following Planas-Iglesias et al. (13), we removed an additional 10 proteins with >50 amino acid amyloid-prone regions, resulting in 22 sequences, which we refer to as AmyPro22. The second dataset consisted of peptides with sequence-level amyloid labels, originally curated by Fernandez-Escamilla et al. to evaluate TANGO (10). The peptides were derived from a total of 24 proteins from multiple publications and measured by CD spectroscopy. In total, this yielded 224 sequences classified as amyloid or non-amyloid. To reduce redundancy to the Waltz training data, sequences were divided into sliding windows of six amino acids and all sequences containing a hexapeptide motif found in WaltzDB were removed. To minimize overlap between evaluation sets, we removed two sequences with greater than 40% sequence similarity to sequences in AmyPro22, as determined by global sequence alignments with the Needleman-Wunsch algorithm (24). This left a total of 158 sequences, which we refer to as Serrano158.

Concurrently with this work, a large dataset of >100,000 peptides tested for aggregation via a massively parallel selection assay became available (23). The sequences are between 1 and 20 amino acids, and were obtained using synthetic NNK oligo pools, resulting in randomized peptides with a high degree of sequence diversity. An aggregation score is reported for each sequence, as determined by an *in-vivo* selection assay with binary labels assigned by the authors. The test set, NNK4, contains 7040 sequences. The training set, NNK1-3, contains 100,730 sequences. The sequences were randomly assigned to five folds for cross-validation.

In an earlier work, a similar selection assay was used to measure aggregation scores for mutants of Aβ42 peptide (25). Of the 753 single amino acid substitutions in this dataset, 13 are recognized to cause familial Alzheimer's disease by driving amyloid formation (26, 27). Following Seuma et al., we define the 13 fAD substitutions as amyloid and the remaining substitutions as non-amyloid. We refer to this dataset as Aβ42 peptide mutants.

Protein language model embeddings

We used the pretrained protein language model, ESM2, to extract embeddings from amino acid sequences (16). ESM2 is a transformer trained with a masked-language modeling (MLM) objective on sequences from UniRef50. To explore the influence of scale on the embeddings, we considered four model sizes: 8M parameters (6 layers), 35M parameters (12 layers), 150M parameters (30 layers), and 650M parameters (33 layers). For each sequence, an embedding tensor of shape $[L, d_{\text{in}}]$, where L is the length of the sequence and d_{in} is the size of the hidden dimension, was extracted from the final hidden layer of the model. The value of d_{in} varies depending on the size of the model. To visualize the embeddings, the embedding vectors were mean-pooled along the length dimension and dimensionality reduction was performed with t-stochastic neighbor embedding (t-SNE).

Aggregation Predictor Module architecture

The Aggregation Predictor Module (APM) was designed to identify local sequence motifs that form amyloid-promoting regions. The APM was adapted from Light Attention (21), which uses convolutional filters to extract local patterns in the residue embeddings generated by pLMs. As described previously, embeddings with dimensions $[L, d_{in}]$, were extracted from ESM2 and passed to the APM. Then two independent, one-dimensional convolutions (kernel size = 5 and stride = 1) were applied along the sequence dimension to yield a value tensor $\mathbf{v}$ and attention tensor $\mathbf{a}$, both with dimensions $[L, d_{in}]$ (the hidden layer dimension size of the original embeddings was preserved). Softmax was applied over the L dimension of the attention tensor to convert the values to attention weights. Dropout was applied with a probability of 0.25 to the feature tensor. The two tensors were then multiplied element-wise to generate a new attention-weighted feature tensor $\mathbf{f}$ with dimensions $[L, d_{in}]$. The tensor $\mathbf{f}$ was passed to a multi-layer perceptron (MLP), which operates along the L dimension. The MLP consisted of two linear layers (output dim=32, output dim=1). Dropout (rate = 0.25), ReLU activation, and batch normalization were applied between these layers. The output was transformed with a sigmoid function to yield a vector $\mathbf{r}$ of length L with values in the range $[0, 1]$. The values in $\mathbf{r}$ were used to compute the sequence aggregation score s by applying a weighted mean operation

$$s = \sum_{i=1}^{L} w_i r_i$$

$$w_i = \frac{r_i}{\sum_{i=1}^{L} r_j}$$

Where w_i is the residue-specific weight computed from $\mathbf{r}$. The residue-wise application of the MLP and the weighted mean are the main modifications to the original Light Attention module. The use of the weighted mean to compute s from the values in $\mathbf{r}$ enhances the interpretability of the model.

Model training

The model was trained to minimize the binary cross-entropy loss function using stochastic gradient descent. The loss was averaged for each batch (n=1000). For a predicted sequence score $\overline{y}$, the loss was defined as:

$$\text{BCEloss}(y, \overline{y}) = -\left(y \cdot \log \overline{y} + (1 - y) \log (1 - \overline{y})\right)$$

To avoid overfitting, the model was trained with early stopping and checkpointing. If the validation loss did not decrease after 200 epochs, the weights from the epoch with the lowest validation loss were selected. Hyperparameter optimization was performed through a manual grid search. Convolutional kernel size (1, 5, 7, 11), learning rates = (0.5, 0.05), and MLP layer count (2, 4) were explored. Hyperparameters that minimally increased performance were minimized to limit model size. The final hyperparameter values that were selected were kernel size = 5, learning rate = 0.05, and MLP layer count = 2.

Model evaluation

We used five-fold cross-validation to evaluate the models. We chose ROC AUC to measure model performance, as it is more robust to differences in label imbalance between the evaluation datasets. ROC AUC values were measured on four evaluation datasets: Aβ42 mutants, NNK4, Serrano158, and AmyPro22. For the datasets annotated at the sequence level (Serrano158, Aβ42 mutants, NNK4), each sequence was taken to be an individual sample. For residue-level annotations (AmyPro22), individual amino acids were considered to be a single sample.

Serrano158 and AmyPro22 were used to perform ablation studies on PALM. The main version of PALM and the ablations were compared using Tukey's Honestly Significant Difference (HSD) test computed using the statsmodels python package (28). The model ablations were grouped into three categories: dataset, embedding, and architecture. HSD tests were performed independently on each group of ablations.

