

AmyloGram: a novel predictor of amyloidogenicity

Michał Burdukiewicz¹, Piotr Sobczyk², Stefan Rödiger³, Paweł Mackiewicz¹ and Małgorzata Kotulska⁴

¹University of Wrocław, Department of Genomics,

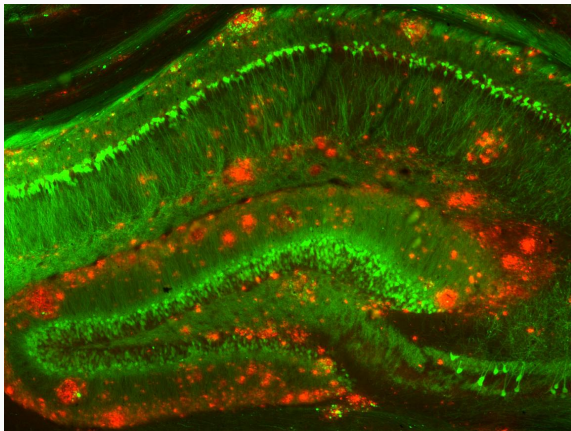
²Wrocław University of Science and Technology, Faculty of Pure and Applied Mathematics,

³Brandenburg University of Technology Cottbus-Senftenberg, Institute of Biotechnology,

⁴Wrocław University of Science and Technology, Department of Biomedical Engineering

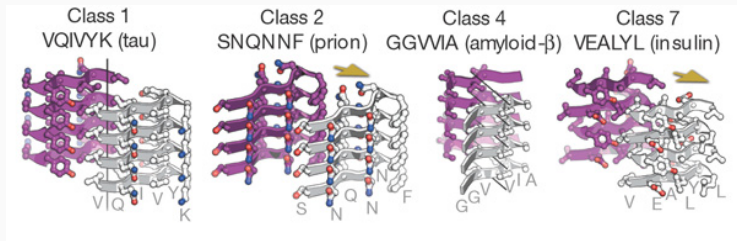
Amyloids

Proteins associated with various neurodegenerative disorders (e.g., Alzheimer's, Parkinson's, Creutzfeldt-Jakob's diseases) creating harmful aggregates.



Amyloid aggregates (red) around neurons (green). Strittmatter Laboratory, Yale University

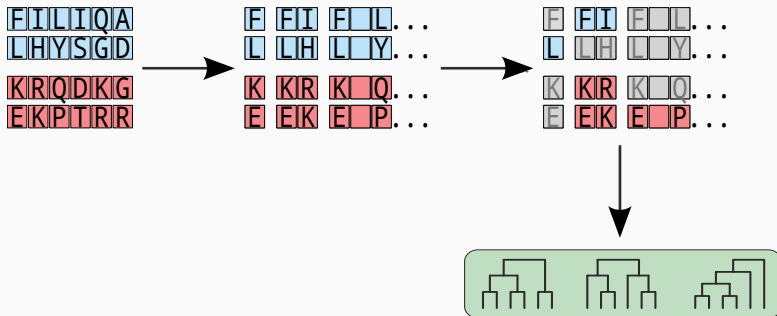
The aggregation of amyloids is initiated by 6- to 15-residue segments called hot spots, diverse subsequences that form unique zipper-like β -structures.



Sawaya et al. (2007)

Amyloidogenic motifs

Which motifs (continuous or gapped subsequences of amino acids) are associated with amyloidogenicity?



Quick Permutation Test

Informative n-grams are usually selected using permutation tests.

During a permutation test we shuffle randomly class labels and compute a defined statistic (e.g. information gain). Values of statistic for permuted data are compared with the value of statistic for original data.

$$\text{p-value} = \frac{N_{T_P > T_R}}{N}$$

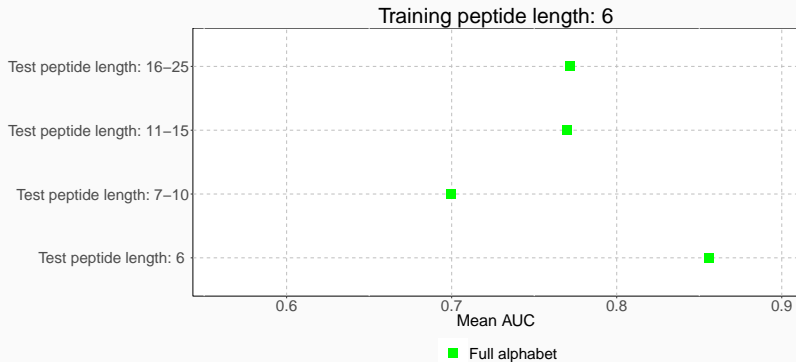
$N_{T_P > T_R}$: number of cases, where T_P (permuted test statistic) has more extreme values than T_R (test statistic for original data).

