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Prediction of amyloidogenicity based on the n-gram analysis

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Abstract

Motivation: Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text Text.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

2 Approach

3 Methods

3.1 Data set

The data used in the study was extracted from AmyLoad data base (Wozniak and Kotulska, 2015). Aside from eight sequences shorter than five residues that were removed from the final data set, we obtained 418 amyloidogenic sequences and 1039 non-amyloidogenic sequences (1457 peptides in total).

Sequences shorter than 6 amino acids and longer than 25 amino acids were removed from data set. The former were too short to be processed in the devised analysis framework and the latter were too diversified and rare, preventing the proper analysis.

The final data set contains 397 amyloidogenic and 1033 non-amyloidogenic sequences (1430 peptides in total).

3.2 Encodings of amino acids

The amyloidogenicity of given peptide may not depend on the exact sequence of amino acids, but on its more general properties. To verify this hypothesis, we created 524 284 reduced amino acid alphabets (encodings) with different lengths (from three to six letters) using Ward's clusterization (Jr, 1963) on the selected physicochemical properties from AAIndex database (Kawashima *et al.*, 2008). We handpicked several measures belonging to more general categories important in process of amyloidogenicity as size, hydrophobicity, solvent surface area, frequency in β-sheets and contactivity. As the rule of thumb, we limited ourselves to properties introduced after 1980 when, thanks to the technological advancements, the measurements were more accurate.

The majority of encodings had at least one duplicate. In such case, only the single representative was included in the cross-validation. After filtering duplicates, we obtained 18 535 unique encodings.

Since highly correlated or, contrarily, uncorrelated measures create very similar encodings, we further reduced the number of properties to 17 by selecting measures with the absolute value of Pearson's correlation coefficient for normalized values larger than 0.95.

3.3 Training of learners

During the training phase, we extracted overlapping hexamers from each sequence. Each hexamer was tagged with the same etiquette (amyloid/nonamyloid) as the original peptide. For example, the sequence of length 6 residues yields only one hexamer and the sequence of 8 residues yields 3 hexamers (Fig. 1 A and B). Assuming that in longer amyloids only

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Category	Property
Contactivity	Average flexibility indices (Bhaskaran and Ponnuswamy, 1988)
Contactivity	14 A contact number (Nishikawa and Ooi, 1986)
Contactivity	Accessible surface area (Radzicka and Wolfenden, 1988)
Contactivity	Buriability (Zhou and Zhou, 2004)
Contactivity	Values of Wc in proteins from class Beta, cutoff 12 A, separation 5 (Wozniak and Kotulska, 2014)
Contactivity	Values of Wc in proteins from class Beta, cutoff 12 A, separation 15 (Wozniak and Kotulska, 2014)
β-frequency	Average relative probability of inner beta-sheet (Kanehisa and Tsong, 1980)
β-frequency	Relative frequency in beta-sheet (Prabhakaran, 1990)
β-frequency	Thermodynamic beta sheet propensity (Kim and Berg, 1993)
Hydrophobicity	Hydrophobicity index (Argos *et al.*, 1982)
Hydrophobicity	Optimal matching hydrophobicity (Sweet and Eisenberg, 1983)
Hydrophobicity	Hydrophobicity-related index (Kidera *et al.*, 1985)
Hydrophobicity	Scaled side chain hydrophobicity values (Black and Mould, 1991)
Polarity	Polarizability parameter (Charton and Charton, 1982)
Polarity	Mean polarity (Radzicka and Wolfenden, 1988)
Size	Average volumes of residues (Pontius *et al.*, 1996)
Stability	Side-chain contribution to protein stability (kJ/mol) (Takano and Yutani, 2001)