We evaluated multiple versions of PALM, with different residue padding strategies and ESM2 model sizes to understand their importance and select the optimal configuration. For these experiments, we used fixed hyperparameters identified during the manual grid search. We selected the non-hydrophobic, $L_{max} = 10$ residue padding strategy and ESM2 8M based on the performance of the model on Serrano158 and AmyPro22. We also performed ablations of the APM, removing sections of the module and retraining the model. For a complete ablation, we replaced the entire APM with a logistic regression (LR) head. The LR head was trained using nested cross-validation over the five folds. The hyperparameters were optimized with grid search in the inner loop of the cross-validation. We explored penalties: (l1, l2) and regularization strength: (0.01, 0.1, 1.0, 10, 100). The comparisons between padding strategies, ESM2 model sizes, and ablations are shown in Figure 3.

We benchmarked PALM against TANGO (10), AggreProt (13), AggreScan (29), ANuPP (12), and PASTA (30). These methods output residue-level aggregation scores, which we

used to compute ROC AUC values AmyPro22. We considered sequences to be classified by Aggrescan as amyloid if the number of hotspots was one or greater. For models that only provide residue values, including PASTA, ANUPP and AggreProt, we obtained sequence-level values by taking the maximum over the residue values. In all cases, taking the maximum residue probability as the overall sequence probability outperformed the mean.

Results

Adding padding residues to WaltzDB sequences results in higher similarity to Serrano158 and AmyPro22

The peptides in WaltzDB are shorter than the majority of natural peptides and the sequences in Serrano158 and AmyPro22 (Fig. 2). We visualized the embedding space of ESM2 8M using t-SNE and observed that the sequences in WaltzDB form a distinct cluster that does not overlap with the sequences in Serrano158 and AmyPro22 (Fig. 2). Notably, the sequences did not cluster by amyloid classification, indicating that local sequence features were more responsible for those differences. We hypothesized that the difference in sequence length was partially responsible for the observed clustering, indicating that padding WaltzDB sequences could create overlap between datasets. Each sequence in WaltzDB was modified by adding up to 10 additional randomly sampled residues at the N- and C-termini to introduce variation in the length of the sequence (see methods). The sequences in WaltzDB-padded (non-hydrophobic, $L_{max} = 10$) formed a cluster that overlapped the Serrano158 cluster, distant from the original WaltzDB cluster. Other padding strategies, which varied in the amino acid composition and maximum length of the padding region, exhibited similar trends (Fig. S3).

WaltzDB residue padding improves performance

The highest performance was observed with WaltzDB-padded(Non-hydrophobic, 10aa), which significantly improved performance on Serrano158 and AmyPro22 relative to the model trained on unaltered WaltzDB sequences (Fig. 3). The difference in ROC AUC was more pronounced on Serrano158, suggesting that padding the training data was particularly important for sequence-level classification. Alternative residue padding strategies performed significantly worse on one or both evaluation datasets, with the exception of non-hydrophobic amino acids with a maximum length of 20 aa. This strategy appeared to be worse than 10 aa non-hydrophobic padding; however, HSD tests indicated that this performance difference was not statistically significant. We inspected the residue level scores of the sequences in the validation set and found that the models trained with alternative padding strategies assigned higher scores to residues from the non-amyloid WaltzDB sequences and padding regions (Fig. S4). The median residue score was found to be higher in the padding and non-amyloid residues for models trained with all other padding strategies.

The smallest ESM2 model provides the best performance

To explore the influence of language model scale on amyloid prediction, we trained the APM using four ESM2 models of increasing size. We observed a negative relationship between the size of the ESM2 model and the performance on both test datasets. Substituting ESM2 embeddings with one-hot encodings significantly lowered performance on Serrano158 and AmyPro22, supporting that embeddings from the protein language model substantially improve performance (Fig. 3). The Serrano158 ROC AUC taken from the ESM2 8M model (PALM) was 0.91 ± 0.005 was significantly better than all other sizes, with the exception of the 35M parameter model. Conversely, AmyPro22 ROC AUC (0.68 ± 0.02) was significantly higher than all other models. The drop in performance with larger language models (ESM2 650M) relative to the default (ESM2 8M) was more pronounced with the AmyPro22 APR prediction task. Between the largest and smallest embedders tested, the ROC AUC dropped by 0.120 on AmyPro22 and 0.031 on Serrano158.

The APM enables residue-level prediction of aggregation-promoting regions

We found that replacing the light attention module with a simple one-dimensional CNN (no attention tensor) resulted in a small, marginally significant drop in performance on Serrano158 (Fig. 3). This change did not impact residue-level prediction performance. Similarly, replacing the weighted-mean reduction operation with the mean over the residue scores did not significantly impact performance on either task. However, when the model was trained with mean-reduction and WaltzDB (no padding), the ROC AUC on AmyPro22 dropped substantially. This suggests that the weighted mean is beneficial for the detection of APRs in sequences with highly variable lengths. Surprisingly, replacing the predictor head with a simple logistic regression (LR) model resulted in equivalent performance on Serrano158. Removing padding and featurizing the sequences with one-hot encodings led to a large decrease in ROC AUC. The drop in ROC AUC observed with the use of one-hot encodings was more pronounced when paired with logistic regression than it was with the APM.

PALM predicts aggregating peptides and aggregation-prone regions

Comparison to existing models

We measured the performance of our model against existing methods for predicting amyloid aggregation using the Serrano158 and AmyPro22 datasets (Table S1). PALM exhibited strong performance on both datasets, with other models performing moderately worse. PASTA (30) performed remarkably well on AmyPro22, but notably failed to predict sequence-level aggregation as assessed by Serrano158. The remaining methods performed similarly on AmyPro22, with ROC AUC values ranging from 0.639 (AggreProt) to 0.649 (ANuPP). On Serrano158 relative to PALM, Aggrescan performed notably worse, ANuPP only moderately worse, and

