Predicting properties of biological sequences using n-gram analysis

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Outline

n-grams

Simplified alphabets

Prediction of amyloidogenicity

Other applications

In silico research allows scientists to more efficiently design experimental studies.

Examples:

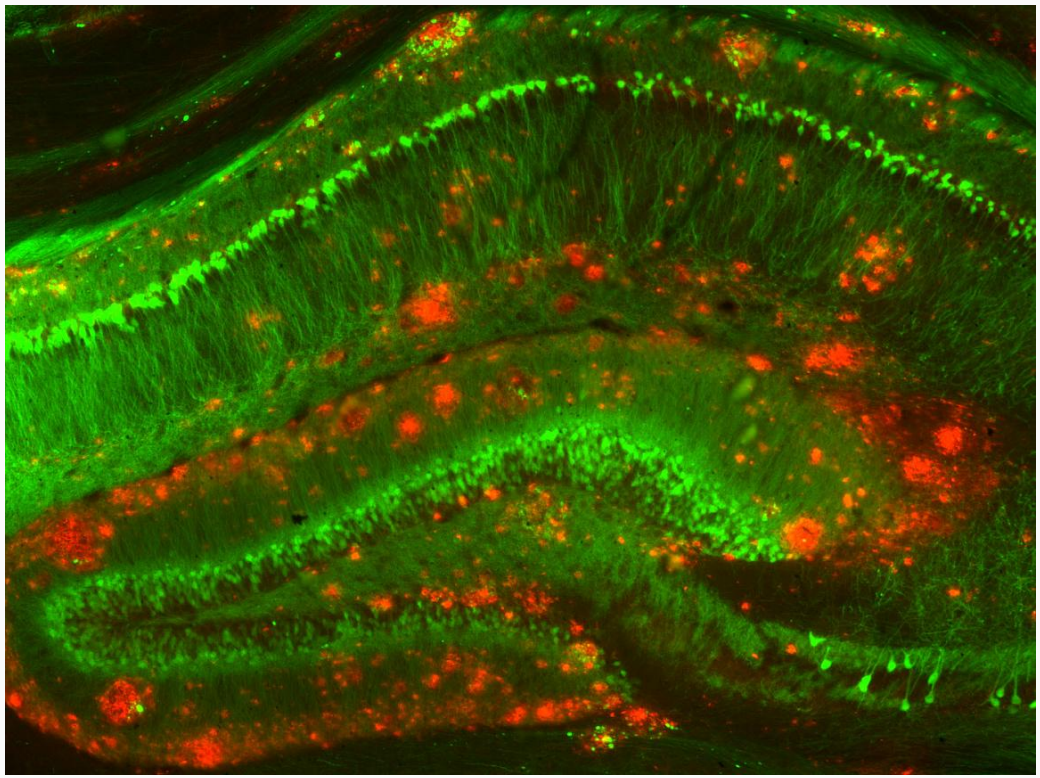
- prediction of protein properties (presence of signal peptides, amyloidogenicity),
- predicting culture conditions of bacteria.

Aim

Create efficient methods for analysis of biological sequences that have human-readable decision rules.

Amyloid proteins

Amyloid are proteins associated with various diseases (e.g., Alzheimer's, Creutzfeldt-Jakob's and Huntington's diseases) which are able to form harmful aggregates.

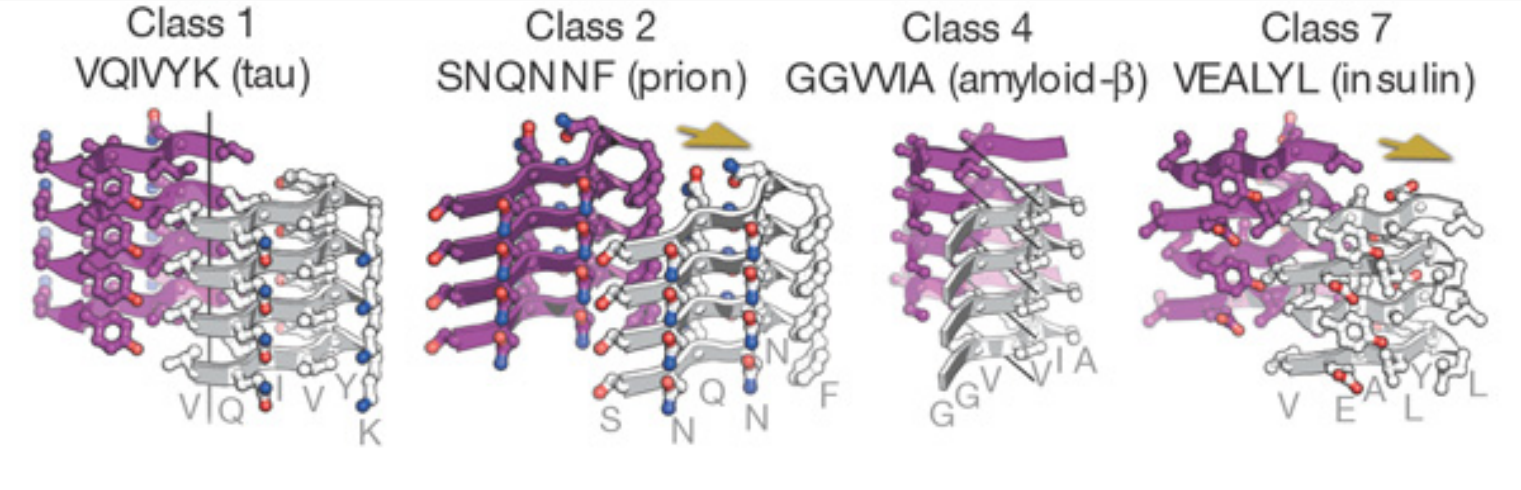


Amyloid aggregates (red) around neurons (green). Strittmatter Laboratory, Yale University.

Amyloid proteins

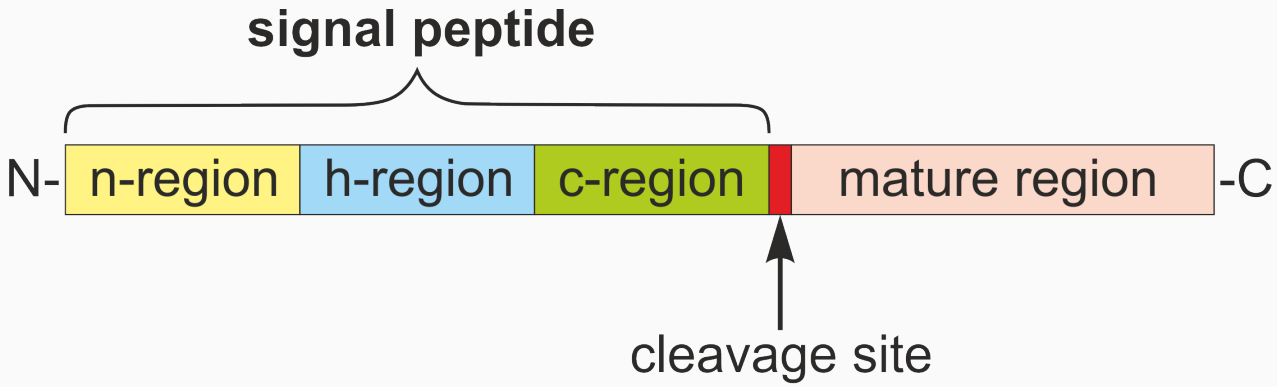
Hot-spots:

- short (6-15 amino acids),
- very high variability of amino acid composition,
- initiate amyloid aggregation,
- create specific "zipper-like" β-structures.



Sawaya et al. (2007)

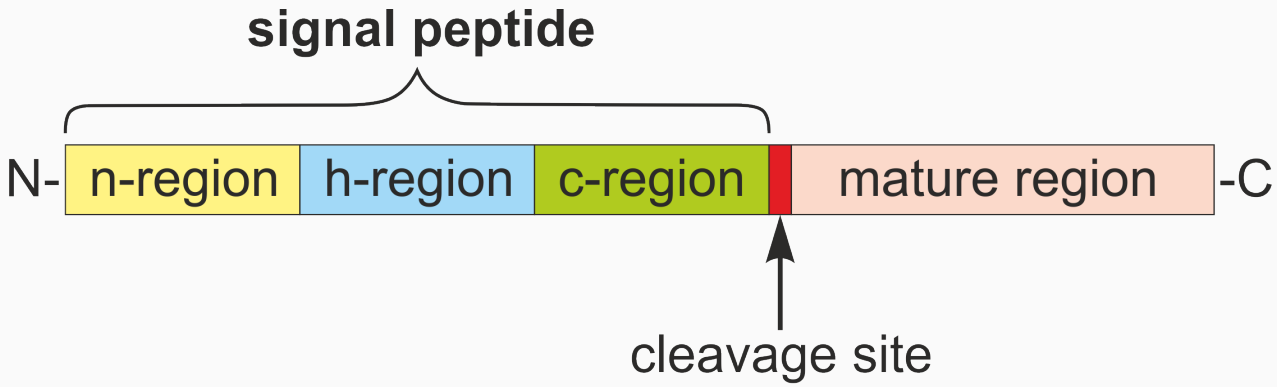
Signal peptides



Signal peptides:

- are short (20-30 residues) N-terminal amino acid sequences forming α-helices,

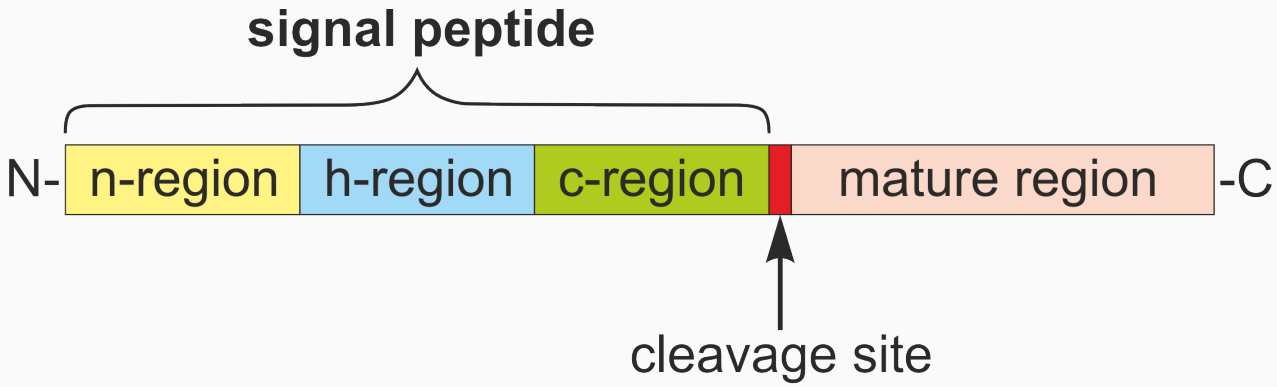
Signal peptides



Signal peptides:

- are short (20-30 residues) N-terminal amino acid sequences forming α-helices,
- direct proteins to the endomembrane system and next to extra- or intracellular localizations,

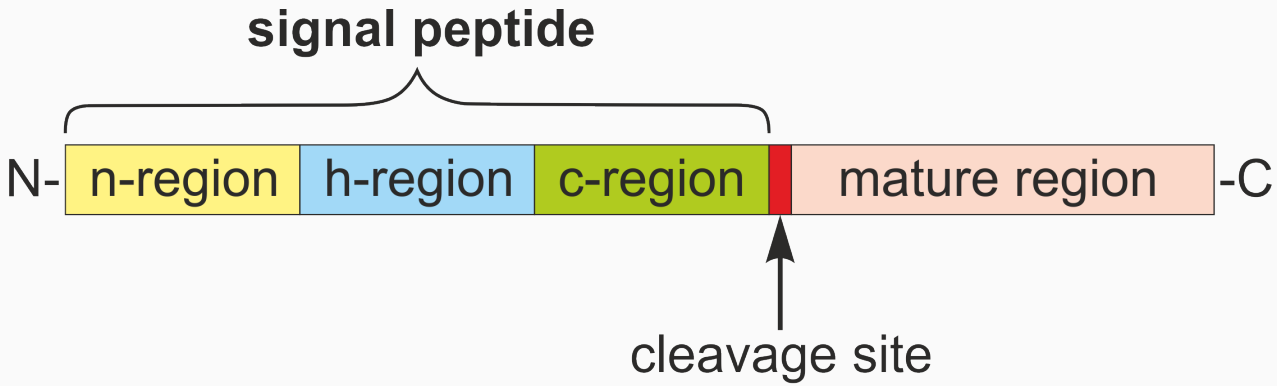
Signal peptides



Signal peptides:

- are short (20-30 residues) N-terminal amino acid sequences forming α-helices,
- direct proteins to the endomembrane system and next to extra- or intracellular localizations,
- are universal enough to direct properly proteins in different secretory systems.