N : number of permutations.

Quick Permutation Test is a fast alternative to permutation tests for n-gram data. It also allows precise estimation of p-value.

QuiPT is available as part of the **biogram** R package.

Cross-validation



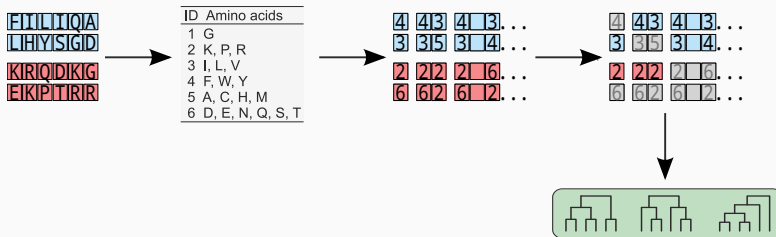
Reduced amino acid alphabets

Does amyloidogenicity depend on the exact sequence of amino acids?

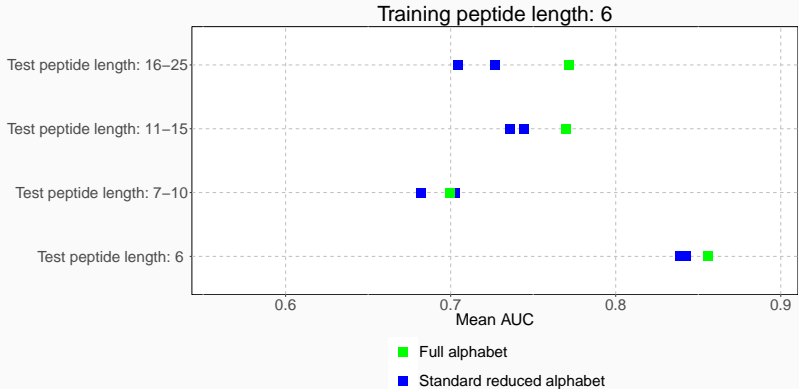
Standard reduced amino acid alphabets

To date, several reduced amino acid alphabets have been proposed, which have been applied to (among others) protein folding and protein structure prediction (Kosiol et al., 2004; Melo and Marti-Renom, 2006).

Standard reduced amino acid alphabets



Cross-validation

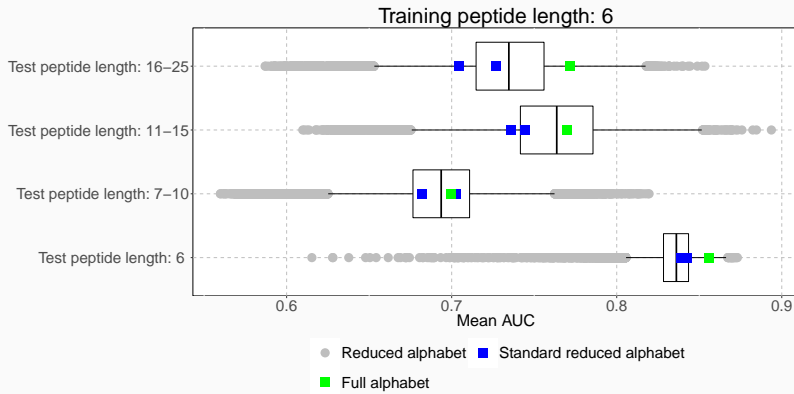


Standard reduced amino acid alphabets do not enhance discrimination between amyloidogenic and non-amyloidogenic proteins.

Novel reduced amino acid alphabets

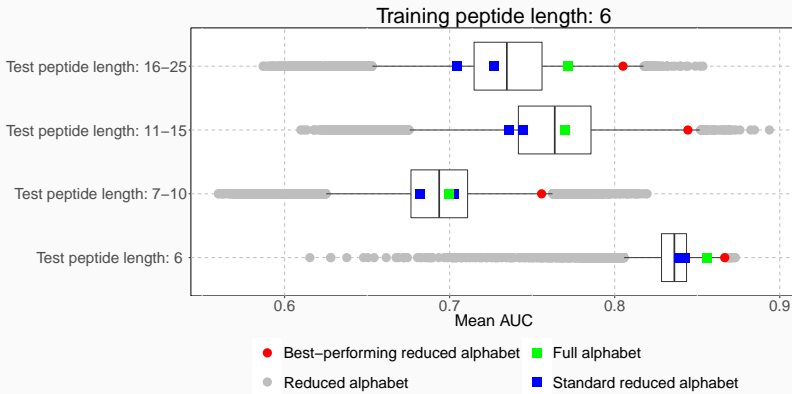
- 17 measures handpicked from AAIndex database:
 - size of residues,
 - hydrophobicity,
 - solvent surface area,
 - frequency in β -sheets,
 - contactivity.
- 524 284 amino acid reduced alphabets with different level of amino acid alphabet reduction (three to six amino acid groups).