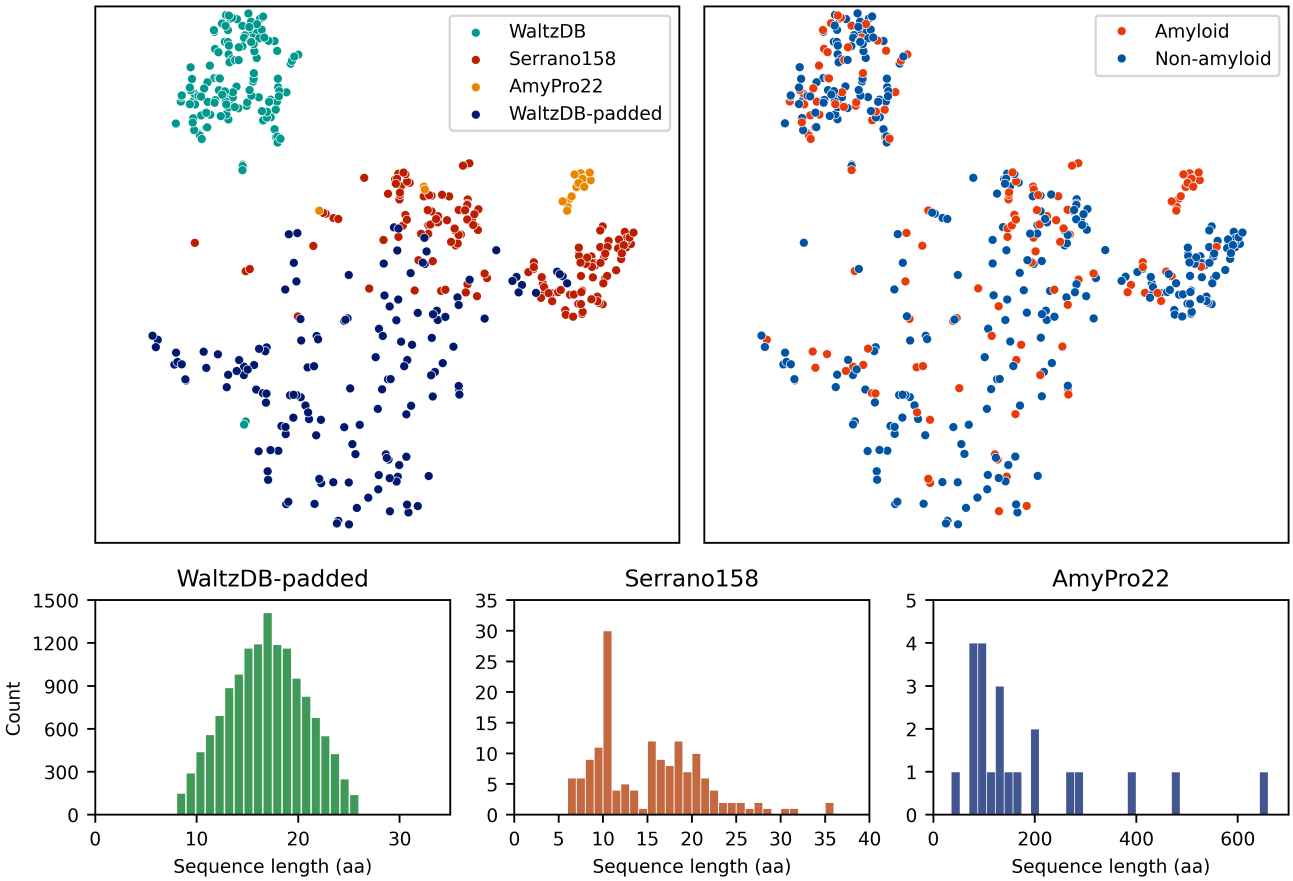

Fig. 2. The sequences in WaltzDB form a distinct cluster from other peptide sequences. Top: The ESM2 8M embeddings for sequences from WaltzDB, Serrano158, AmyPro22, and WaltzDB-padded (non-hydrophobic, $L_{max} = 10$) are visualized using t-SNE dimensionality reduction. Left: The sequences are colored by dataset, right: the sequences are colored by whether they are labeled as Amyloid/Non-amyloid. The WaltzDB and WaltzDB-padded (non-hydrophobic, $L_{max} = 10$) datasets were sub-sampled to 200 sequences for visualization. Bottom: The distribution of sequence lengths for the evaluation datasets.

TANGO and Aggreprot performed comparably.

Model	ROC AUC	
	AmyPro22	Serrano158
PALM	0.678 ± 0.02	0.908 ± 0.005
TANGO	0.645	0.894
AggreProt	0.639	0.888
ANuPP	0.649	0.857
AggreScan	0.645	0.801
PASTA	0.707	0.327

Table 1. Benchmark ROC AUC values for different models. The AmyPro22 dataset is labeled at the residue level, while Serrano158 is labeled at the sequence level.

Aggregation score distributions

As expected, distributions of the predicted scores on Serrano158 and AmyPro22 separated based on their labels, with AmyPro22 exhibiting more overlap than Serrano158 (Fig. 4). The sequence scores on Serrano158 were bimodal. While the majority of the non-amyloid sequences had scores close to 0, some were erroneously assigned scores higher than 0.5. In contrast, almost all of the amyloid sequences were assigned scores close to 1. The residue scores distributions on AmyPro22 were unimodal and centered around 0.78. The amyloid sequences exhibited a tail to the right with higher scores. The non-amyloid sequences did as well, but to a lesser degree. In comparison, there was a much higher degree of separation of scores between classes in the training set.

Visualizing residue score profiles

The residue score profiles of representative peptides highlight aggregation-prone regions within the sequence. First, we considered the 42 amino acid isoform of human amyloid beta peptide (Aβ42), which contains an APR between residues 12-42. The residue scores were highest in two regions between residues 13-23 and 30-40 (Fig. 4). Both of these regions match sequences labeled as amyloid in WaltzDB. The residue scores were notably high for all five models trained with cross-validation, indicating that the high aggregation scores persist even when those sequences are held out in the validation set. We also considered the viral protein PB1-F2, which contains a C-terminal region (i.e., residues 53-90) that has been shown to form amyloid fibrils (31). The residue score profile of PB1-F2 displayed multiple peaks, with the highest