Signal peptides

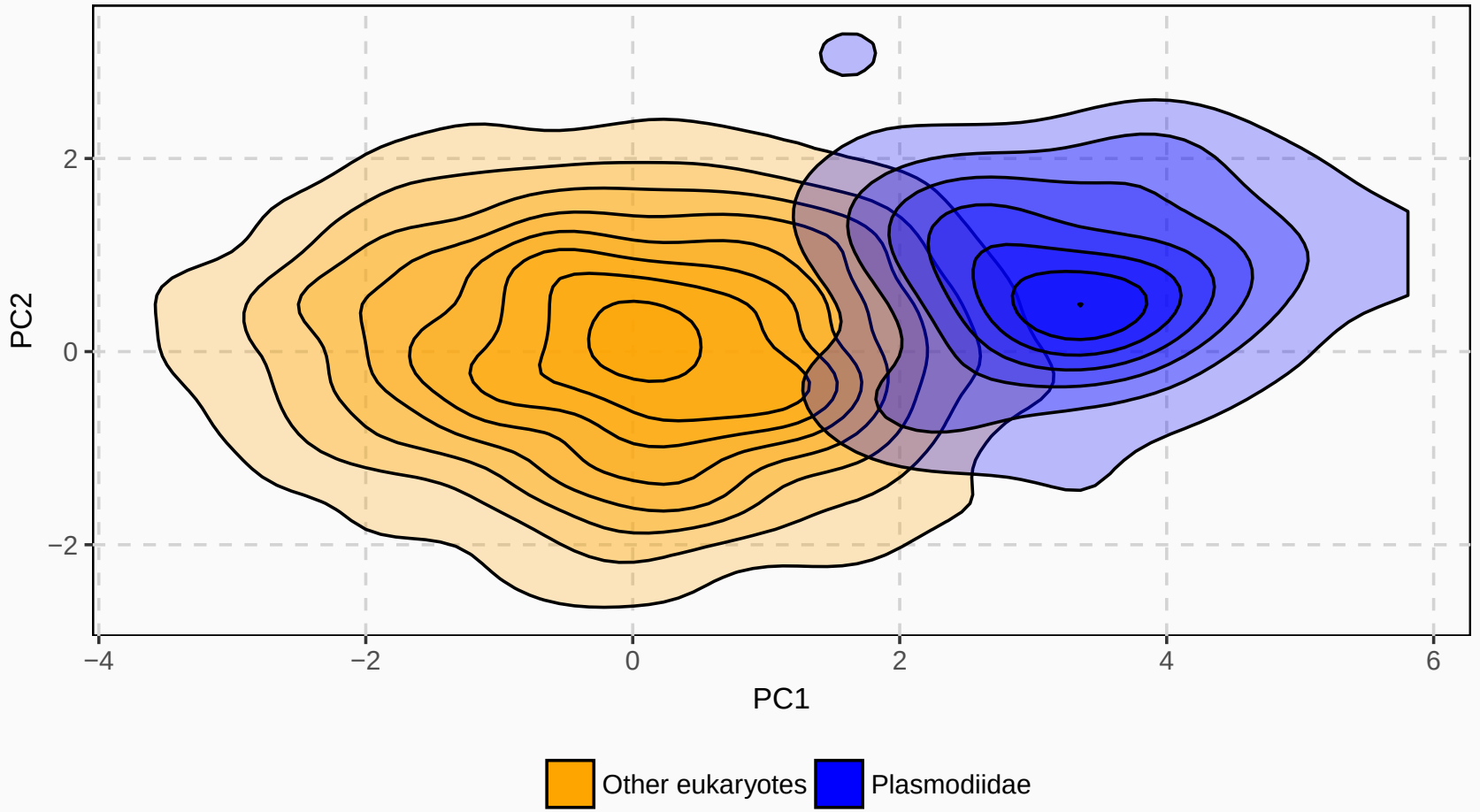


Signal peptides possess three distinct domains with variable length and characteristic amino acid composition (Hegde and Bernstein, 2006):

- n-region: mostly basic residues (Nielsen and Krogh, 1998),
- h-region: strongly hydrophobic residues (Nielsen and Krogh, 1998),
- c-region: a few polar, uncharged residues.

Signal peptides

Amino acid composition of signal peptides differ between Plasmodium sp. and other eukaryotes. Therefore, predictors of signal peptides do not detect malarial signal peptides accurately.



n-grams

Computational analysis of biological sequences requires converting them to features understandable by machines.

The optimal conversion of information:

- loss-less,
- concise.

n-grams (k-tuples, k-mers):

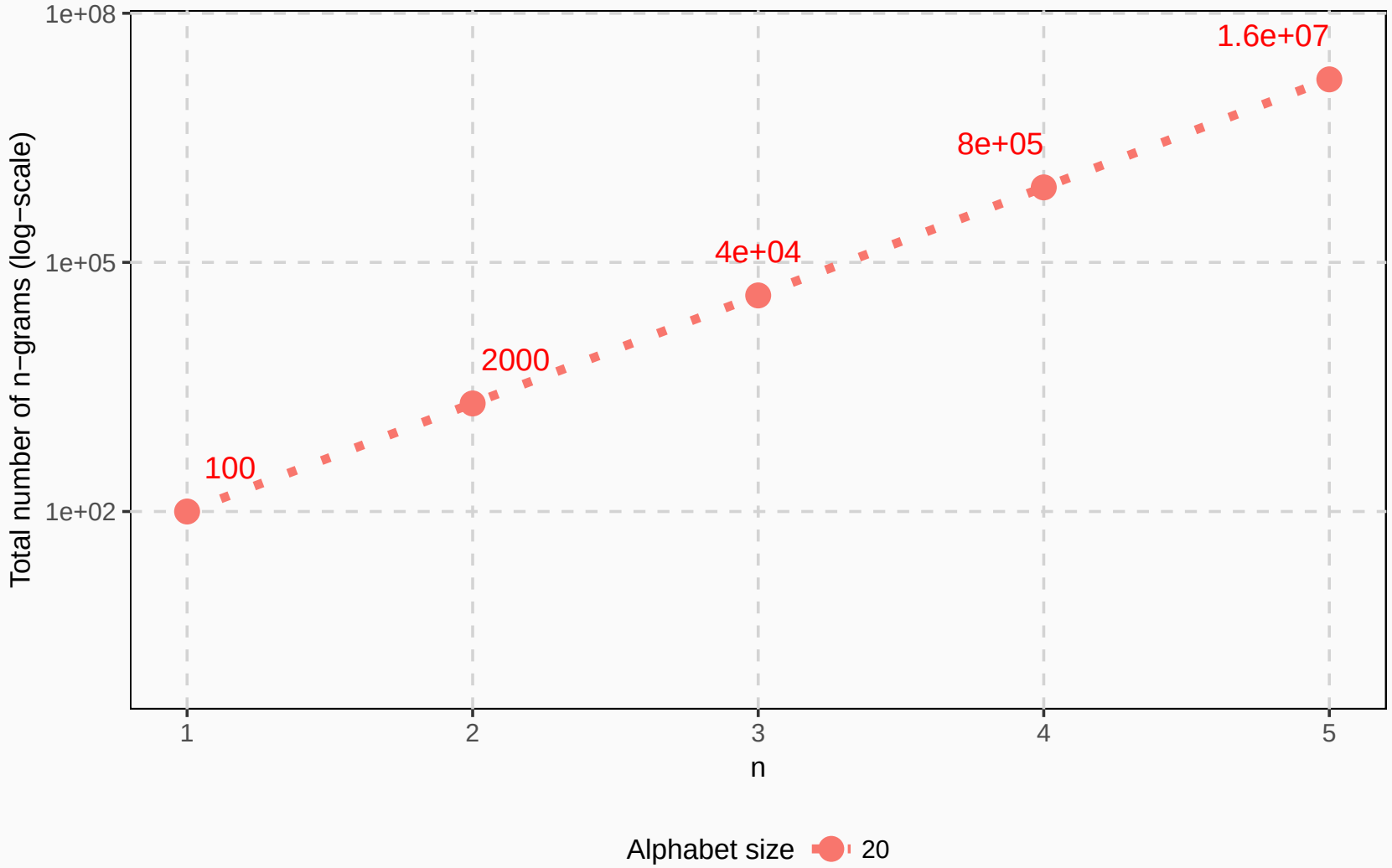
- subsequences (continuous or gapped) of *n* residues,
- considers the context of a specific residue.

	P1	P2	P3	P4	P5
S1	M	R	K	L	Y

2-grams: MR, RK, KL, LY

2-grams (gap 1): M – K, R – L, K – Y

3-grams: MRK, RKL, KLY



Longer n-grams are more informative, but create larger feature spaces, which are hard to process and analyze.

Permutation Tests

Informative n-grams are usually selected using permutation tests.

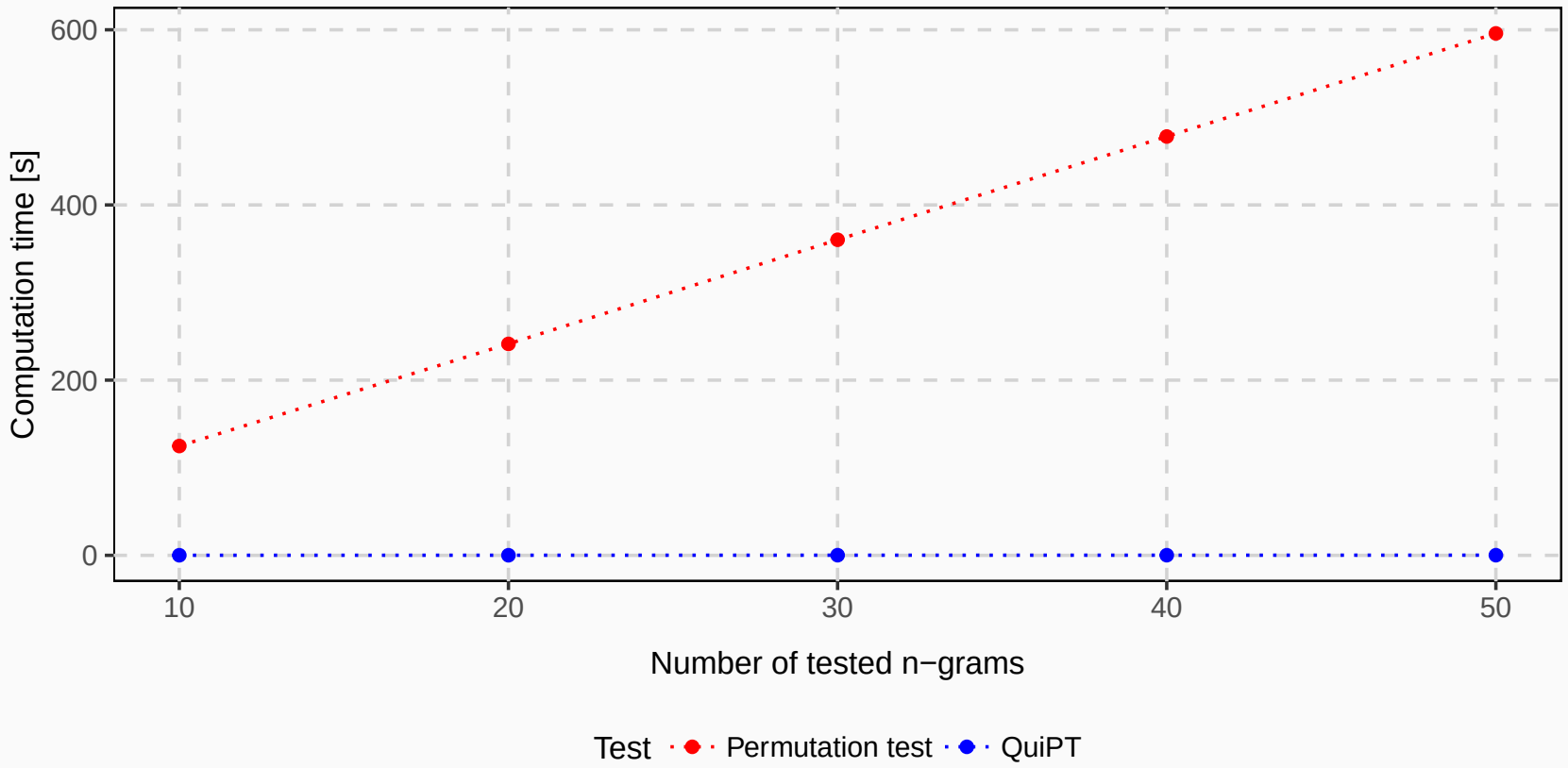
During a permutation test we shuffle randomly class labels and compute a defined statistic (e.g. information gain). Values of statistic for permuted data are compared with the value of statistic for original data.

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

$N_{T_P > T_R}$: 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.

QuiPT



QuiPT (available as part of the **biogram** R package) is faster than classical permutation tests and returns exact p-values.

Simplified alphabets

Simplified alphabets:

- are based on grouping amino acids with similar physicochemical properties,
- ease computational analysis of a sequence (Murphy et al., 2000),
- create more explicite models.