Table 1. Physicochemical properties used in the creation of reduced amino acid alphabets.
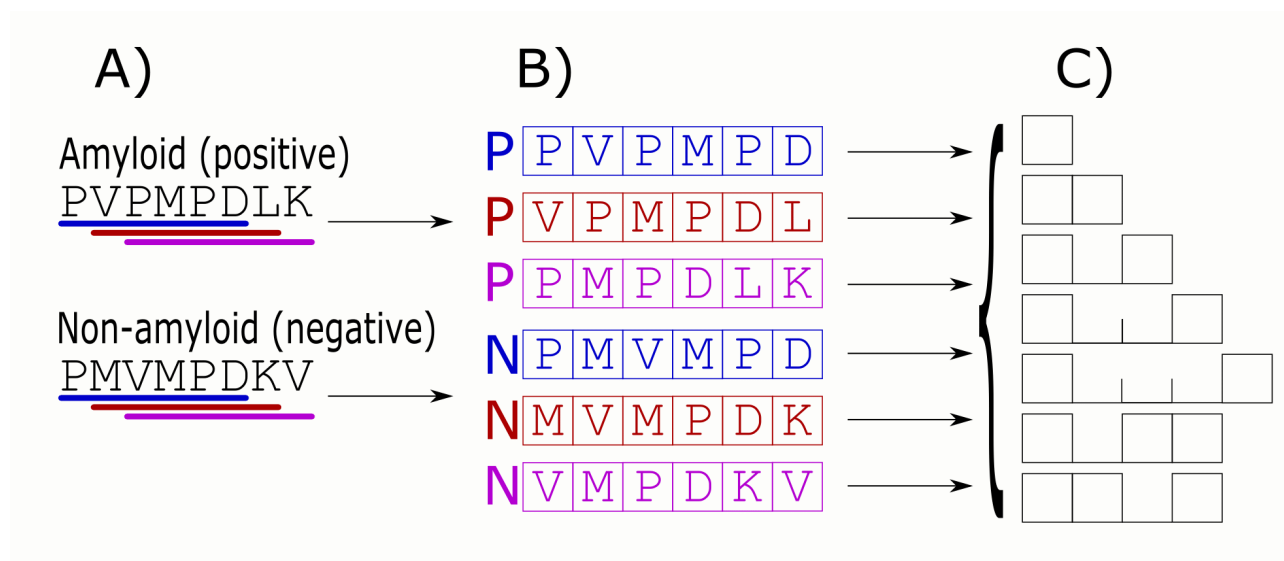
Fig. 1. The scheme of n-gram extraction. A) Input data - peptides with a known amyloidogenicity status. B) Each peptide sequence was divided into overlapping hexamers. The amyloidogenicity status of a source sequence was used as the amyloidogenicity status of a derived hexamer. C) From each hexamer we extracted continuous 1-, 2- and 3-grams. We selected also gapped 2-grams with the length of gap equal from 1 to 3 residues and gapped 3-grams with a single gap between the first and the second or the second and the third element of the n-gram.

a short part of the sequence is responsible for amyloidogenicity, our method might results in many false positives in the training data set. To evade this problem, we restricted the maximum length of peptides in training data set to fifteen amino acids to easy the extraction of probable hot-spots.

From each hexamer we extracted n-grams of the following length: 1, 2 and 3. In the case of 2- and 3-grams, we separately counted both gapped and continuous n-grams. For 2-grams we counted n-grams with gaps of lengths from 1 to 3 and for 3-grams with a single gap between the first and the second or the second and the third element (see Fig. 1).

The study the length of amyloidogenicity signal, we trained three classifiers for each encoding on the sequences of different length. We considered separately six-residue sequences, shorter of equal to ten residues and shorter or equal to fifteen residues.

All n-grams extracted from the hexamers in the training data set were filtered using described below Quick Permutation Test with the information gain (mutual information) as the criterion of the importance of a specific n-gram. In the next step, we used n-grams with the p-value smaller than 0.05 to build a random forest (Breiman, 2001) classifier using ranger **R** package (Wright and Ziegler, 2015).

Furthermore, we repeated the procedure described above on two typical reduced alphabets of amino acids derived from the literature to check if the process of amyloidogenicity does require nonstandard groupings of amino acids. We also added the full amino acid alphabet to assess the advantages of the amino acid encoding.

3.4 Quick Permutation Test (QuiPT)

3.5 Cross-validation and selection of the best-performing encoding

The ability to correctly predict amyloidogenicity was assessed during the five-fold cross-validation. Since the data set was very heterogeneous, we repeated the cross-validation fifteen times for each classifier to obtain more precise estimates of performance measures for each classifier.

To evaluate if our classifiers are able to use decision rules extracted from sequences of given length to correctly classify longer or shorter sequences, we calculate performance measures separately for four ranges of lengths of sequences: 6, 7-10, 11-15 and 16-25. The number of sequences from the given length range was roughly comparable between folds of cross-validation.