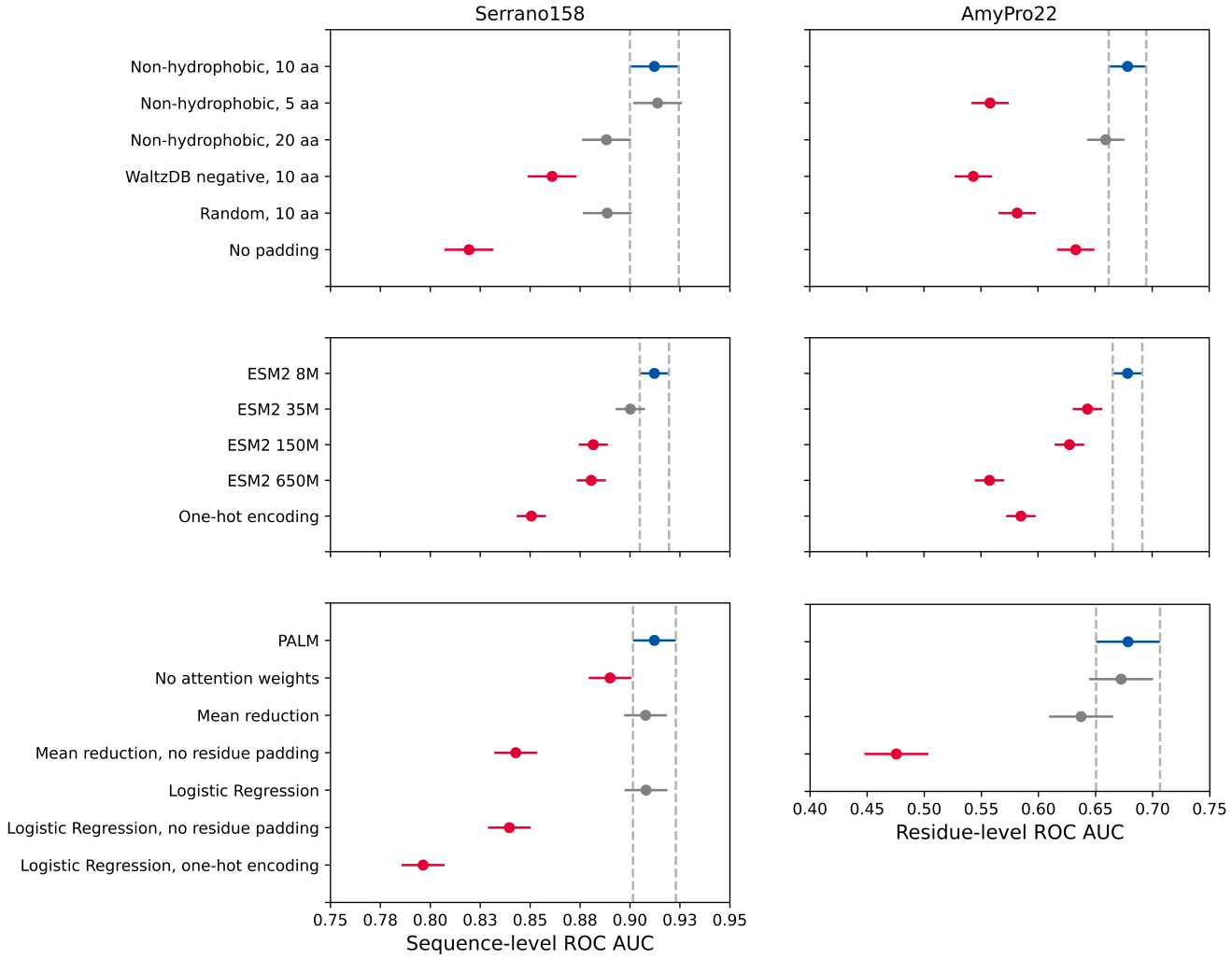

Fig. 3. Model ablations reveal key elements of the model. Left column: ROC AUC values on Serrano158, right column: ROC AUC values on AmyPro22. First row: alternate WaltzDB padding strategies, second row: ESM2 model sizes, third row: architectural ablations in the APM. ROC AUC values across cross-validation folds were compared using Tukey's Honestly Significant Difference (HSD) test. PALM is shown in blue with a dashed confidence interval surrounding it. Models that performed comparatively to PALM are shown in grey, and models that fall outside of the confidence interval are shown in red.

between residues 68-73, part of the region that is annotated. Some residues outside of the C-terminal region also showed higher scores, but overall the values were similar to the average score across the AmyPro22 dataset (0.78). Prediction variability between five cross-validation folds was generally observed to be at its lowest in amyloid-prone regions. Scores across all five folds exhibited near-perfect agreement in the aggregating regions of the 42-residue amyloid beta peptide. Interestingly, the residue scores were lower between residues 24-29, despite the fact that this contains a third WaltzDB sequence labeled as amyloid, potentially because it plays less of a role in aggregation. Regions of the PB1-F2 APR exhibit a similar lack of variability across cross-validation folds, including residues 68 to 72.

Evaluating PALM on additional datasets

PALM classifies diverse sequences, but struggles to predict the effect of amino acid substitutions

We evaluated PALM on a diverse set of additional datasets to assess its practical utility. First, we considered NNK4 (Table 2), (23)) a random library of peptides experimentally labeled as amyloid/non-amyloid by a high-throughput parallel selection assay. PALM showed some predictive power, showing a slightly higher ROC AUC than Tango and AggreProt, but lagged behind CANYA, a model trained on a large dataset obtained with the same high-throughput experimental assay.

Next, we considered single amino acid substitutions of amyloid beta peptide (Aβ42). We followed Seuma et al. (25), evaluating the ability of the model to classify 13 amino acid substitutions of Aβ42 known to cause familial Alzheimer's Disease (fAD) by increasing the rate of aggregation (Table 2). Surprisingly, we found that PALM and AggreProt completely failed to identify these mutations (Table 2). CANYA