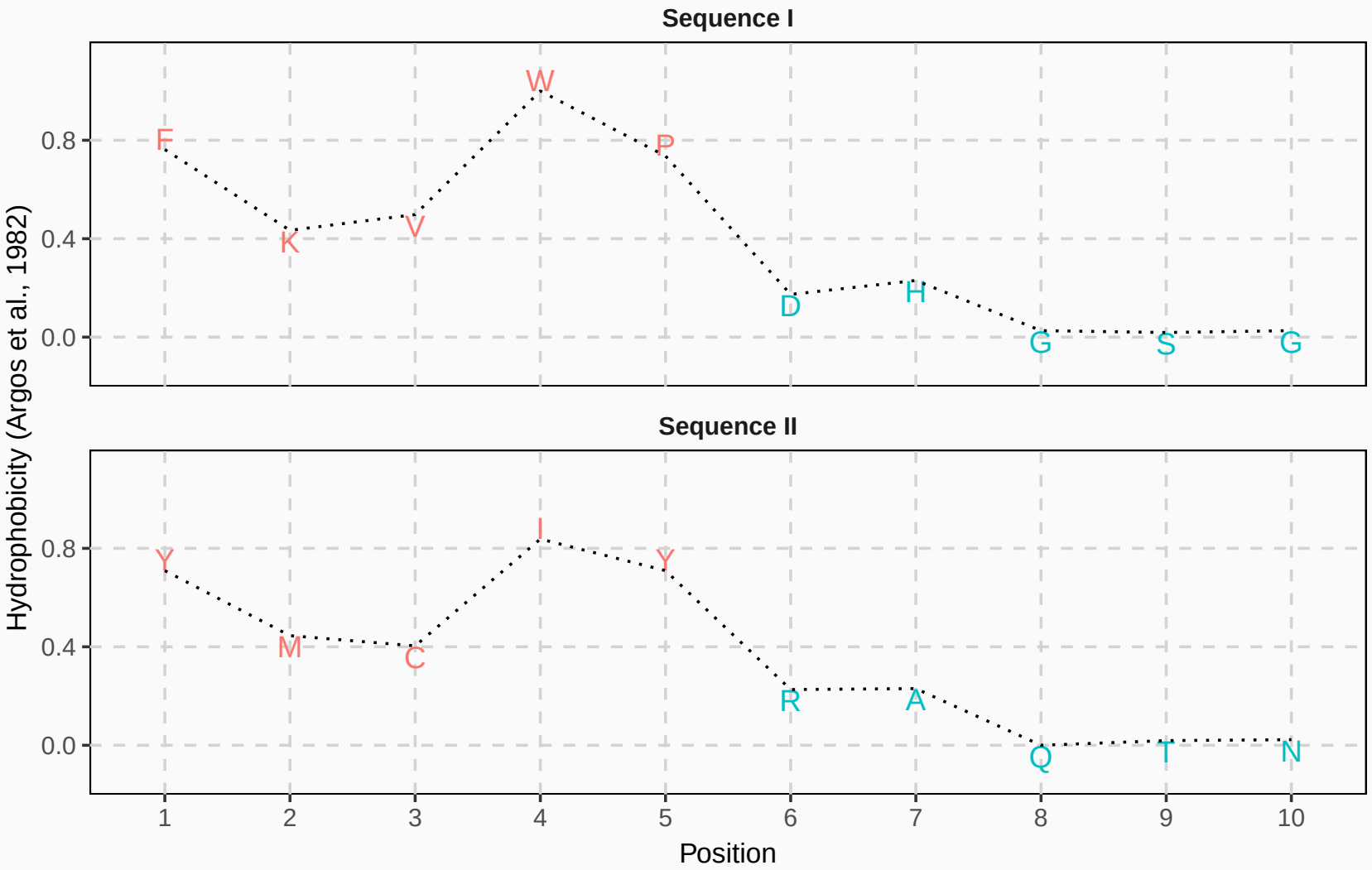
Two sequences that are drastically different considering their amino acids composition can have the same physicochemical properties.

Sequence I:

`FKVWPDHGSG`

Sequence II:

`YMCIYRAQTN`


Sequence I
Sequence II
Hydrophobicity (Argos et al., 1982)
0.0
0.4
0.8
F
K
V
W
P
D
H
G
S
G
Y
M
C
I
Y
R
A
Q
T
N
1
2
3
4
5
6
7
8
9
10
Position

Subgroup	Amino acid
1	C, I, L, K, M, F, P, W, Y, V
2	A, D, E, G, H, N, Q, R, S, T

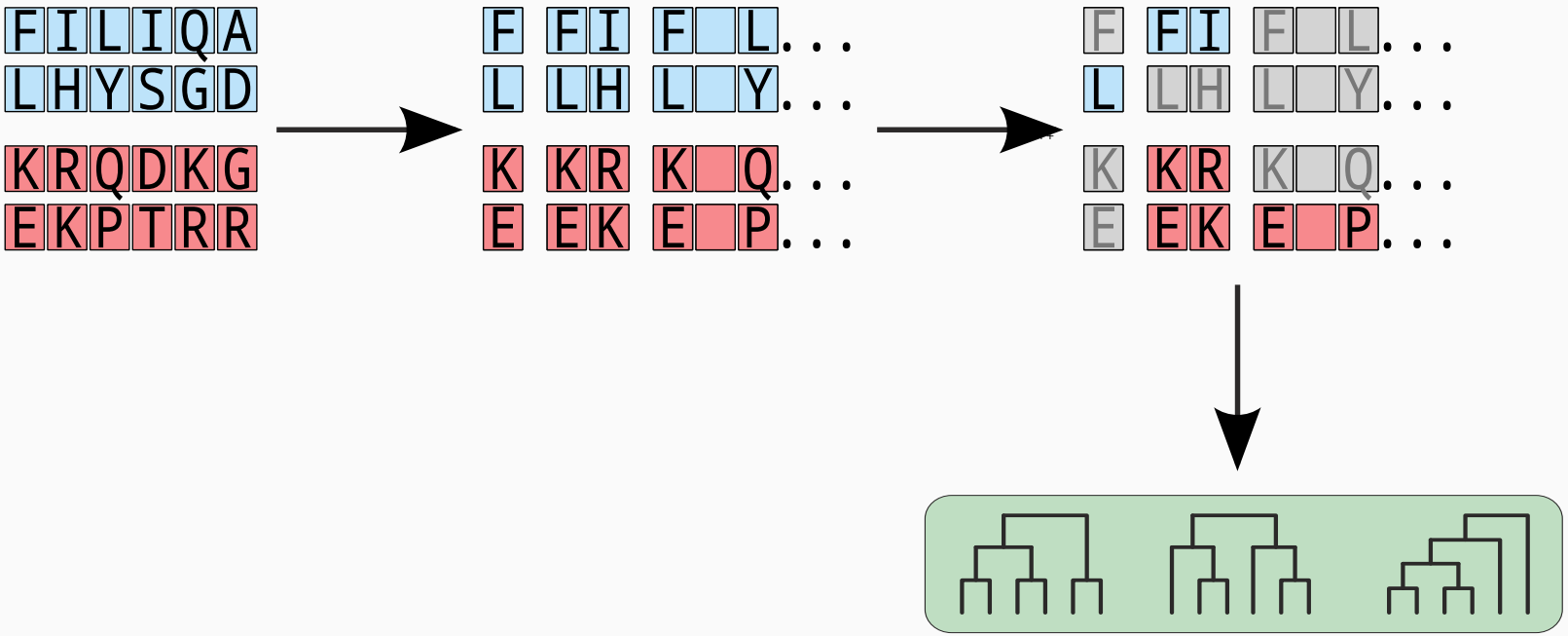
Sequence I: `FKVWPDHGSG` `1111122222`

Sequence II: `YMCIYRAQTN` `1111122222`

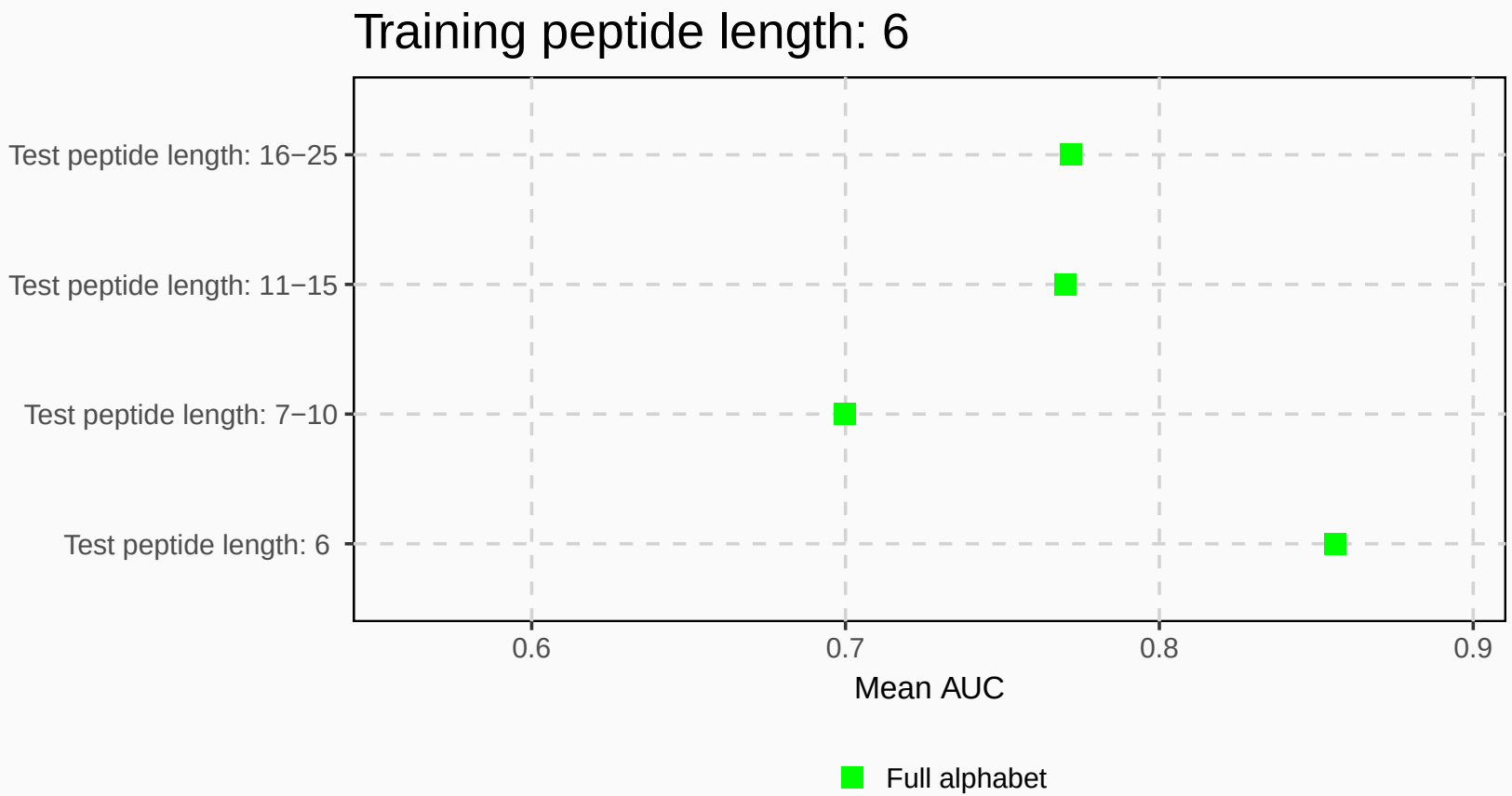
Prediction of amyloidogenicity

AmyloGram

AmyloGram: n-gram based tool for prediction of amyloid proteins (Burdukiewicz et al., 2016).


FILIQA
LHYSGD
KRQDKG
EKPTRR
F FI F L...
L LH L Y...
K KR K Q...
E EK E P...
F FI F L...
L LH L Y...
K KR K Q...
E EK E P...

Cross-validation



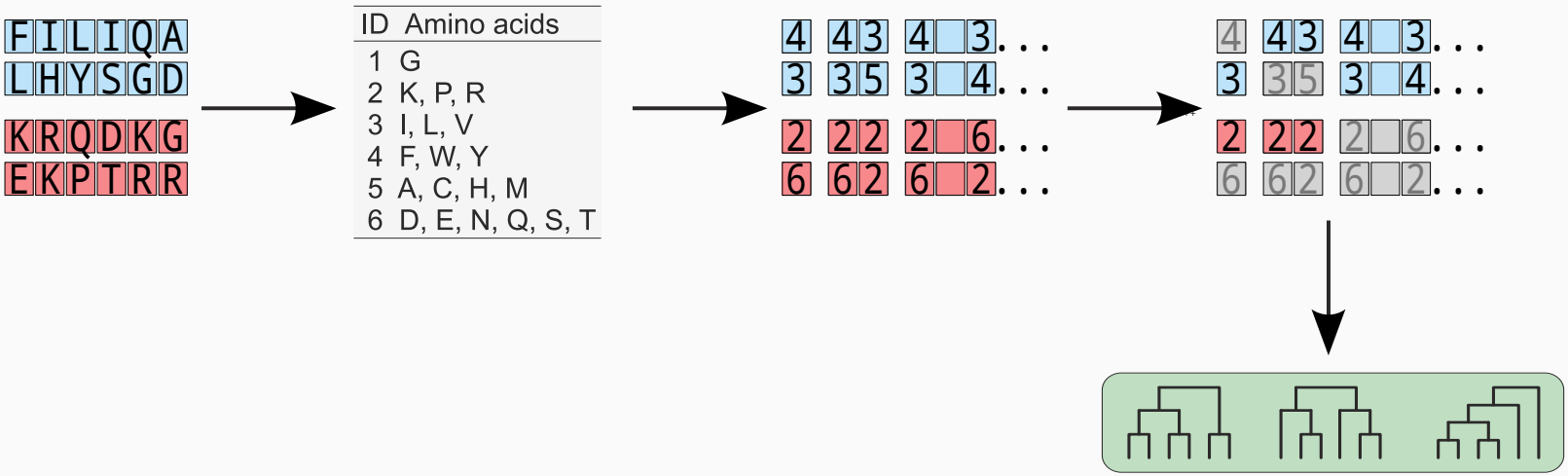
AUC (Area Under the Curve) measures the performance of a classifier (1 - classifier always properly recognizes amyloid proteins, 0 - classifier never properly recognizes amyloid proteins).