To choose the most adequate reduced amino acid alphabet, we ranked the values of AUC (with rank 1 for the best AUC, rank 2 for the second best AUC and so on) for each range of sequence length in the testing data set. The encoding with the lowest sum of ranks from all sequence length categories was selected as the best one. For this encoding, we choose the range of peptides length in the training set providing the best AUC in cross-validation.

3.6 Encoding distance

The encoding distance is a measure defining the similarity between two encodings. It has zero value for identical encodings and grows with the diffences between encodings. It was introduced to verify if the reduced alphabets very similar to the best-performing encoding will also have good prediction performance.

We define the encoding distance as the minimum number of amino acids that have to be moved between subgroups of encoding to make a identical to b (the order of subgroups in the encoding and amino acids in a group is unimportant). This measure is further scaled by a factor reflecting how much moving amino acids between groups altered mean group properties.

To compute the scale factor s for the encoding distance between encoding a with n subgroups and encoding b with m subgroups we firstly calculate p_i and p_j, mean values of physicochemical properties of all amino acids separately for each subgroup. The factor between a and b is equal to:

$$s_{ab} = \sum_{i=1}^{n} \min \left(\sum_{j=1}^{m} \left(\sqrt{\sum^{l} p_i^2} - \sqrt{\sum^{l} p_j^2} \right) \right)$$

where l is equal to the number of physicochemical properties of concern.

3.7 Benchmark of AmyloGram

The best-performing reduced amino acid alphabet chosen during the cross-validation was later used to train AmyloGram, n-gram based predictor of amyloidogenicity.

We used *pep424* data set (Walsh *et al.*, 2014) to compare the performance of AmyloGram and other predictors of amyloidogenicity. Since the model of AmyloGram does not covers peptides shorter than five amino acids, we removed them from the total benchmark data set. It should not affect the comparison as only five sequences were eliminated (around 1% of the original data set). Additionally, we also benchmarked three predictors learned on the n-grams extracted from sequences of different length ranges without any amino acid encoding.

All benchmarked classifiers were trained on sequences used during the cross-validation. Since some peptides were common for both *pep424* and AmyLoad, we removed them from the training data set. After purification, the learning data set had 269 positive sequences and 746 negative sequences longer than five residues and shorter than fifteen residues. Aside from the preparation of the training data, we exactly repeated the procedure of n-gram extraction as described above (see Fig. 1).

4 Results and discussion

4.1 Selection of the best-performing encoding

The selected encoding performed better than other reduced alphabets considering all sequence length ranges in training and testing data set. It has the AUC always in the fourth quartile (Fig. 2). For the best-performing encoding the most problematic was correct prediction of the amyloidogenicity in the longest peptides 16-25 not when learned on very short sequences, but longer ones (6-10 and 6-15). Such behavior was typical for most of the analyzed encodings.

The highest values of AUC the chosen encoding reaches in the relatively the simplest cases of predicting amyloidogenicity of the shortest sequences (6 residues). Of course, the decision rules were the easiest to extract when also the learning set has only sequences of the same lengths, but results were quite comparable even if the training set contained longer sequences. Also in this situation, majority of reduced amino acid alphabets behaved like the selected encoding and also have higher AUC than in other scenarios.

Considering only the AUC value, the most problematic testing data were sequences longer than 15 amino acids. Surprisingly, the classifiers trained on the shortest sequences available (six residues) were performing the best. That might indicate that our n-gram approach extracts the important features the better, the shorter are sequences in training data set. For example, in the amyloidogenic peptide of length 15, only a very specific region of residues might be responsible for the creation of harmful aggregates. In this case, when in our framework overlapping n-grams are extracted, only part of them may carry the true signal of amyloidogenicity, but all are marked as amyloids. Despite this problem, the overall prediction of classifiers learned on the long sequences was still adequate, with the median values of AUC higher than 0.7 for every testing set.

In addition to the high AUC, the best encoding has also very good sensitivity and specificity regardless of the length of sequences in training and testing set (Fig. 3). The classifiers trained on the peptides of length 6 tend to have the best specificity, while predictors learned on the long sequences have the best sensitivity. Albeit the classifiers trained on six-residue-long sequences have generally better AUC, training on the sequences ranging from six to ten residues seem to yield most balanced classifiers with optimal sensitivity and specificity.