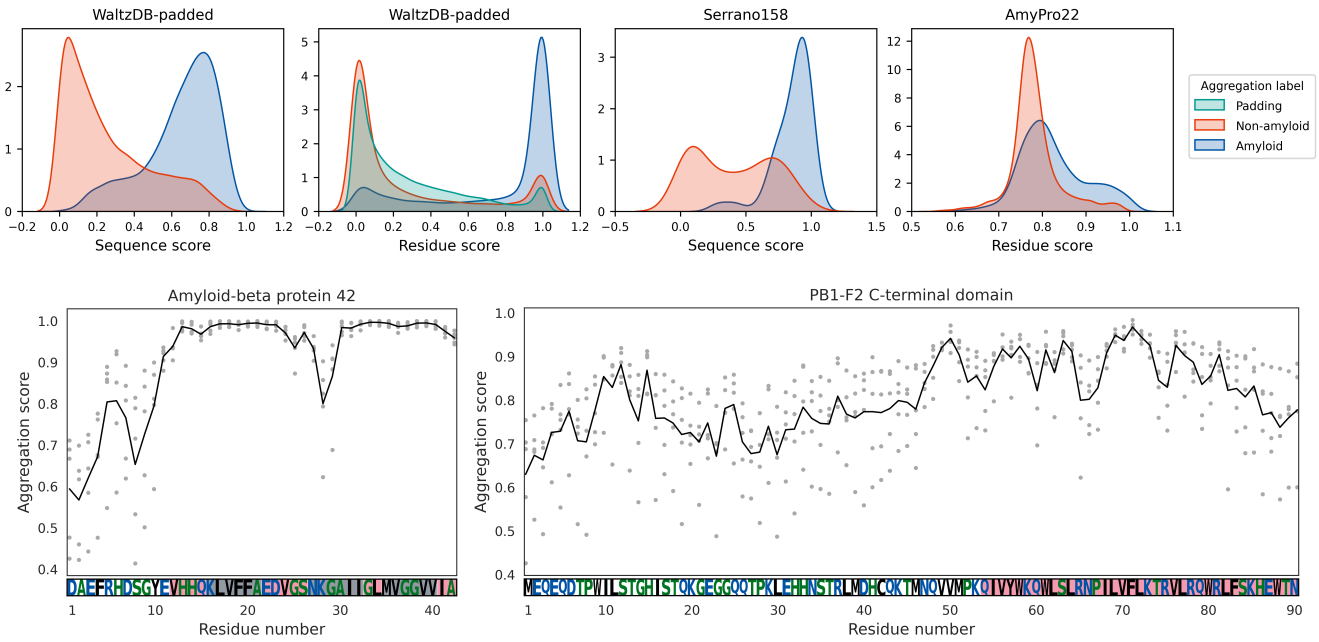

Fig. 4. PALM learns to identify aggregation-prone regions in peptide sequences. Top row: comparison of sequence and residue score distributions. From left to right: the sequence score for amyloid/non-amyloid sequences in the training set, the residue score of amyloid/non-amyloid/padding residues in the training set, the sequence score for Serrano158, and the residue score for AmyPro22. The amyloid/non-amyloid distributions of the test set sequences show separation, with some overlap. Bottom row: residue score profiles for human amyloid beta peptide 42 (left) and PB1-F2 influenza A (right). The APR residue range from AmyPro22 is highlighted in red. The hexapeptides present in the WaltzDB2.0 training set are highlighted in green.

also struggled, with a relatively low ROC AUC. TANGO performed well on this task, which is consistent with the fact that its scores are intended to be used to compare between closely related sequences.

Retraining on CANYA data improves performance across prediction tasks

We retrained the PALM architecture with the NNK1-3 dataset. PALM (NNK1-3) matched the ROC AUC of CANYA on the NNK4 test set and showed an improved ability to predicted dominant fAD mutations (Table 2). Interestingly, PALM (NNK1-3) trained with one-hot encodings replacing language model embeddings was less effective on the NNK4 dataset, but showed improved performance on the fAD mutation prediction task.

We visualized the residue scores of WT Aβ42 and the 13 dominant fAD mutants predicted by PALM and the variants trained on NNK1-3 (Fig. 5). The residue scores predicted by PALM for each of the mutants were similar to the wild-type sequence, which already predicted values close to 1, despite the fact that the mutations are known to further increase the rate of aggregation. The residue scores predicted by the PALM (NNK1-3) were lower overall (Fig. S5), but displayed sharp peaks in similar regions in the sequence (Fig. 5). In particular, Val18 and Phe19 were respectively predicted by PALM (CANYA) and PALM (NNK1-3, one-hot) to be among the most aggregation prone residues of the sequence. The difference in residue scores between the mutants and the wild-type predicted by PALM (NNK1-3, one-hot) were localized to within 1-3 residues, as expected by the size of the convolutional filter. In contrast, some single mutations were predicted by PALM (NNK1-3) to have a broader effect. For example, the residue score for the E22G Arctic mutant at position 22 was slightly lower than WT, but increased from residues 25-42. One of the mutations that was not predicted significantly increase aggregation, A21G, was experimentally determined as having the smallest positive effect on aggregation of the 13 mutants (25).

Model	ROC AUC	
	NNK4	Aβ42 mutants
PALM	0.72 ± 0.01	0.51 ± 0.03
PALM (NNK1-3)	0.82 ± 0.002	0.62 ± 0.02
PALM (NNK1-3, one-hot)	0.78 ± 0.001	0.70 ± 0.01
CANYA	0.81	0.61
TANGO	0.68	0.75
AggreProt	0.68	0.45

Table 2. The ROC AUC values for models evaluated on two additional datasets. Both the CANYA NNK4 and Aβ42 mutants datasets are evaluated at the sequence level.

Discussion

PALM classifies peptides using embeddings from ESM2

We present a model trained on WaltzDB-2.0 that leverages ESM2 8M embeddings to achieve robust classification of amyloid peptides in our benchmark dataset, Serrano158. Replacing the sequence embeddings with one-hot encoding results in a large drop in performance, supporting that the em-