Does amyloidogenicity depend on the exact sequence of amino acids?

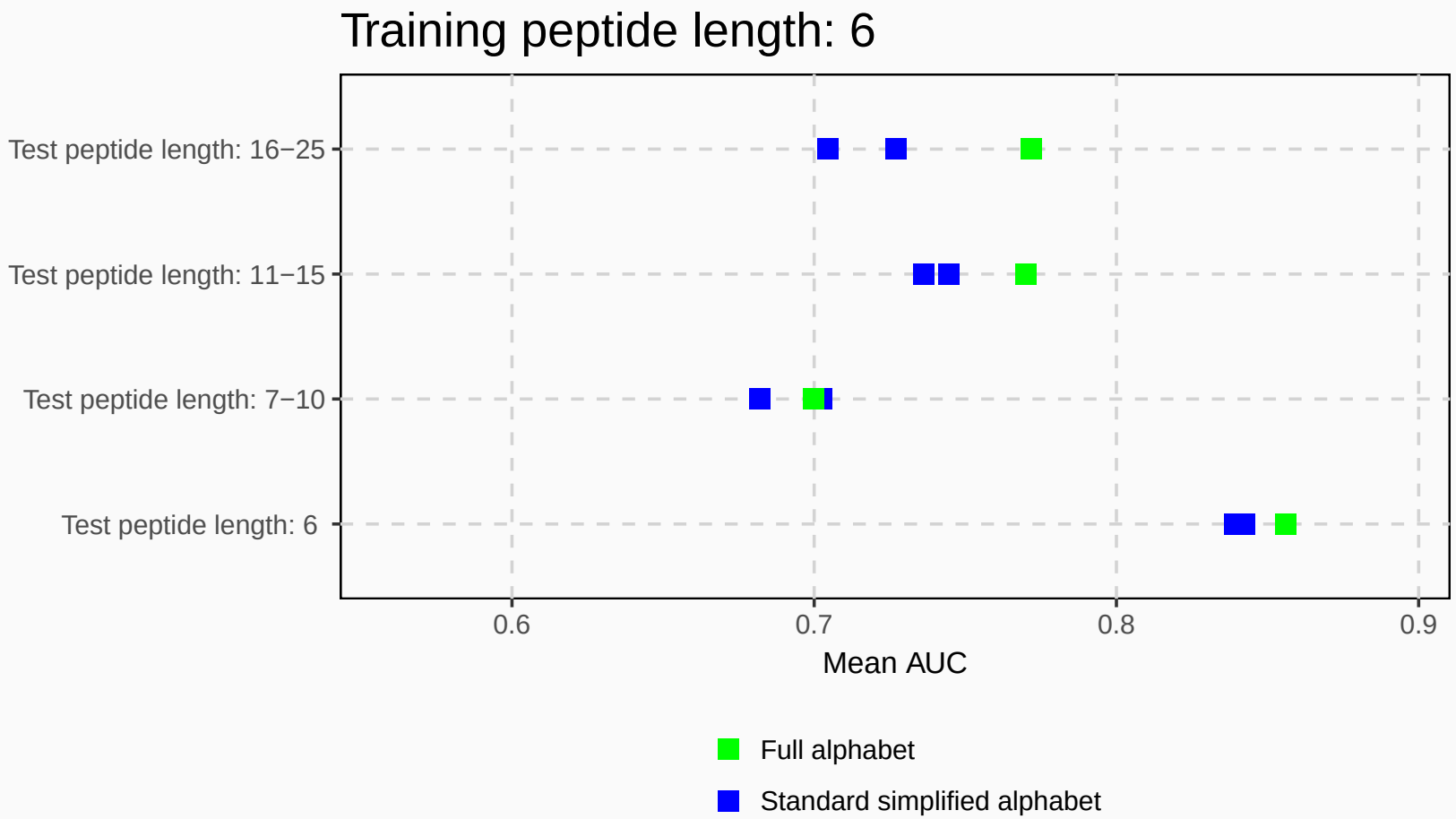
Standard simplified amino acid alphabets

To date, several simplified 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 simplified amino acid alphabets



Cross-validation

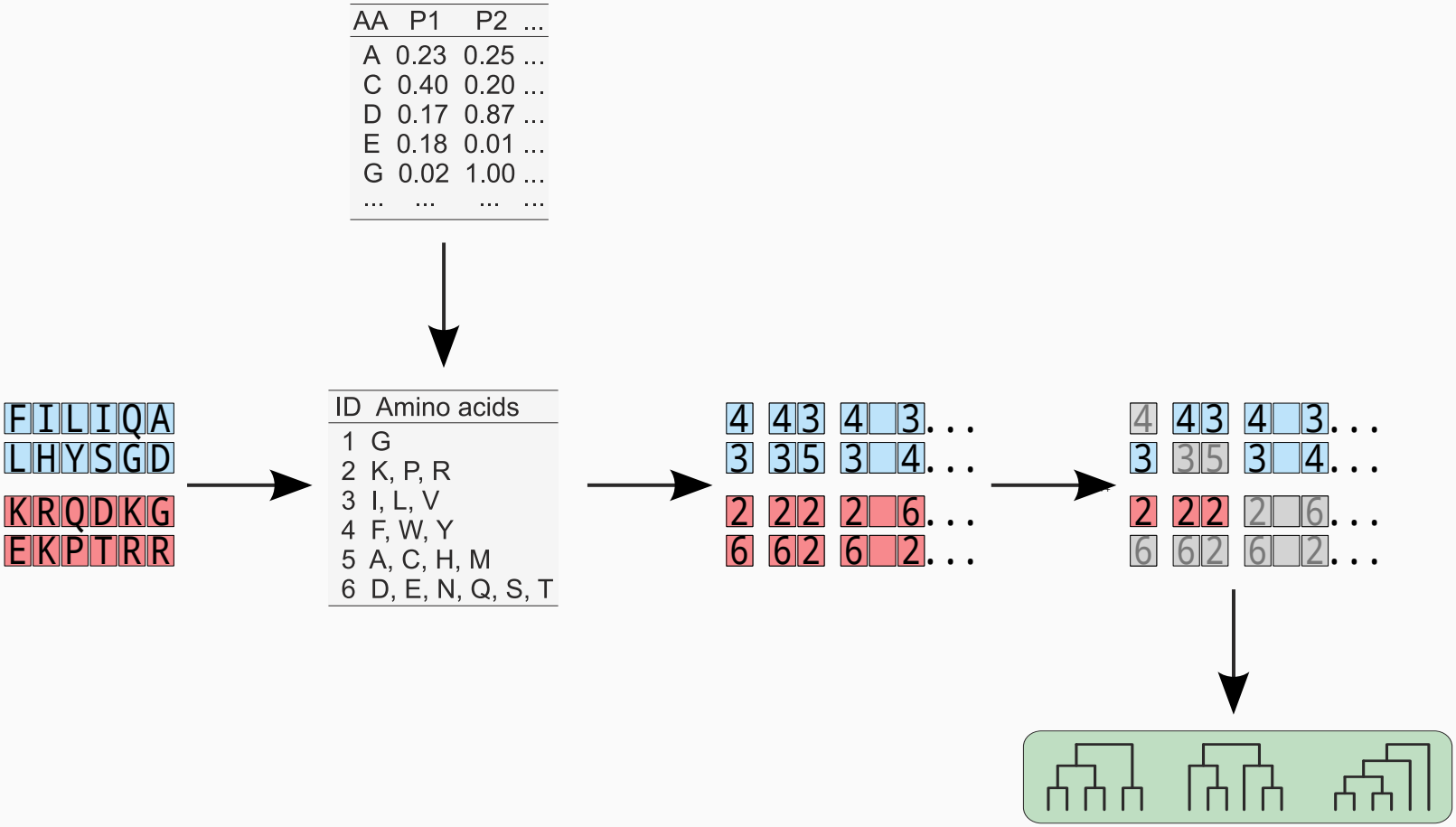


Standard simplified amino acid alphabets do not enhance discrimination between amyloidogenic and non-amyloidogenic proteins.