Cross-validation



Hinges of boxes correspond to the 0.25 and 0.75 quartiles. The bar inside the box represents the median. The gray circles correspond to the reduced alphabets with the AUC outside the 0.95 confidence interval.

The best-performing reduced alphabet



The reduced amino acid alphabets with the lowest sum of ranks from all sequence length categories (assuming the rank 1 for the best AUC, rank 2 for the second best AUC and so on) was selected as the best one.

The best-performing reduced alphabet

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 best-performing reduced alphabet

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

Group 3 and 4 - hydrophobic amino acids.

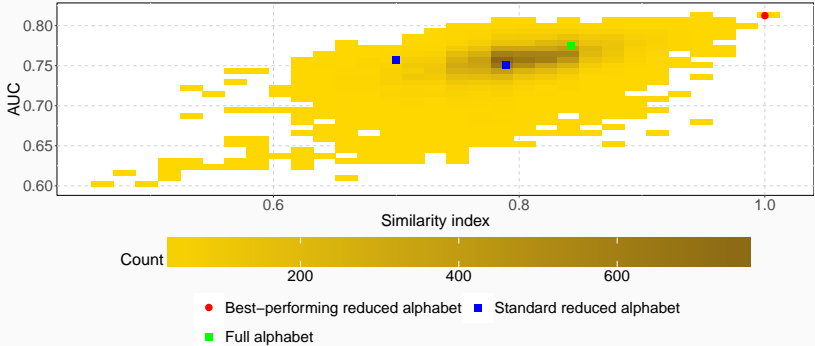
The best-performing reduced alphabet

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

Group 2 - charged breakers of β -structures.

Is the best-performing reduced amino alphabet associated with amyloidogenicity?

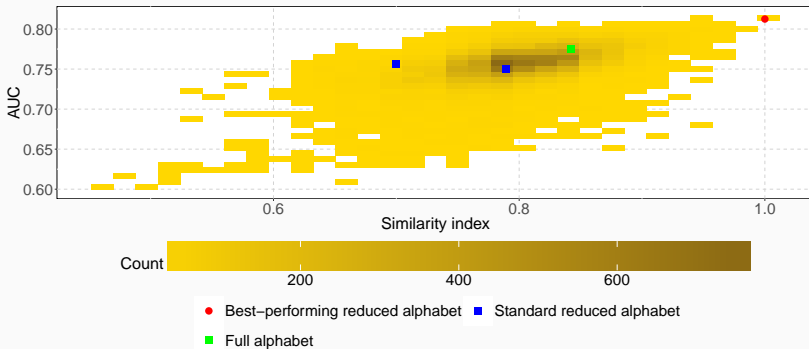
Similarity index



Similarity index (Stephenson and Freeland, 2013) measures the similarity between two reduced alphabets (1 - identical, 0, totally dissimilar).

The color of a square is proportional to the number of reduced alphabets in its area.

Similarity index

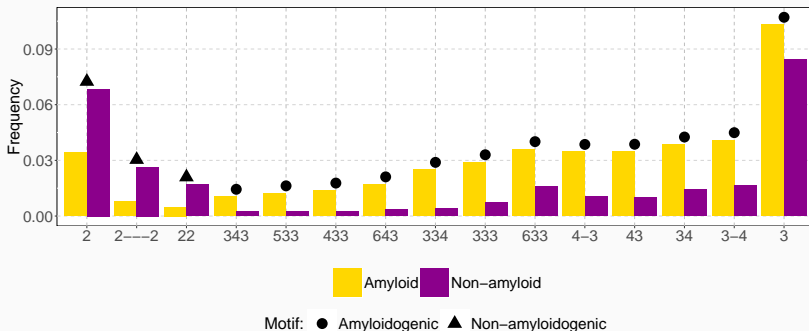


The correlation between mean AUC and similarity index is significant ($p\text{-value} \leq 2.2^{-16}$; $\rho = 0.51$).

Knowledge-discovery

Are informative n-grams found by QuiPT associated with amyloidogenicity?