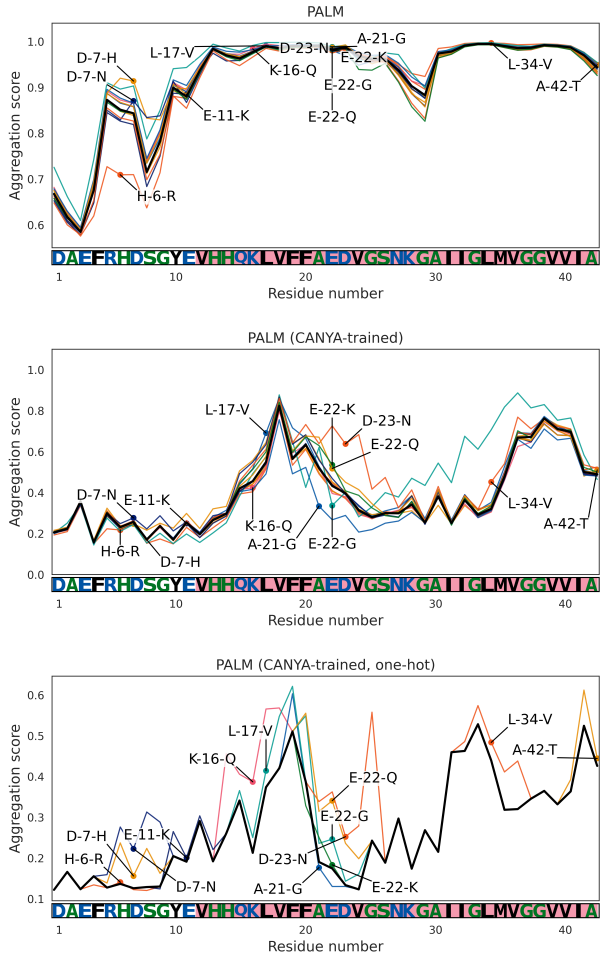

Fig. 5. Training PALM with NNK1-3 leads to improved detection of mutations that promote aggregation in fAD. The residue aggregation scores for the wild-type Aβ42 sequence are shown as a black line and the aggregation scores for the sequences with aggregation promoting substitutions are shown as colored lines. Top row: PALM, middle row: PALM trained on NNK1-3, bottom row: PALM trained on NNK1-3 with one-hot encodings. The mutated residue is highlighted with a dot and label. The wild-type sequence and residue number is shown below. The residue range annotated as the APR in AmyPro is highlighted in red.

beddings are key to the model's performance. Despite the importance of pLM embeddings, increasing the scale of the ESM2 model leads to decreased performance. This is consistent with other work, which has shown that embeddings generally lead to improved performance in transfer learning, but that the benefit of increased scale is not observed for tasks that are not closely aligned with the masked language modeling pretraining task (32). The addition of padding residues was critical to the performance of the model, likely because the sequences that are classified by the model during inference are substantially longer than the six amino acid sequences in WaltzDB-2.0. The performance of PALM on Serrano158 and NNK4 is robust relative to previous methods, with only TANGO and Aggreprot close to matching its performance. However, there is still a gap between PALM and CANYA, which was trained on NNK1-3 data. This is likely explained by the limited sequence diversity in WaltzDB-2.0, which is 100 times smaller than NNK1-3.

The ability to identify APRs emerges without training on residue-level labels

Identification of APRs within sequences is critical for understanding how individual residues contribute to aggregation. Due to practical considerations and limitations in data, many works in the field have focused on short windows of longer peptide sequences when predicting APRs (10–12, 20). In contrast, PALM generates embeddings of the complete sequence at once, allowing for long-range interactions to be captured. PALM outperformed all of the existing models, except for PASTA, when identifying APRs in AmyPro22, and this ability emerges from the model without explicitly being trained to predict aggregation-promoting residues. The performance on AmyPro22 was more sensitive to the padding strategy, with only non-hydrophobic 20 aa padding providing to a comparable ROC AUC value. As with Serrano158, the ESM2 8M embeddings were critical for performance, but increasing the ESM2 model scale had a negative effect. The ablations indicate that the weighted mean and residue padding serve similar functions; removing either results in a minor decrease, whereas removing both causes a significantly larger decline. It is important to note that the residue-level annotations in AmyPro22 are compiled using different methods and therefore may be of limited accuracy. Visual inspection of Aβ42 and the PB1-F2 residue profiles shows that there is wide range of residue scores within the regions labeled as APR, suggesting that finer-grained resolution of residues contributing to aggregation may be possible. As PALM can be trained with sequence-level labels, which are readily obtained from experiments, it can continue to improve at APR identification as new data becomes available.

More data is required to predict single amino acid substitutions that increase aggregation rate

The ability to predict the impact of single mutations on aggregation is important for guiding the design of new sequences. Given the strong performance of PALM on Serrano158 and NNK4, we assessed whether it could identify single amino acid substitutions in Aβ42 that lead to dominant familial Alzheimer's disease by increasing the aggregation rate. We found that PALM was unable to identify that these mutations increase aggregation, in part because the residue scores at these positions were already close to the maximum value of 1. Training PALM with NNK1-3, a larger and more diverse dataset than WaltzDB-2.0, resulted in substantially improved performance on this task. The residue score profiles of PALM (NNK1-3) reveal that the values are not already saturated for the WT sequence, and that the aggregation-inducing mutations consistently increase the scores at multiple positions in the sequence. When training PALM with NNK1-3, one-hot encodings performed well compared to ESM2 8M embeddings. On NNK4, the drop in ROC AUC with one-hot encodings was small, and on the Aβ42 mutants, the model using one-hot encodings performed substantially better. This

shows that the benefit of using transfer learning with pLM embeddings is less substantial as more labeled data becomes available. Future work can explore the potential of fine-tuning pLMs with larger labeled datasets.

Conclusion

PALM leverages protein language model embeddings to achieve state-of-the-art prediction of aggregation in peptide sequences. The architecture allows for residue-level identification of aggregation-promoting regions within protein sequences, without explicitly being trained on this task. As new data becomes available, PALM can be improved, as demonstrated by training on the NNK1-3 dataset, which enabled the identification of aggregation-promoting mutations in amyloid-beta peptide. These insights will support therapeutic peptide development and the identification of aggregation-promoting mutations relevant to disease.

CODE AVAILABILITY

Code and model weights will be made available at a future date.

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Supplementary Information

Dataset	Type	Sequence count	Sequence length range	Amyloid (%)	Annotation level
WaltzDB-padded	Train	14,148	[8, 26]	36.2	Sequence
AmyPro22	Test	22	[35, 660]	18.3	Residue (APR)
Serrano158	Test	158	[6, 36]	24.6	Sequence
Aβ42 mutants	Test	753	[42]	1.7	Sequence
NNK1-3	Train	100,730	[1, 20]	20.7	Sequence
NNK4	Test	7,394	[1, 20]	23.8	Sequence

Table S1. A summary of experimental datasets used for model training and evaluation. The curation of the datasets is described in the methods.