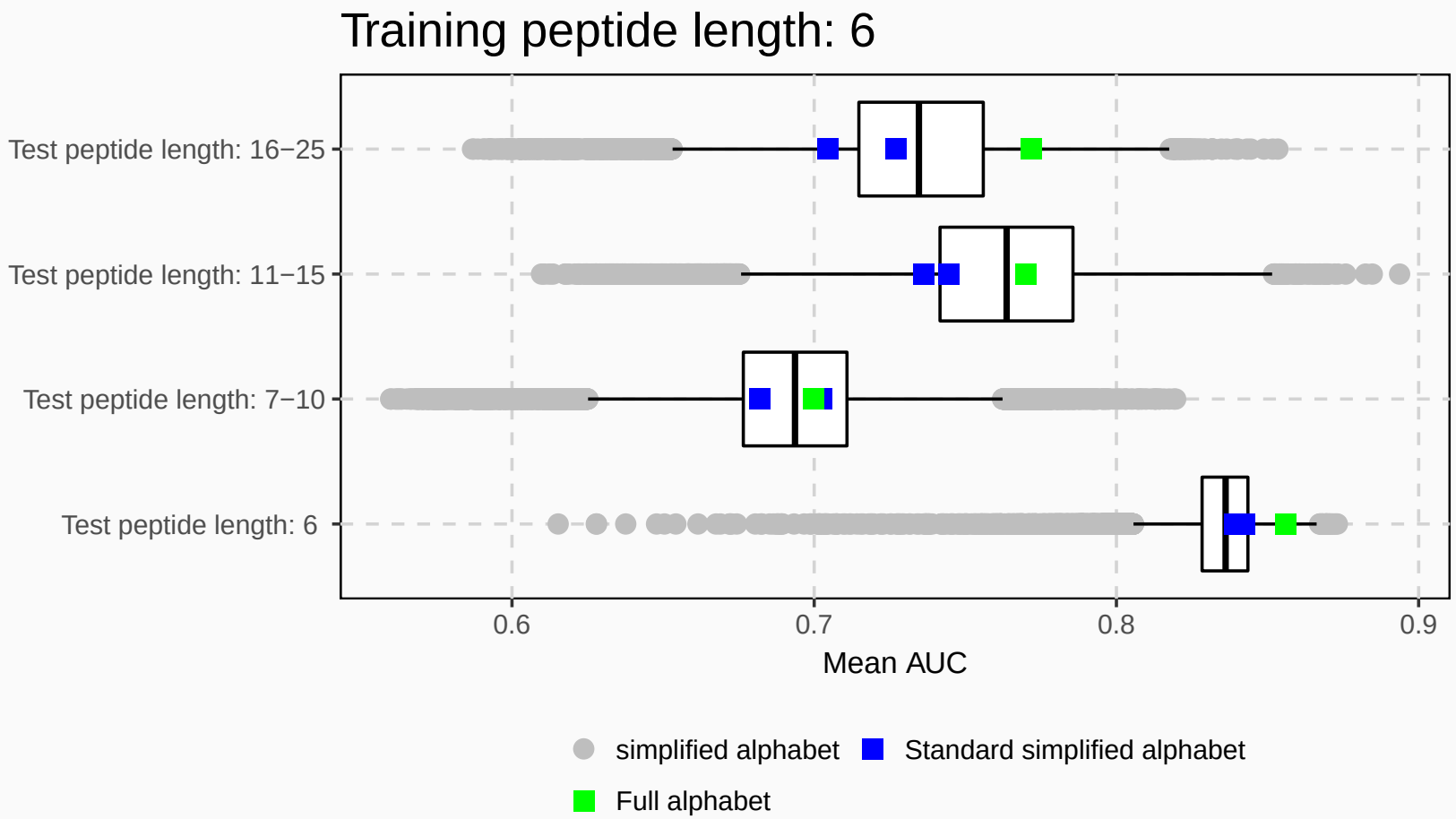
Novel simplified amino acid alphabets

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

Novel simplified amino acid alphabets

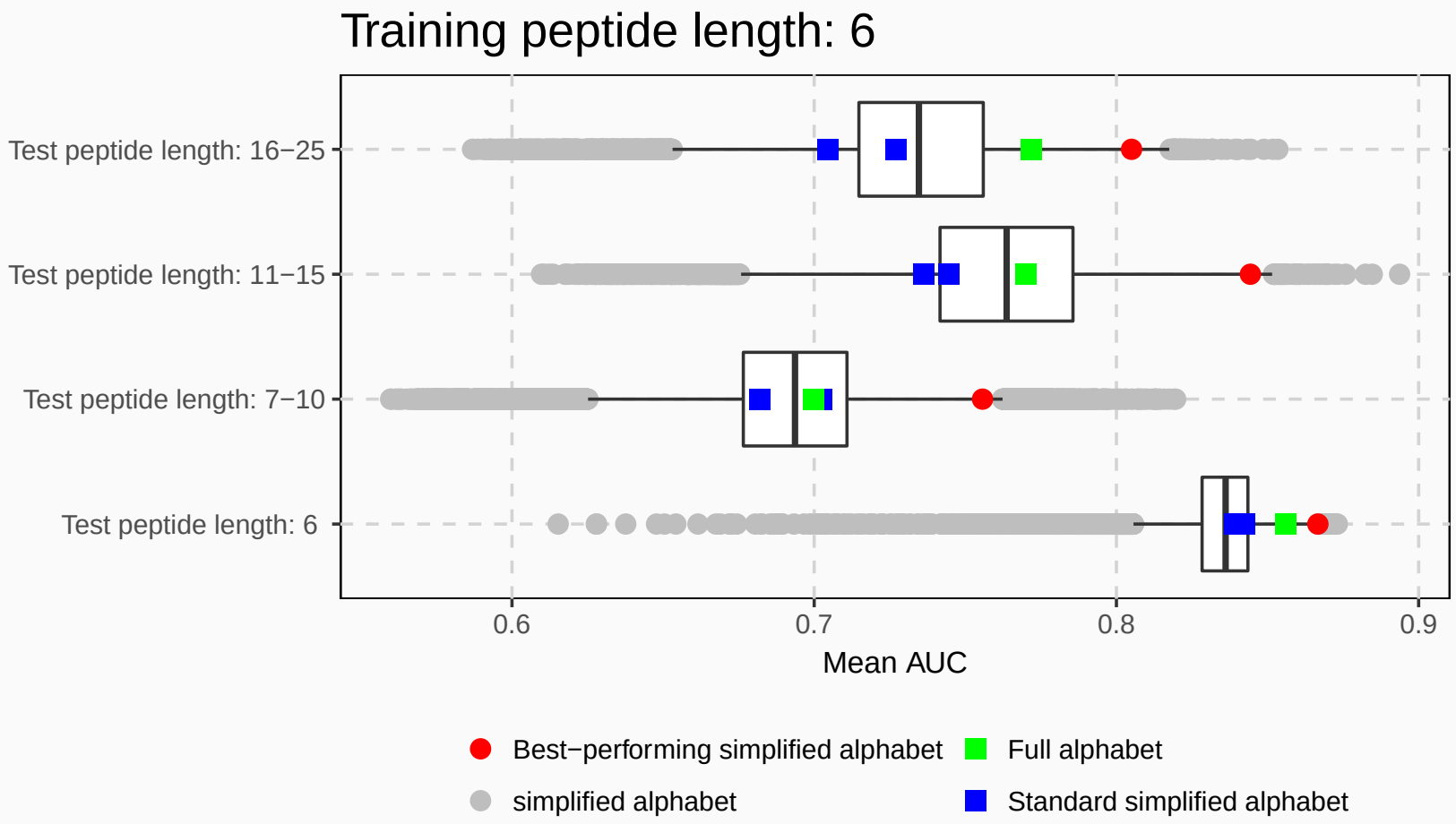


Cross-validation



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 simplified alphabets with the AUC outside the 0.95 confidence interval.

The best-performing simplified alphabet



The best-performing simplified 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

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1	G
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Group 3 and 4 - hydrophobic amino acids.

The best-performing simplified alphabet

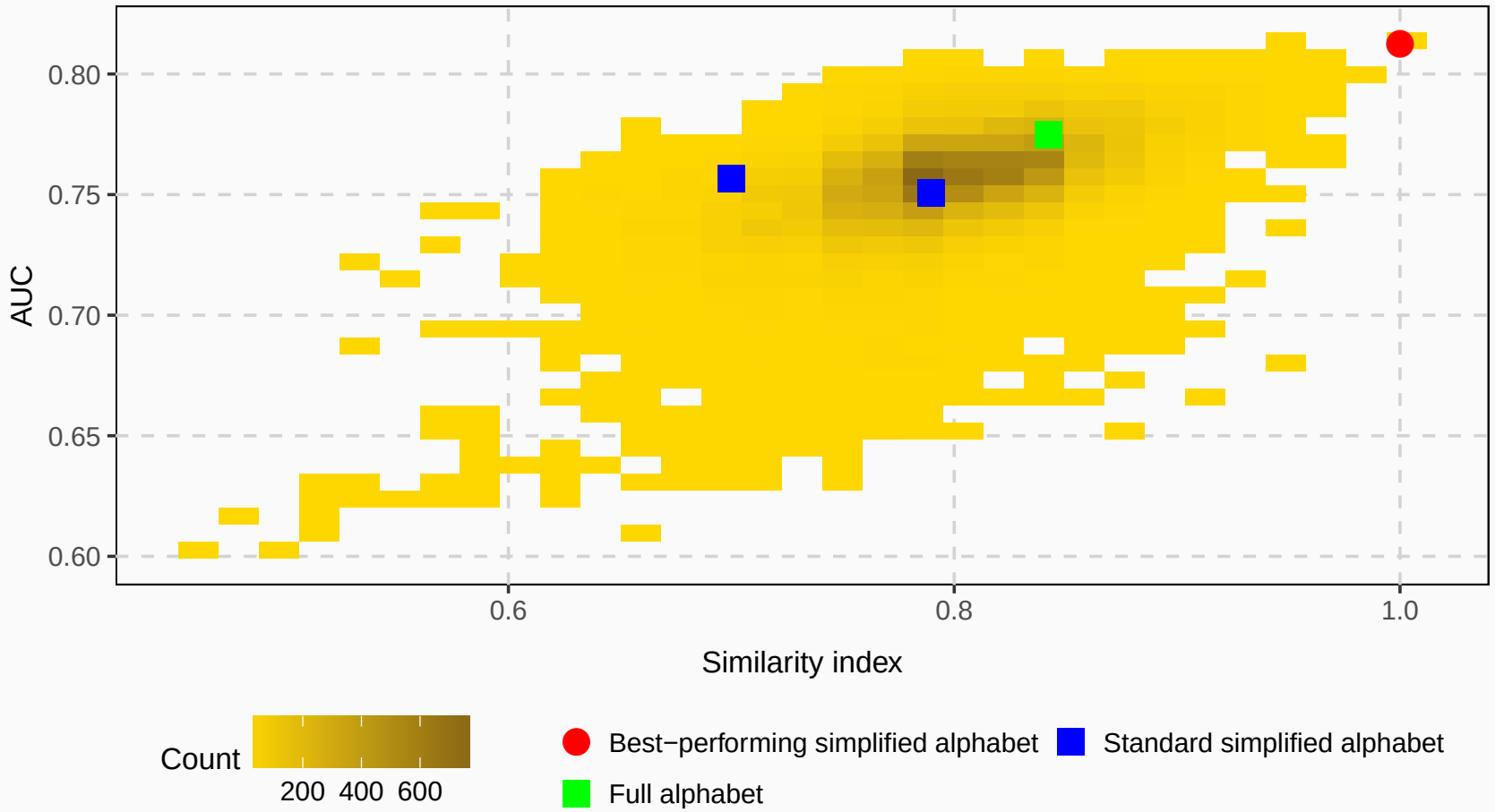
Subgroup ID	Amino acids
1	G
2	K, P, R
3	I, L, V
4	F, W, Y
5	A, C, H, M
6	D, E, N, Q, S, T

Group 2 - charged breakers of β-structures.

Alphabet similarity and performance

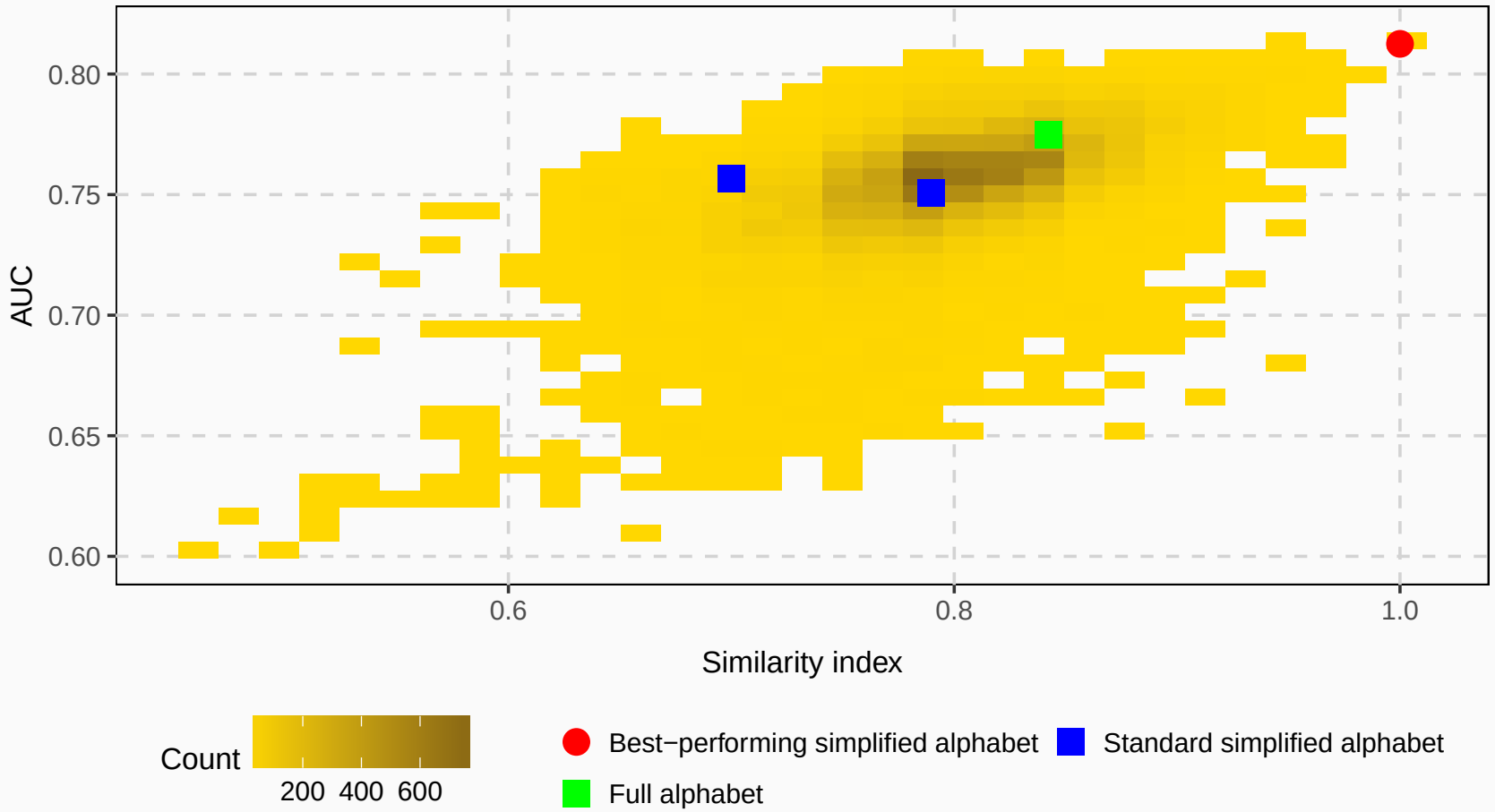
Is the best-performing simplified amino alphabet associated with amyloidogenicity?