We also evaluated classifiers based on the full amino acid alphabet. In most cases they also places in the fourth quartile considering their AUC value (Fig. 2). Nevertheless, they never predict amyloidogenicity better than the selected encoding. It suggests that despite the predictive power of n-grams based on full alphabet, the encoding allows better generalization of the prediction rules and in the consequence better performance.

Similarly to the best-performing encoding, the sensitivity of classifiers based on the full amino acid alphabet seems to decrease with the length of sequences in the training data set (Fig. 3). Furthermore, these classifiers seem to always have one of the worst sensitivities among all analyzed predictors, especially when tested on longer amyloids. It means that using the full amino acid alphabet it easier to point the non-amyloidogenic sequences instead of recognizing amyloids.

The encodings found in the literature performs substantively worse than other analyzed amino acid alphabets in all categories. It indicates that classical divisions of amino acids do not create groups suitable for the recognition of amyloids. This observation is well-supported by the specificity-sensitivity plot (Fig. 3), where classifiers trained with this encodings of amino acids groups with the worst performers.
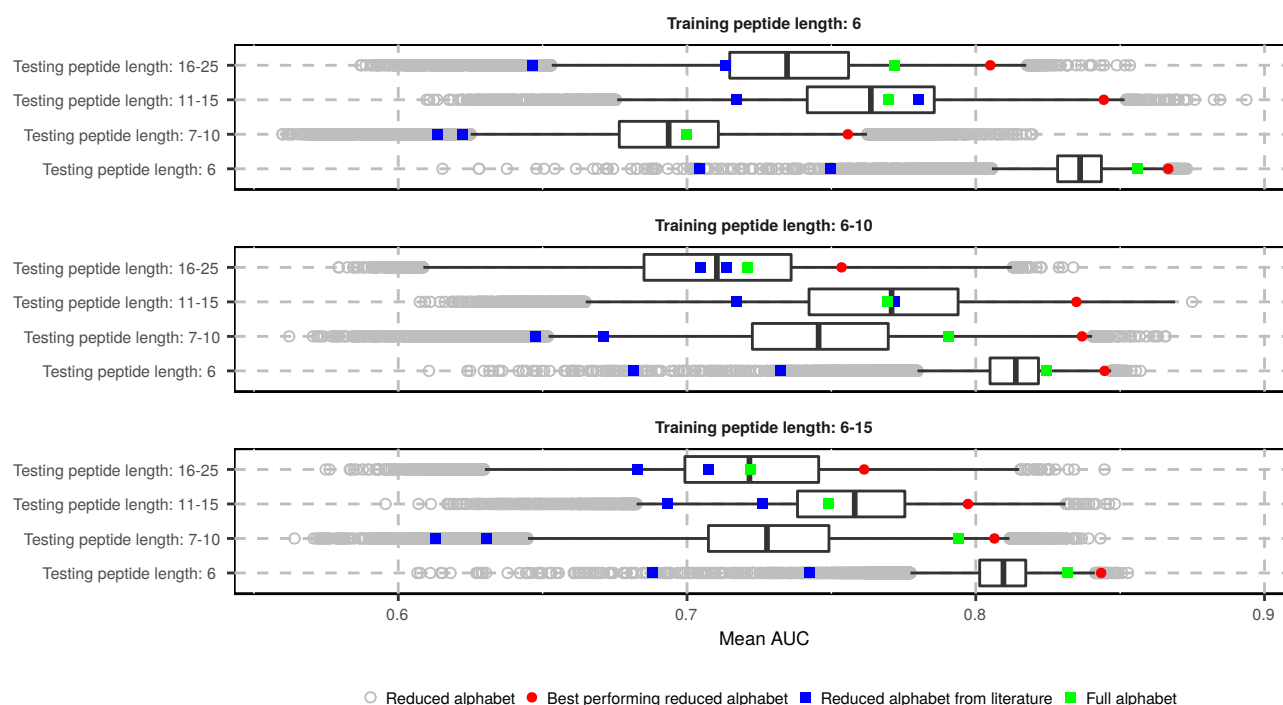
Fig. 2. Distribution of AUC values of different reduced amino acid alphabets for different lengths of sequences in the training and testing data sets.