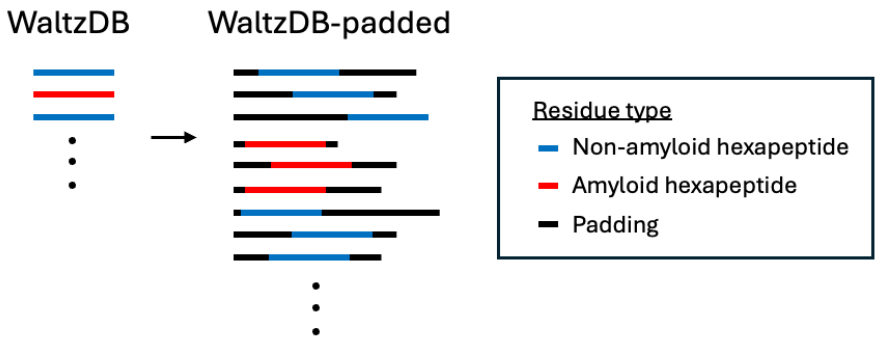

Fig. S1. A diagram illustrating how padding was appended to WaltzDB sequences. A hexapeptide sequence is selected from WaltzDB and then two distinct padding sequences are appended to the beginning and end of the sequence. Amyloid sequences are shown in red, non-amyloid sequences are shown in blue, and padding sequence is shown in black.

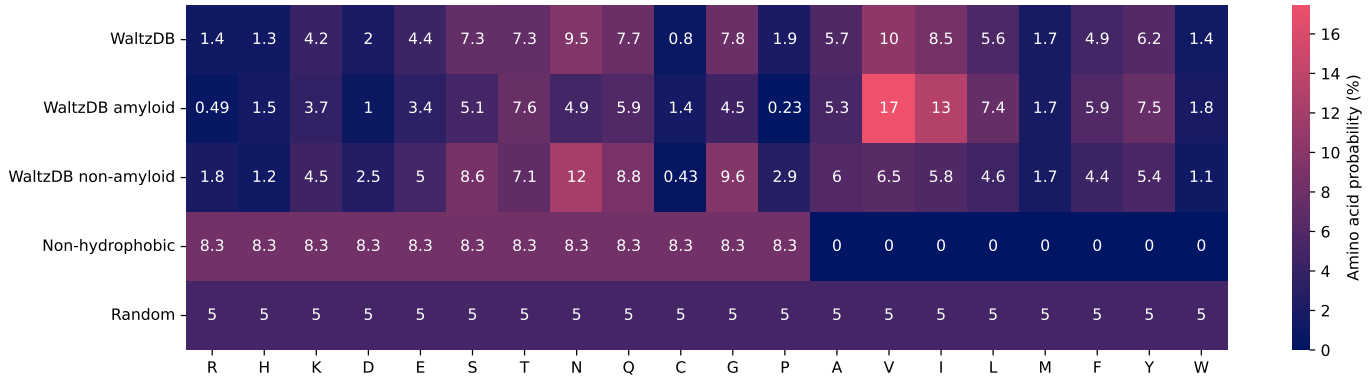

Fig. S2. Amino acid probability distributions for datasets used in training and residue padding. Random and non-hydrophobic padding strategies are evenly distributed among subsets of amino acids, while distributions measured on subsets of the WaltzDB dataset are taken from experimentally measured proteins.