Similarity index



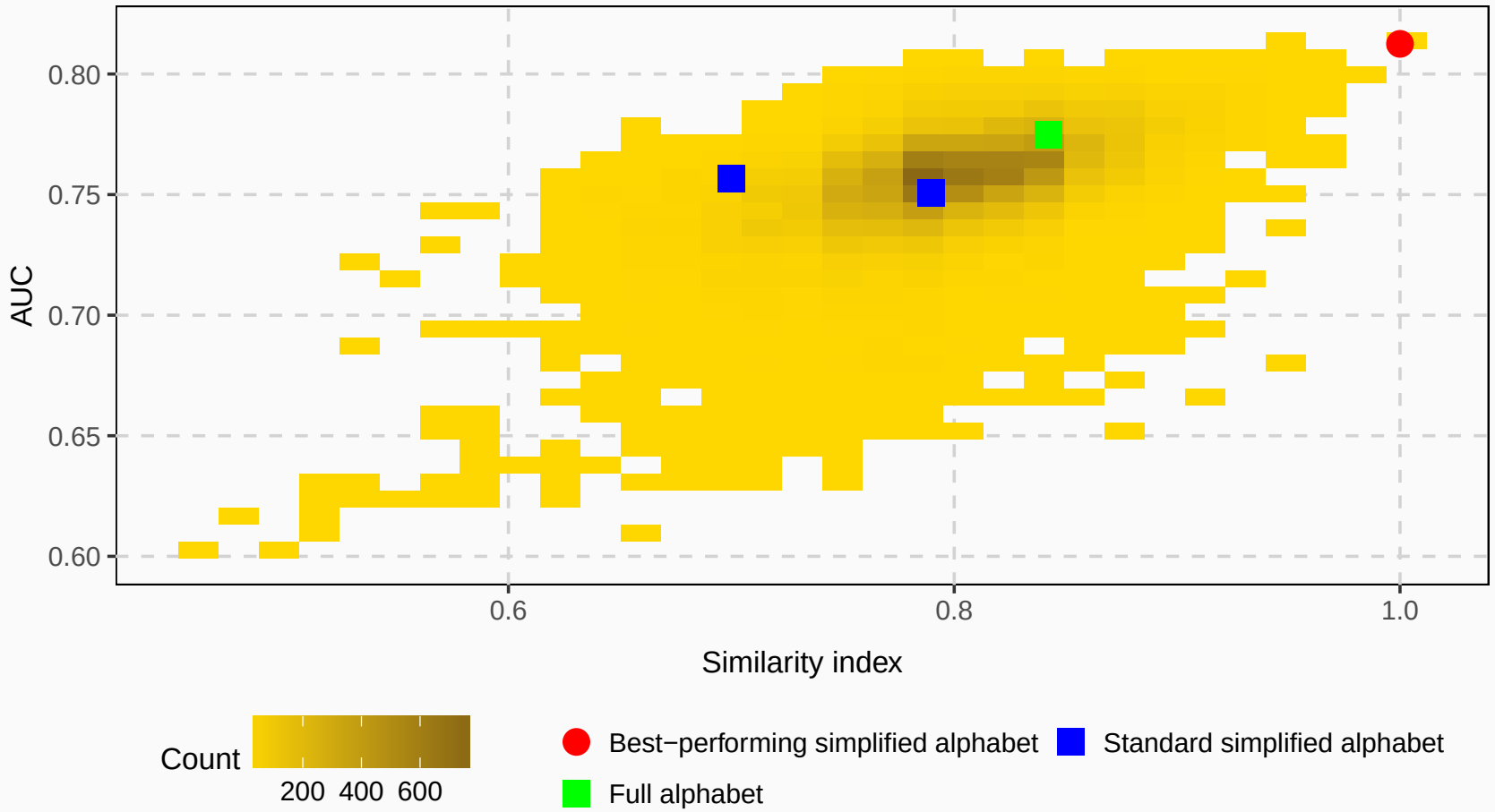
Similarity index (Stephenson and Freeland, 2013) measures the similarity between two simplified alphabets (1 - identical, 0, totally dissimilar).

Similarity index



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

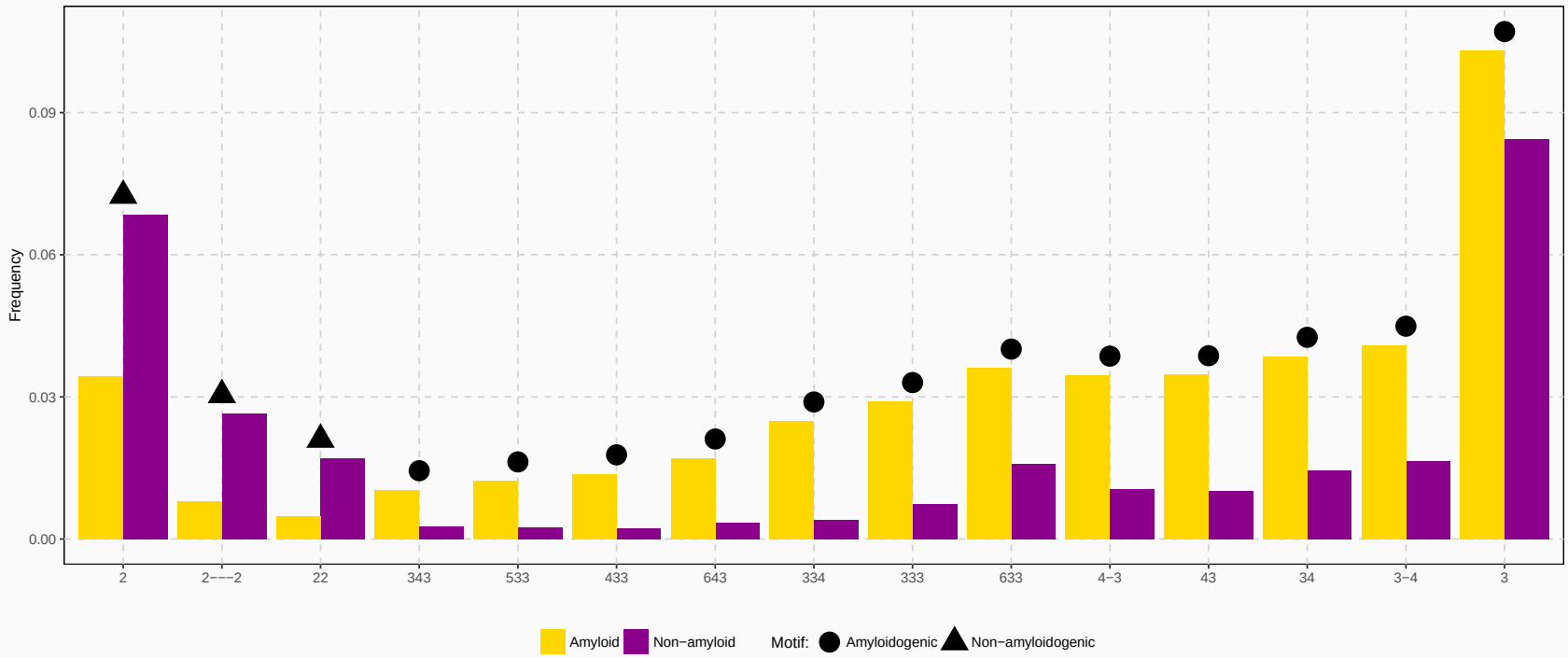
Similarity index



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

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 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*.

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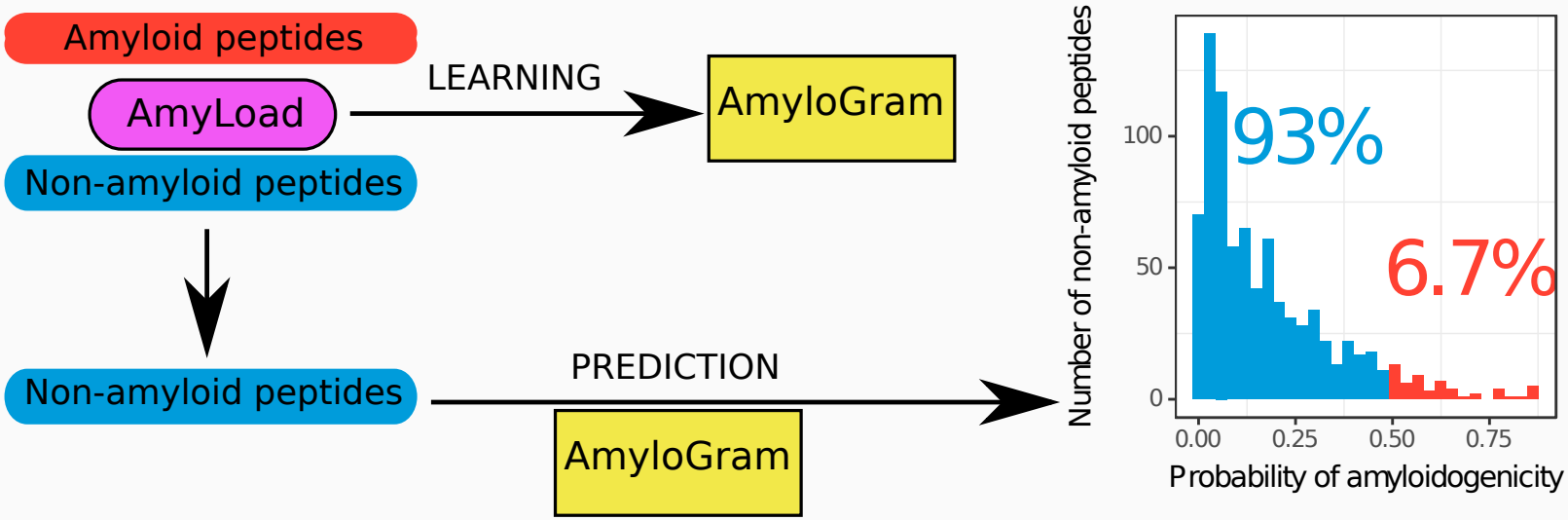
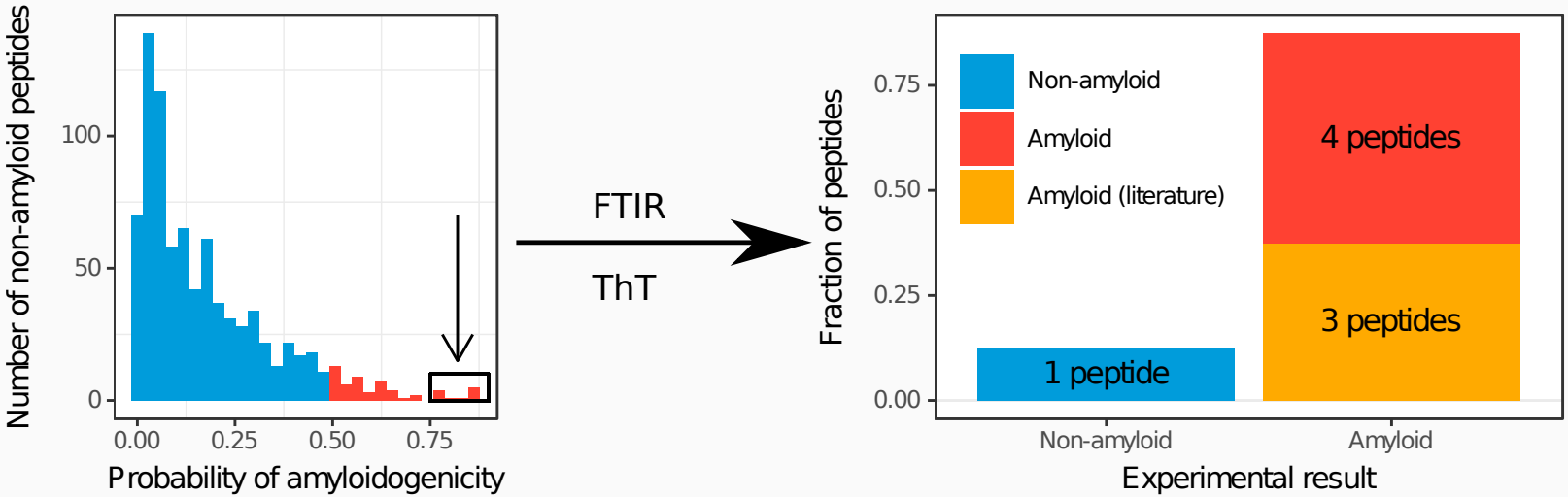
APPNN (Família et al., 2015)	0.8343	0.5823

MCC (Matthew's Correlation Coefficient) measures the performance of a classifier (1 - classifier always properly recognizes amyloid proteins, -1 - classifier never properly recognizes amyloid proteins).