4.2 The best-performing encoding and important n-grams

Table 2. The best-performing encoding.

Subgroup ID	Amino acids
1	G
2	K, P, R
3	I, L, V
4	F, W, Y
5	A, C, H, M
6	D, E, N, Q, S, T

The reduced amino acid alphabet chosen during the analysis has six subgroups (Tab. 2). Two subgroups, 3 and 4 contain mostly hydrophobic amino acids, while 1 and 4 are mostly hydrophilic. In addition to this, subgroup 1 and 4 have amino acid with the highest average flexibility. The least flexible amino acids are in subgroup 5. The most polarizable amino acids belong to subgroup 4, while the lowest polarizability is typical for glycine, the only amino acid in subgroup 1. The glycine has also the highest thermodynamic beta sheet propensity, which has the lowest values for subgroups 3 and 4.

Identical reduced amino acid alphabets were created through ten other combinations of physicochemical properties. Only four features appeared in all eleven combinations: hydrophobicity index (Argos *et al.*, 1982), average flexibility indices (Bhaskaran and Ponnuswamy, 1988), polarizability parameter (Charton and Charton, 1982) and thermodynamic beta sheet propensity (Kim and Berg, 1993).

We calculated encoding distances between the best-performing reduced amino acid alphabet and other encodings (Fig. 4). To compute the scale factor, we used described above features common for all
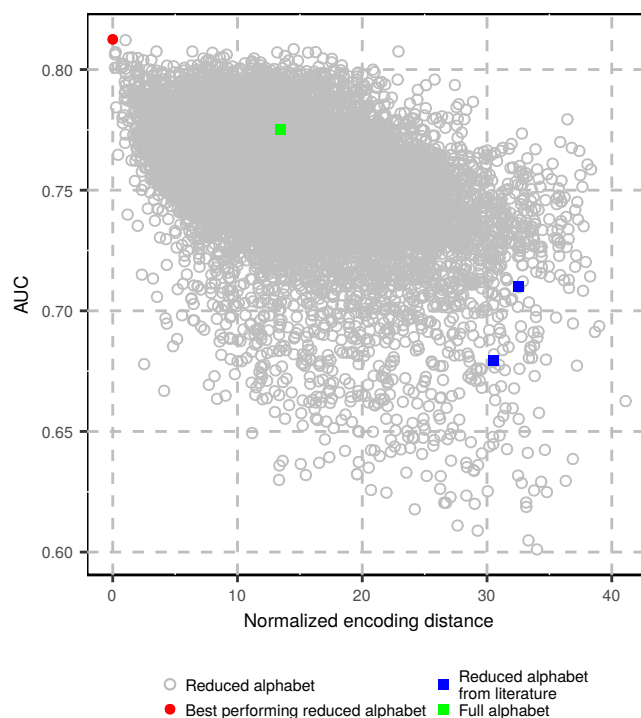
Fig. 4. The encoding distance and AUC of reduced alphabets studied in the cross-validation. Classifiers having the smallest encoding distance to the best classifier have the highest AUC.
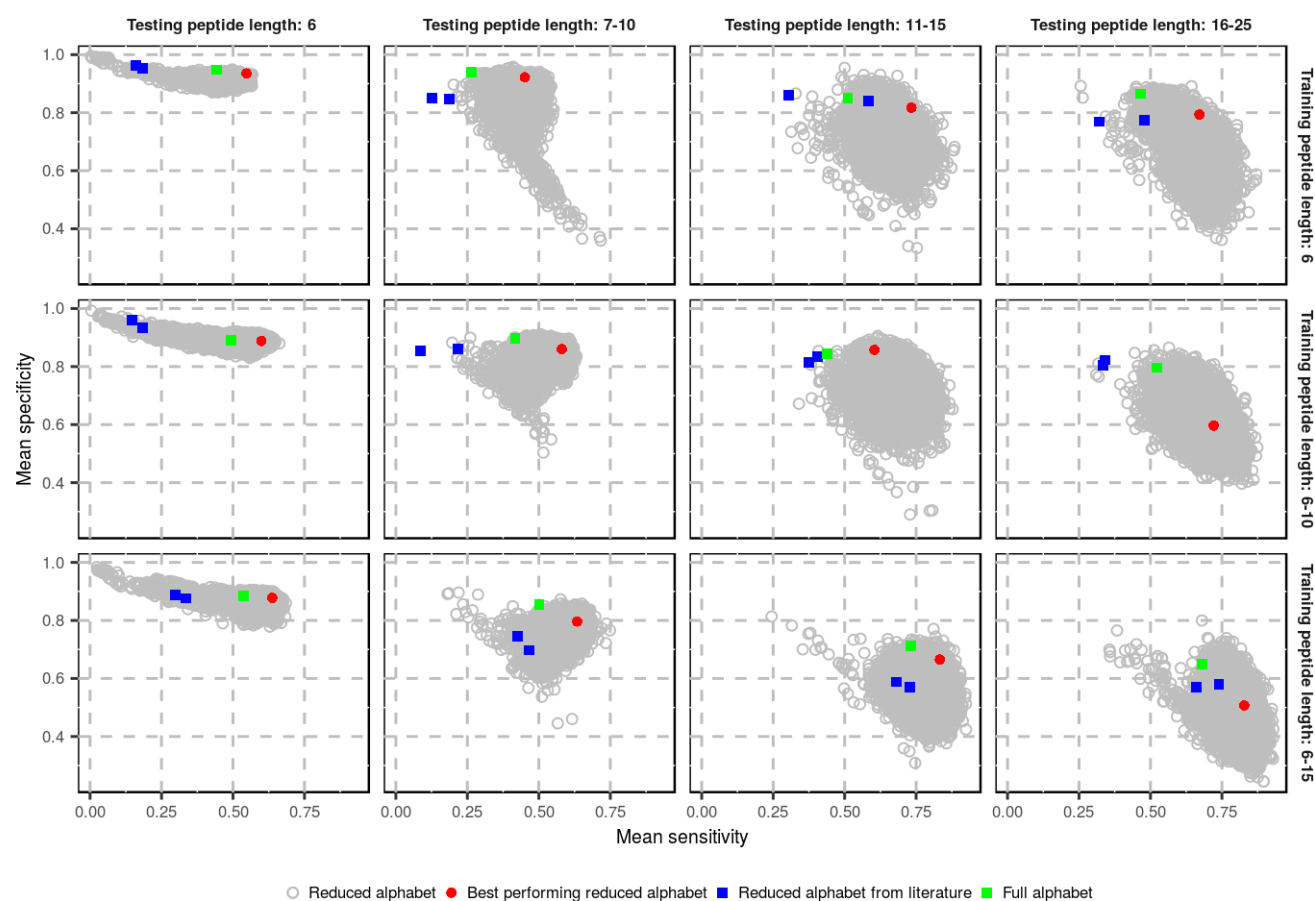
Fig. 3. Sensitivity and specificity of classifiers in cross-validation for different lengths of sequences in the training and testing data sets. The classifier based on the best-performing encoding always have good specificity and sensitivity.