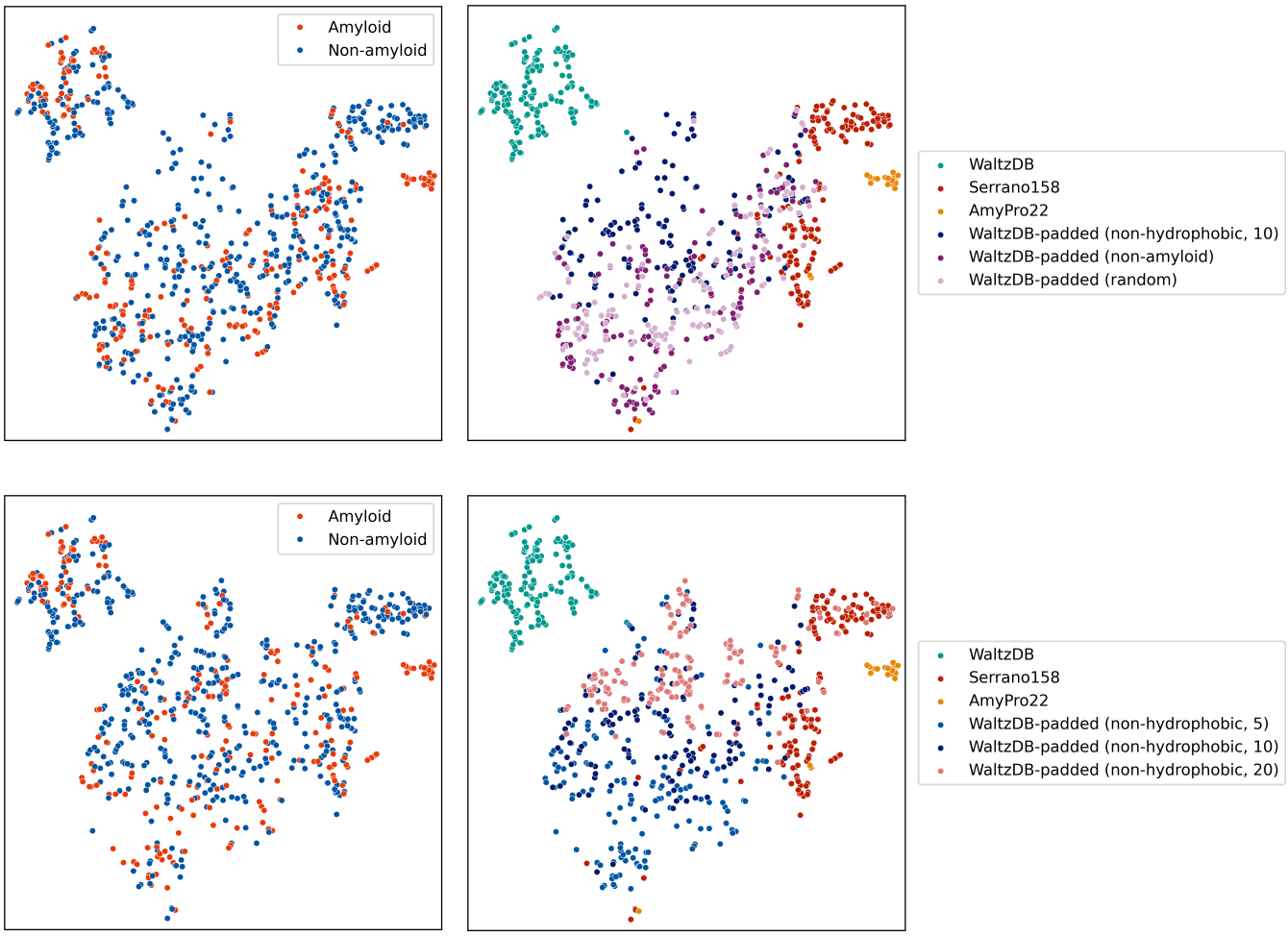

Fig. S3. ESM2 embeddings for sequences generated with different padding strategies visualized using t-SNE, colored by label (left column) and dataset (right column). Top row: comparing sequences generated with different residue padding distributions, all with max length 10 aa. Bottom row: comparing sequences generated with the non-hydrophobic residue padding strategy, with different max lengths. Datasets with different maximum padding lengths occupy different regions of embedding space.

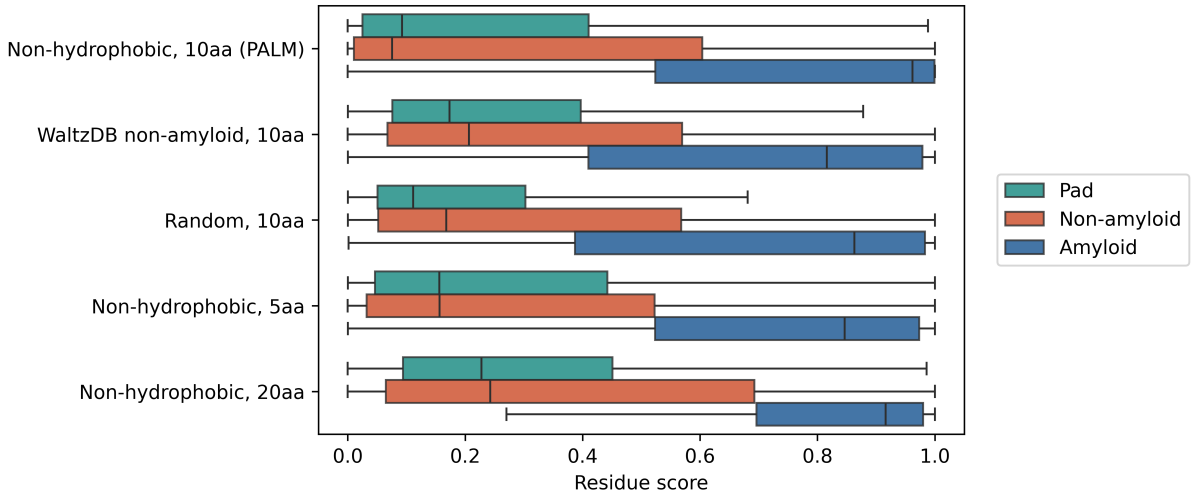

Fig. S4. Residue score distributions from models trained on WaltzDB with different padding strategies. Distributions were computed using the WaltzDB training set. Padding is shown in green, while fibrillating residues are shown in blue and non-fibrillating residues are shown in red.

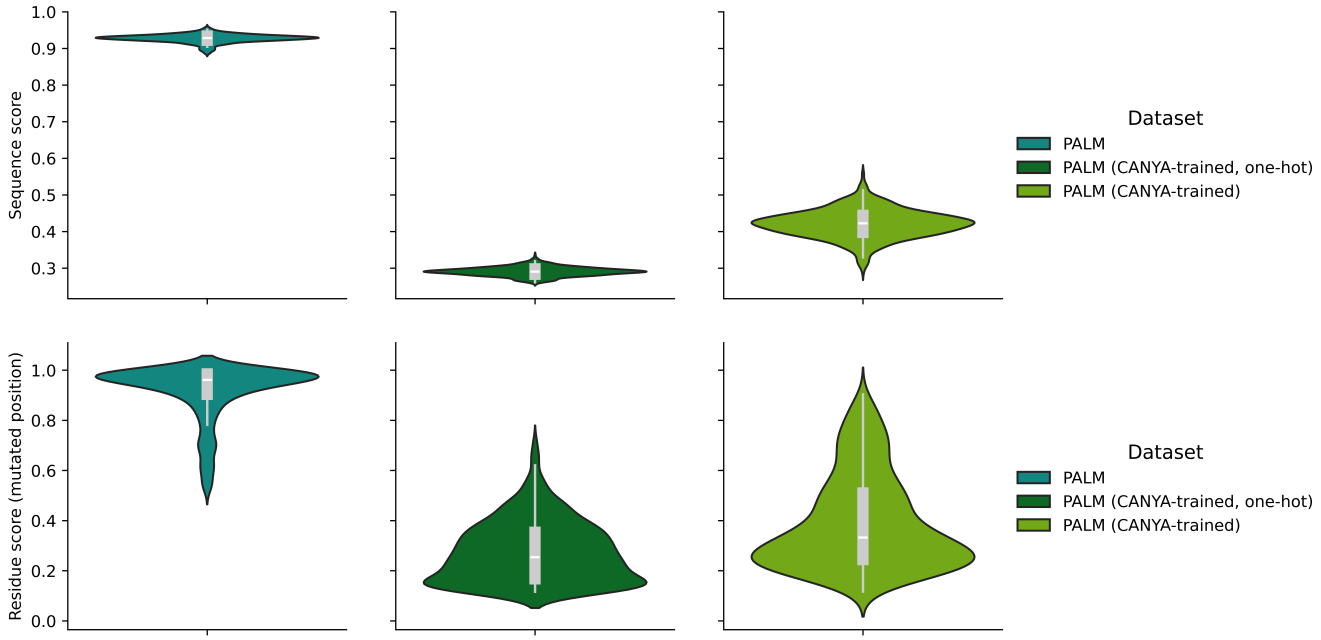

Fig. S5. Violin plots of sequence and residue scores taken from the amyloid beta mutant dataset. Scores are taken from three variants of PALM: base, CANYA-trained, and CANYA-trained with one-hot embeddings. Top: sequence-level scores for all 753 amyloid beta mutants. Bottom: residue scores taken from the mutated position of each sequence.