Experimental validation

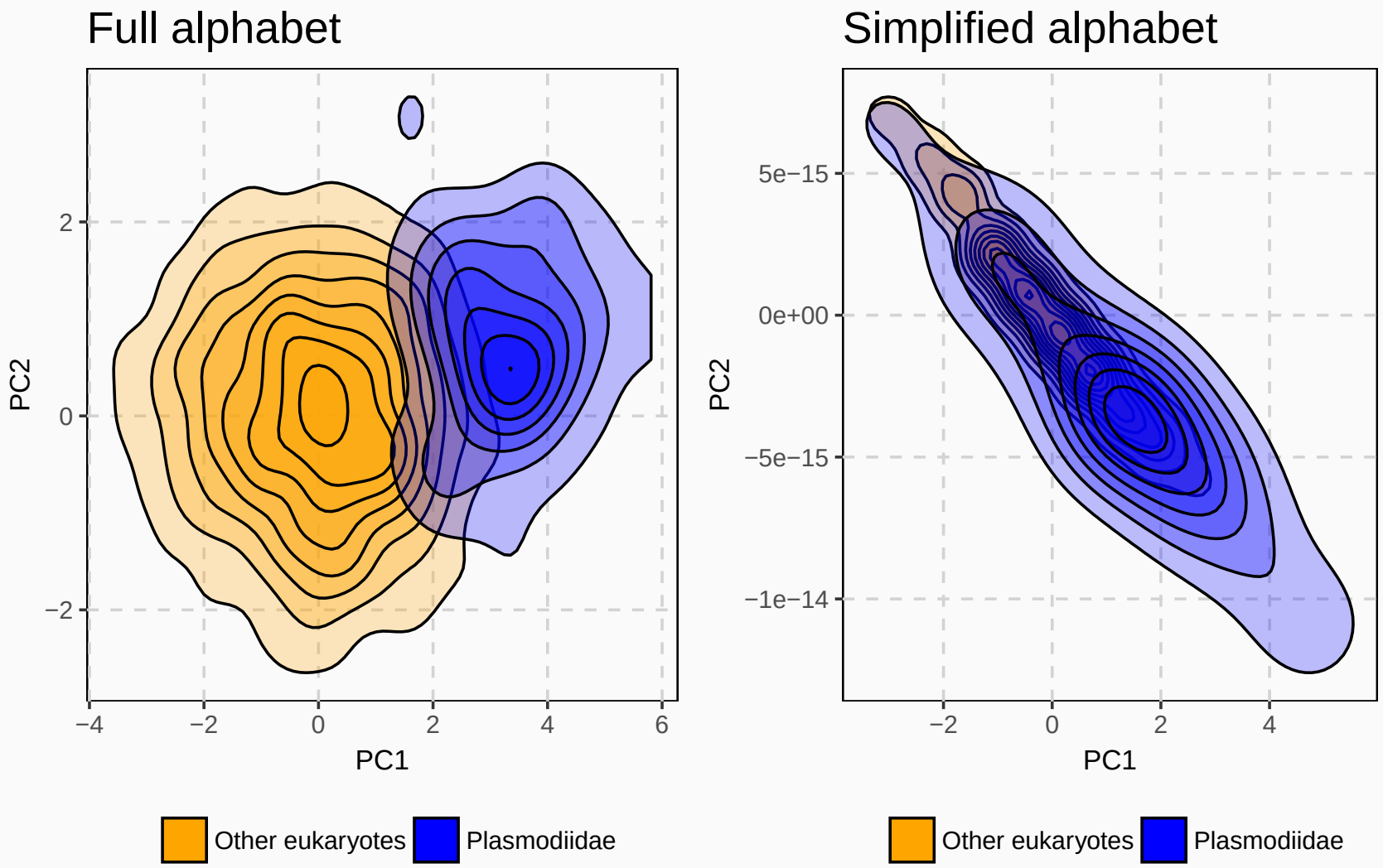


Experimental validation



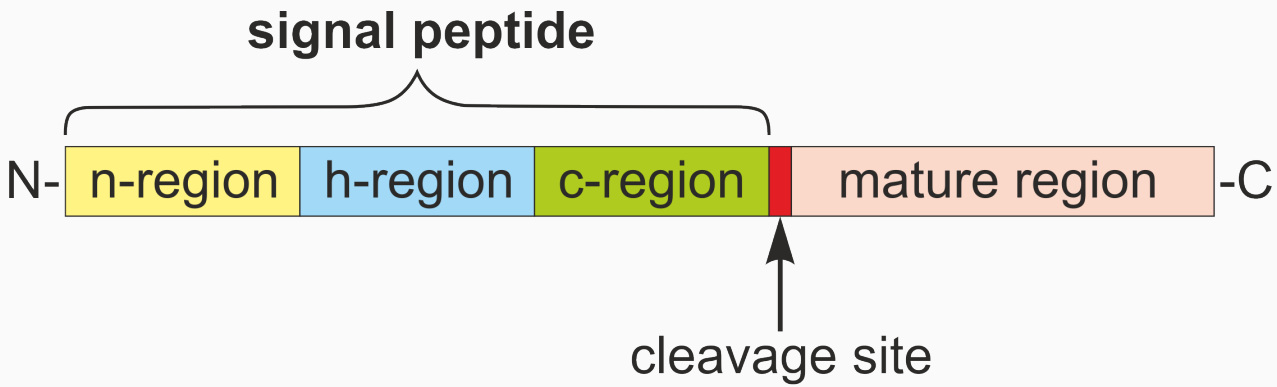
Other applications

Signal peptide prediction



PCA of amino acid frequency in signal peptides.

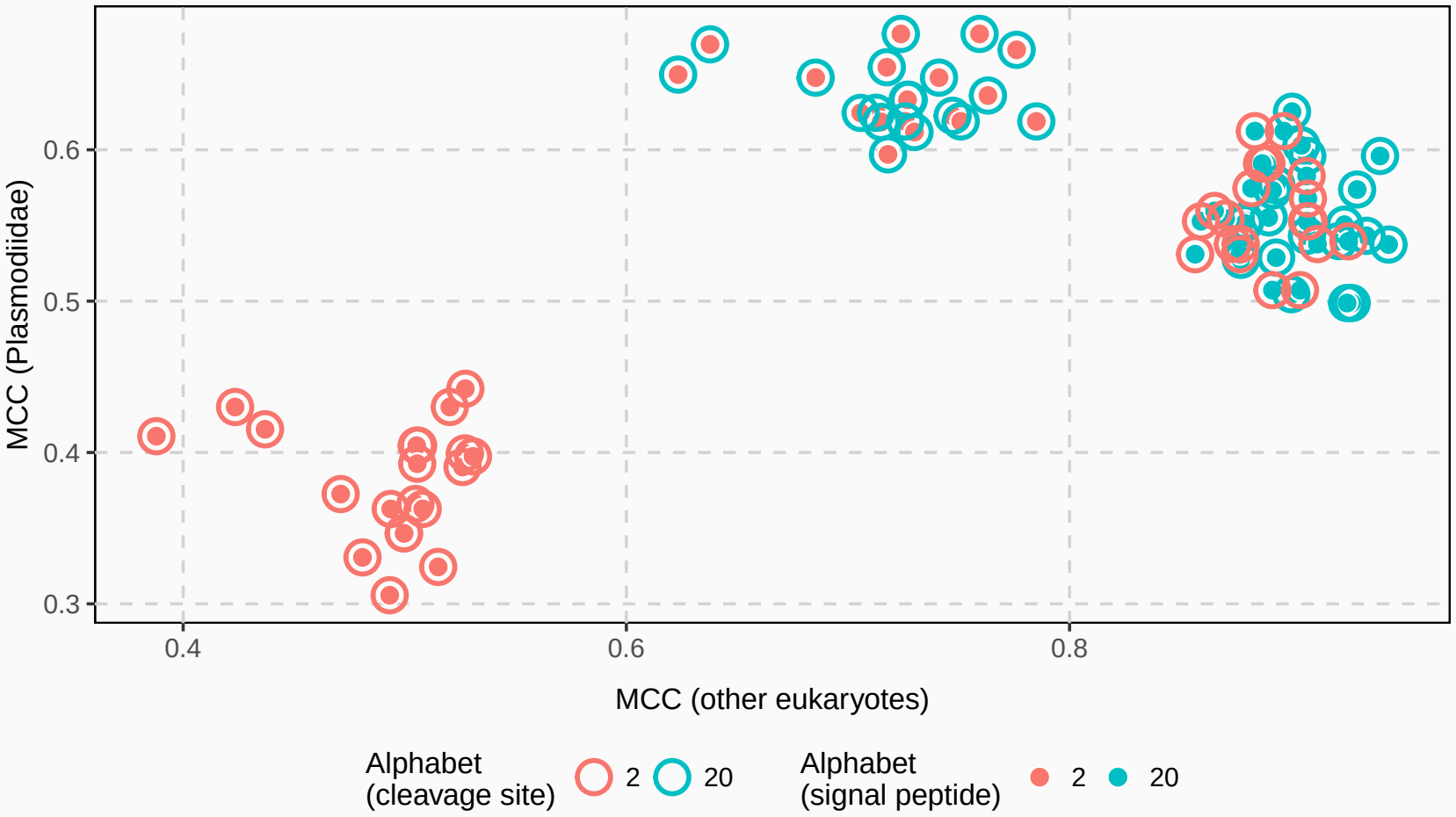
Signal peptide prediction



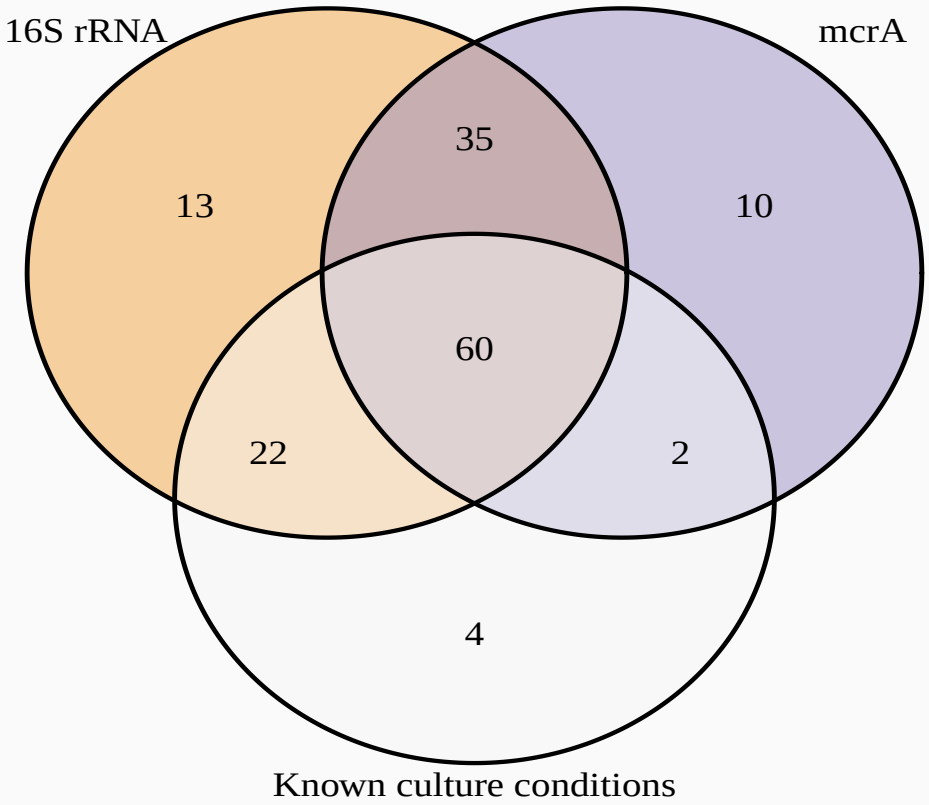
SignalP 4.1 (Petersen et al., 2011) combines output of two separate predictors:

- cleavage site,
- signal peptide.

Signal peptide prediction



Prediction of culturing conditions



`metanogen.biotech.uni.wroc.pl` (Jabłoński et al., 2015)

Prediction of culturing conditions

Results of nested cross-validation of MethanoGram.

Culturing condition	Mean error
Growth rate [h^{-1}]	0.35
Growth doubling time [h]	27.19
Optimal growth temp. [°C]	8.89
Optimal growth pH	0.47
Optimal growth NaCl [mol/dm^3]	0.21

Summary

1. Created algorithms effectively filtering n-grams.
2. Introduced new methods for search of simplified amino acids.
3. Implemented novel algorithms in the **R** package *biogram*.
4. Applied the n-gram analysis framework to:
    - prediction of amyloids (AmyloGram) (to appear in Scientific Reports),
    - prediction of atypical signal peptides,
    - prediction of culture conditions of methanogenes (MethanoGram).

Summary

Web servers:

- **AmyloGram**: `http://www.smorfland.uni.wroc.pl/shiny/AmyloGram/`.
- **MethanoGram**: `http://www.smorfland.uni.wroc.pl/shiny/MethanoGram/`.
- **signalHsmm**: `http://www.smorfland.uni.wroc.pl/shiny/signalHsmm/`.

Software packages:

- **biogram**: `https://cran.r-project.org/package=biogram`.
- **AmyloGram**: `https://cran.r-project.org/package=AmyloGram`.

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Publications I

1. **Burdukiewicz, M.**, Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports (accepted).

2. Rödiger, S., **Burdukiewicz, M.**, Spiess, A.-N., and Blagodatskikh, K. (2017). Enabling reproducible real-time quantitative PCR research: the RDML package. Bioinformatics.

3. Schiebel, J., Böhm, A., Nitschke, J., **Burdukiewicz, M.**, Weinreich, J., Ali, A., Roggenbuck, D., Rödiger, S., and Schierack, P. (2017). Genotypic and phenotypic characteristics in association with biofilm formation in different pathotypes of human clinical Escherichia coli isolates. Appl. Environ. Microbiol. AEM.01660-17.

Publications II

4. **Burdukiewicz, M.**, Rödiger, S., Sobczyk, P., Menschikowski, M., Schierack, P., and Mackiewicz, P. (2016a). Methods for comparing multiple digital PCR experiments. Biomolecular Detection and Quantification 9, 14–19.