Informative n-grams



Out of 65 the most informative n-grams, 15 (23%) were also found in the motifs validated experimentally (Paz and Serrano, 2004).

Benchmark and summary

Is performance of the AmyloGram, the classifier based on the best-performing reduced amino acid alphabet, also adequate on the independent dataset?

Benchmark results

Classifier	AUC	MCC
AmyloGram	0.8972	0.6307
PASTA 2.0 (Walsh et al., 2014)	0.8550	0.4291
FoldAmyloid (Garbuzynskiy et al., 2010)	0.7351	0.4526
APPNN (Família et al., 2015)	0.8343	0.5823

The predictor based on the best-performing alphabet, called AmyloGram, was benchmarked against the most popular tools for the detection of amyloid peptides using an external data set *pep424*.

Summary

We identified a group of reduced amino acid alphabets which capture properties of amyloids.

Our algorithm was also capable of extracting n-gram associated with amyloidogenicity, partially confirming experimental results.

Our software is available as a web-server:

`smorfland.uni.wroc.pl/amylogram`.

n-gram analysis workflow is implemented in the R package

biogram: <https://cran.r-project.org/package=biogram>.

Acknowledgements and funding

This research was partially funded by the KNOW Consortium and National Science Center (2015/17/N/NZ2/01845).

- Małgorzata Kotulska.
- Paweł Mackiewicz,
- Stefan Rödiger,
- **biogram** software
(<https://cran.r-project.org/package=biogram>):
 - Piotr Sobczyk,
 - Chris Lauber,
- **AmyLoad** database (comprec-lin.iia.pwr.edu.pl/amyload):
 - Paweł Woźniak,

References

Família, C., Dennison, S. R., Quintas, A., and Phoenix, D. A. (2015). Prediction of Peptide and Protein Propensity for Amyloid Formation. *PLOS ONE*, 10(8):e0134679.

Garbuzynskiy, S. O., Lobanov, M. Y., and Galzitskaya, O. V. (2010). FoldAmyloid: a method of prediction of amyloidogenic regions from protein sequence. *Bioinformatics (Oxford, England)*, 26(3):326–332.

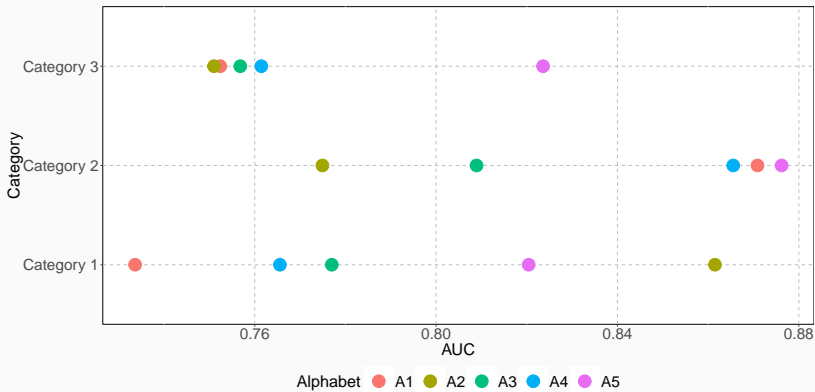
References II

- Kosiol, C., Goldman, N., and Buttimore, N. H. (2004). A new criterion and method for amino acid classification. *Journal of Theoretical Biology*, 228(1):97–106.
- Melo, F. and Marti-Renom, M. A. (2006). Accuracy of sequence alignment and fold assessment using reduced amino acid alphabets. *Proteins*, 63(4):986–995.
- Paz, M. L. d. I. and Serrano, L. (2004). Sequence determinants of amyloid fibril formation. *Proceedings of the National Academy of Sciences*, 101(1):87–92.

References III

- Sawaya, M. R., Sambashivan, S., Nelson, R., Ivanova, M. I., Sievers, S. A., Apostol, M. I., Thompson, M. J., Balbirnie, M., Wiltzius, J. J. W., McFarlane, H. T., Madsen, A. , Riek, C., and Eisenberg, D. (2007). Atomic structures of amyloid cross-spines reveal varied steric zippers. *Nature*, 447(7143):453–457.
- Stephenson, J. D. and Freeland, S. J. (2013). Unearthing the root of amino acid similarity. *Journal of Molecular Evolution*, 77(4):159–169.
- Walsh, I., Seno, F., Tosatto, S. C. E., and Trovato, A. (2014). PASTA 2.0: an improved server for protein aggregation prediction. *Nucleic Acids Research*, page gku399.

Ranking alphabets



Ranking alphabets