encodings identical to the best-performing one. The value of AUC is significantly lower for more distant encodings (-0.4370 Pearson's correlation coefficient, p-value smaller than 2.2×10^{-16}). Such relationship between AUC and encoding distance confirms that only reduced alphabets sufficiently similar to the best-performing encoding are able to remove unnecessary diversity while preserving information important for recognition of amyloids.

We discovered that 65 n-grams have p-values smaller than 0.05 in the every fold of repetitions of cross-validation regardless of the training set.

4.3 Benchmark of AmyloGram

The benchmark covered Amylogram as well as two best-performing peer-reviewed predictors of amyloidogenicity (PASTA2 (Walsh *et al.*, 2014) and FoldAmyloid (Garbuzynskiy *et al.*, 2010)). We analyzed Area Under the Curve (AUC), Matthew's Correlation Coefficient (MCC), Sensitivity and Specificity (see Tab. 3).

Interestingly, the n-gram extraction method was efficient enough to produce classifiers able to outperformed published methods. Two of three n-gram based classifiers trained on the full alphabet have AUC higher than PASTA2 and all three were more successful than FoldAmyloid. They also maintained they high Specificity as seen previously during cross-validation.

Although the proposed n-gram extraction creates efficient classifiers, the encoding of amino acids further increases the prediction of amyloidogenicity. AmyloGram has the highest AUC, MCC and Sensitivity among all tested classifiers. Is has lower specificity than two classifiers trained on the full alphabet, but still outperforms other published method in this category. It is important to highlight that AmyloGram is the most balanced of analyzed classifiers, having the best Specificity/Sensitivity balance, as indicated by the value of MCC.

Table 3. Results of benchmark on pep424 data set for AmyloGram, PASTA2, FoldAmyloid and random forest predictor learned on n-grams extracted without any amino acid encoding from the sequences of the length specified in the brackets.

Classifier	AUC	MCC	Sensitivity	Specificity
AmyloGram	**0.8972**	**0.6307**	**0.8658**	0.7889
PASTA2	0.8550	0.5227	0.7987	0.7444
FoldAmyloid	0.7351	0.4526	0.7517	0.7185
full alphabet (6)	0.8411	0.5427	0.4966	**0.9593**
full alphabet (6-10)	0.8581	0.5698	0.7517	0.8259
full alphabet (6-15)	0.8610	0.5490	0.8188	0.7519
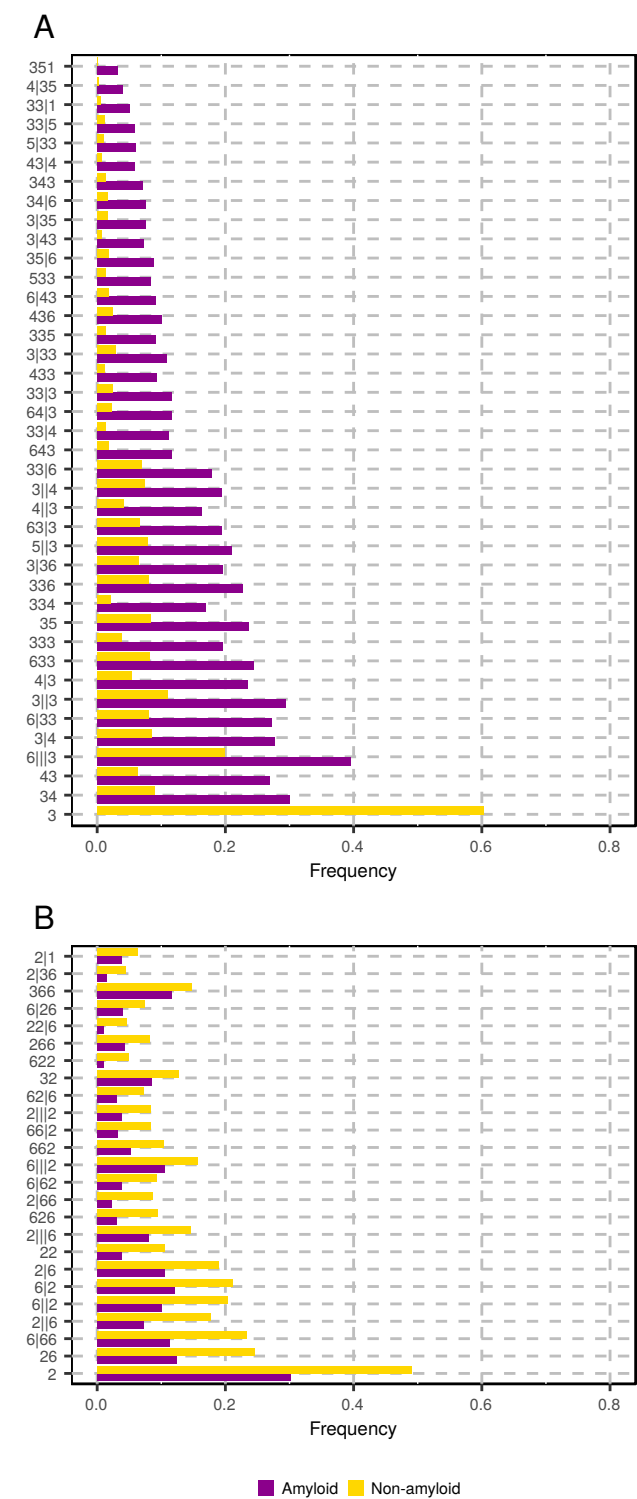
A

B

■ Amyloid   ■ Non-amyloid

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Fig. 5. Blabla