5. **Burdukiewicz, M.**, Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2016b). Prediction of amyloidogenicity based on the n-gram analysis. PeerJ Preprints.

6. Spiess, A.-N., Rödiger, S., **Burdukiewicz, M.**, Volksdorf, T., and Tellinghuisen, J. (2016). System-specific periodicity in quantitative real-time polymerase chain reaction data questions threshold-based quantitation. Scientific Reports.

7. Rödiger, S., **Burdukiewicz, M.**, Blagodatskikh, K., Jahn M., and Schierack, P. (2016). R as an Environment for Reproducible Analysis of DNA Amplification Experiments (R Journal).

Publications III

8. Rödiger, S., **Burdukiewicz, M.**, and Schierack, P. (2015). chipPCR: an R package to pre-process raw data of amplification curves. Bioinformatics 31, 2900–2902.

9. Spiess, A.-N., Deutschmann, C., **Burdukiewicz, M.**, Himmelreich, R., Klat, K., Schierack, P., and Rödiger, S. (2015). Impact of Smoothing on Parameter Estimation in Quantitative DNA Amplification Experiments. Clinical Chemistry 61, 379–388.

10. Kolenda, R., **Burdukiewicz, M.**, and Schierack, P. (2015). A systematic review and meta-analysis of the epidemiology of pathogenic Escherichia coli of calves and the role of calves as reservoirs for human pathogenic E. coli. Frontiers in Cellular and Infection Microbiology 5.

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References

Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2016). Prediction of amyloidogenicity based on the n-gram analysis. Technical Report e2390v1, PeerJ Preprints.

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.

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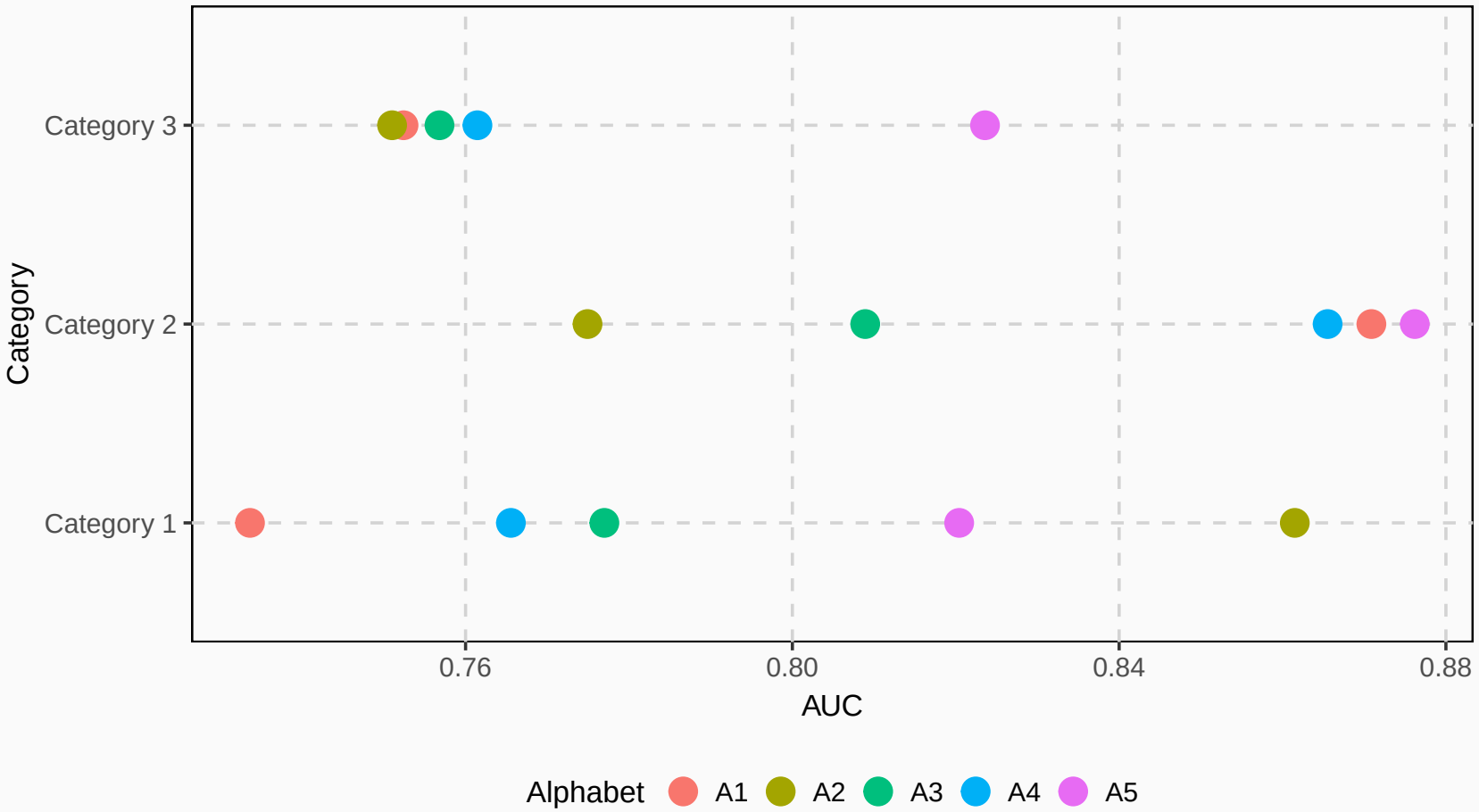
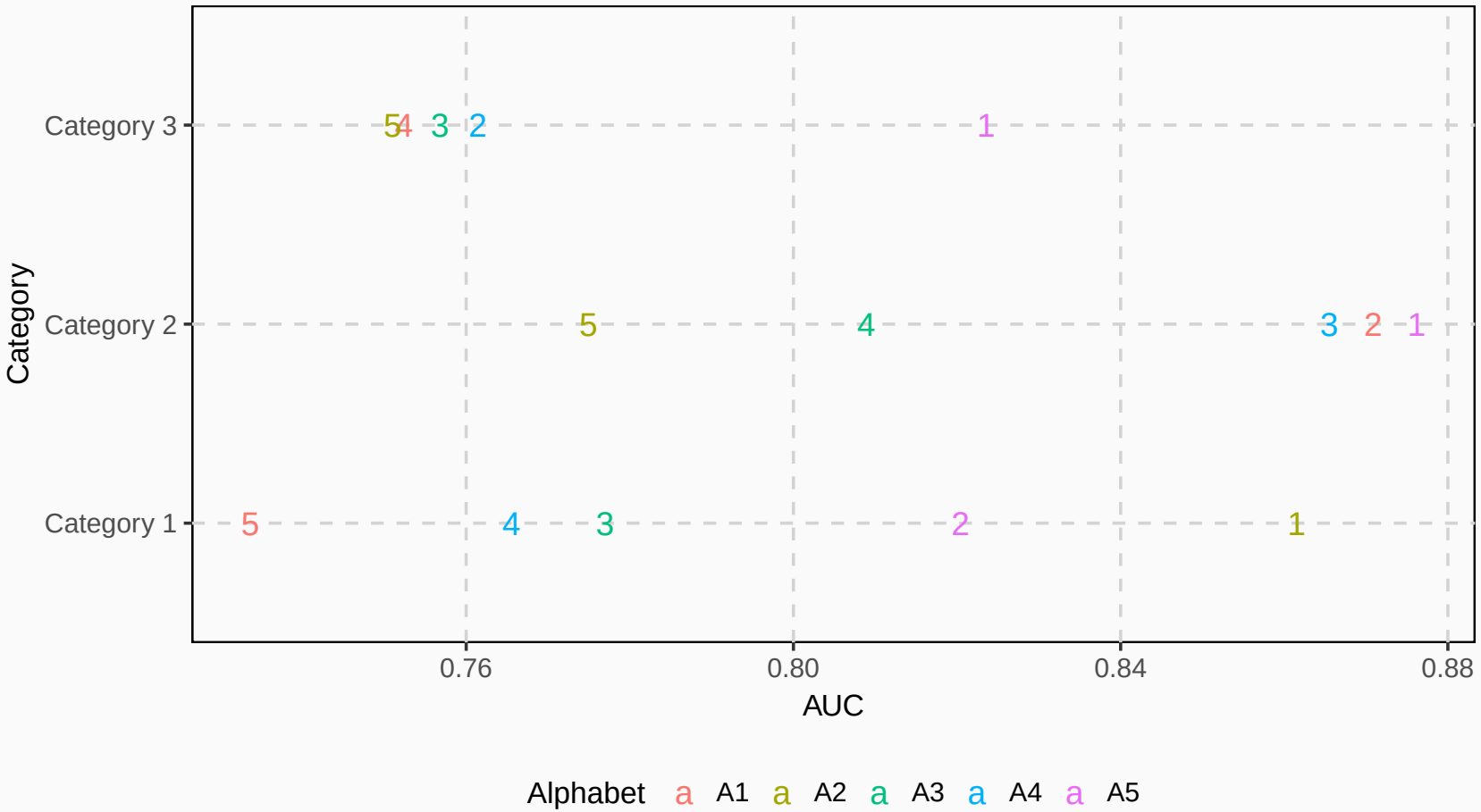
Ranking alphabets

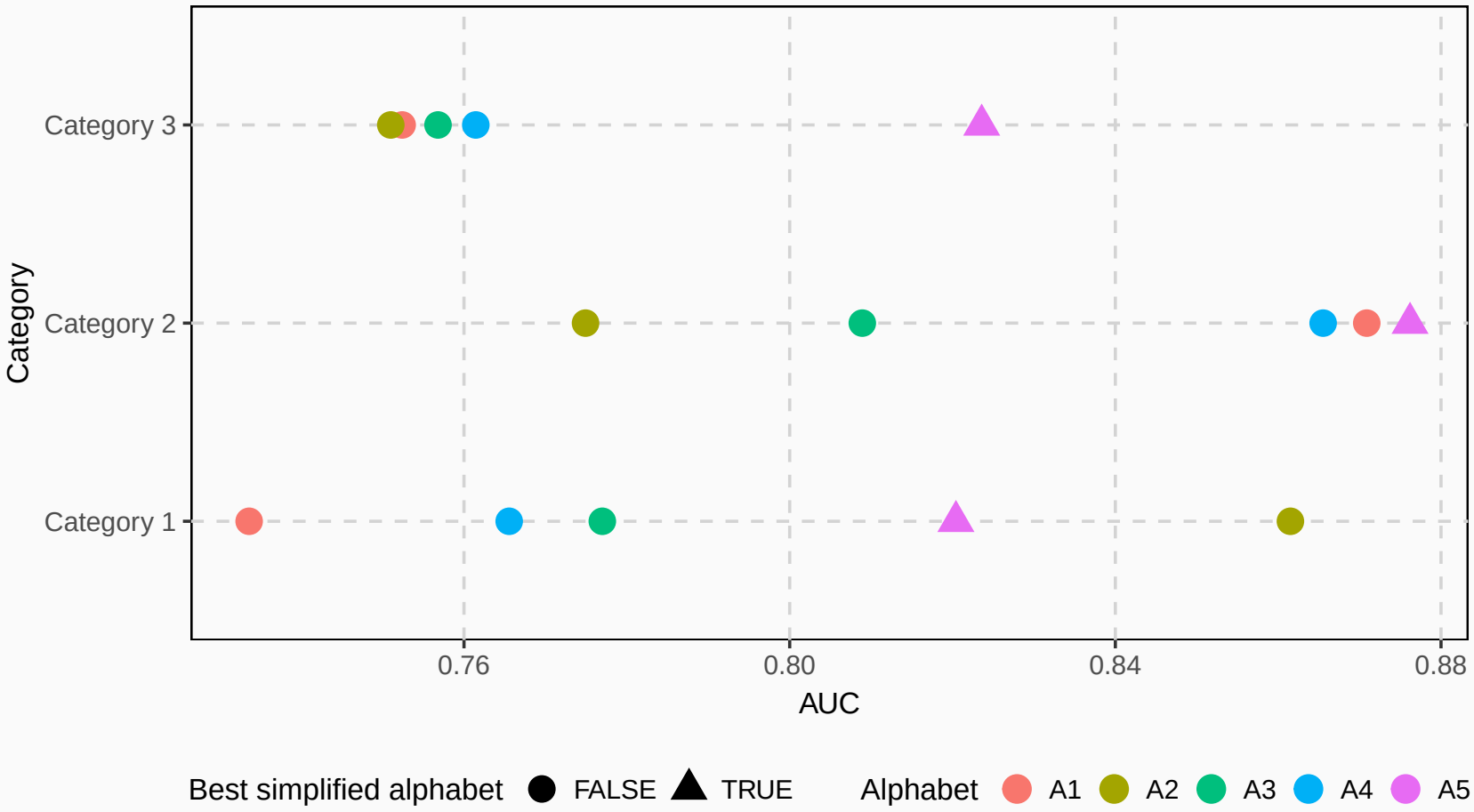


Ranking alphabets



We rank alphabets separately in all length categories assuming the rank 1 for the best AUC, rank 2 for the second best AUC and so on.

Ranking alphabets



The best-performing alphabet has the lowest sum of ranks.