Alphabet of life: analysis of proteins in R

Michał Burdukiewicz, PhD

Faculty of Mathematics and Computer Science

About me

- bioinformatician (Warsaw University of Technology, Brandenburg University of Technology Cottbus-Senftenberg, .prot),
- founder of the Why R? Foundation and Wrocław R User Group,
- facilitator of McKinsey Tech Meetup.

Presentation plan

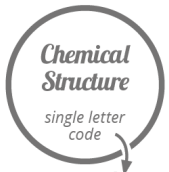
1. Amino acids and proteins
2. n-grams and reduced alphabets
3. Amyloid prediction
4. Shiny web server
5. Other applications

Amino acids and proteins

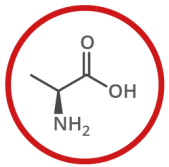
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

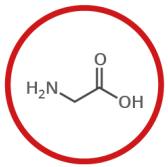
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL



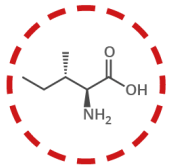
NAME (A)
three letter code
DNA codons



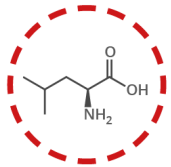
ALANINE (A)
Ala
GCT, GCC, GCA, GCG



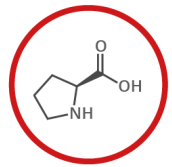
GLYCINE (G)
Gly
GGT, GGC, GGA, GGG



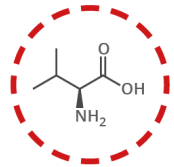
ISOLEUCINE (I)
Ile
ATT, ATC, ATA



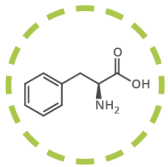
LEUCINE (L)
Leu
CTT, CTC, CTA, CTG, TTA, TTG



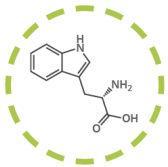
PROLINE (P)
Pro
CCT, CCC, CCA, CCG



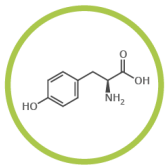
VALINE (V)
Val
GTT, GTC, GTA, GTG



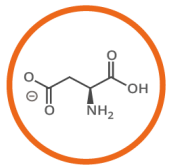
PHENYLALANINE (F)
Phe
TTT, TTC



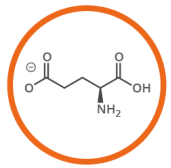
TRYPTOPHAN (W)
Trp
TGG



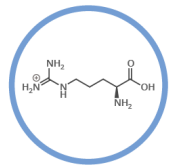
TYROSINE (Y)
Tyr
TAT, TAC



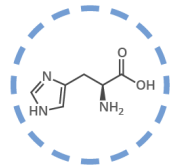
ASPARTIC ACID (D)
Asp
GAT, GAC



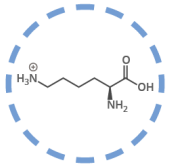
GLUTAMIC ACID (E)
Glu
GAA, GAG



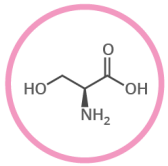
ARGININE (R)
Arg
CGT, CGC, CGA, CGG, AGA, AGG



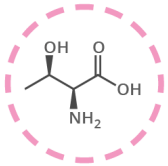
HISTIDINE (H)
His
CAT, CAC



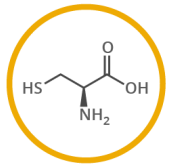
LYSINE (K)
Lys
AAA, AAG



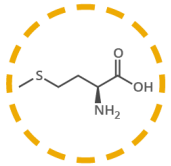
SERINE (S)
Ser
TCT, TCC, TCA, TCG, AGT, AGC



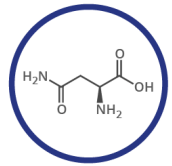
THREONINE (T)
Thr
ACT, ACC, ACA, ACG



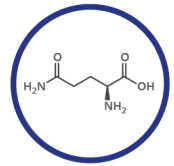
CYSTEINE (C)
Cys
TGT, TGC



METHIONINE (M)
Met
ATG



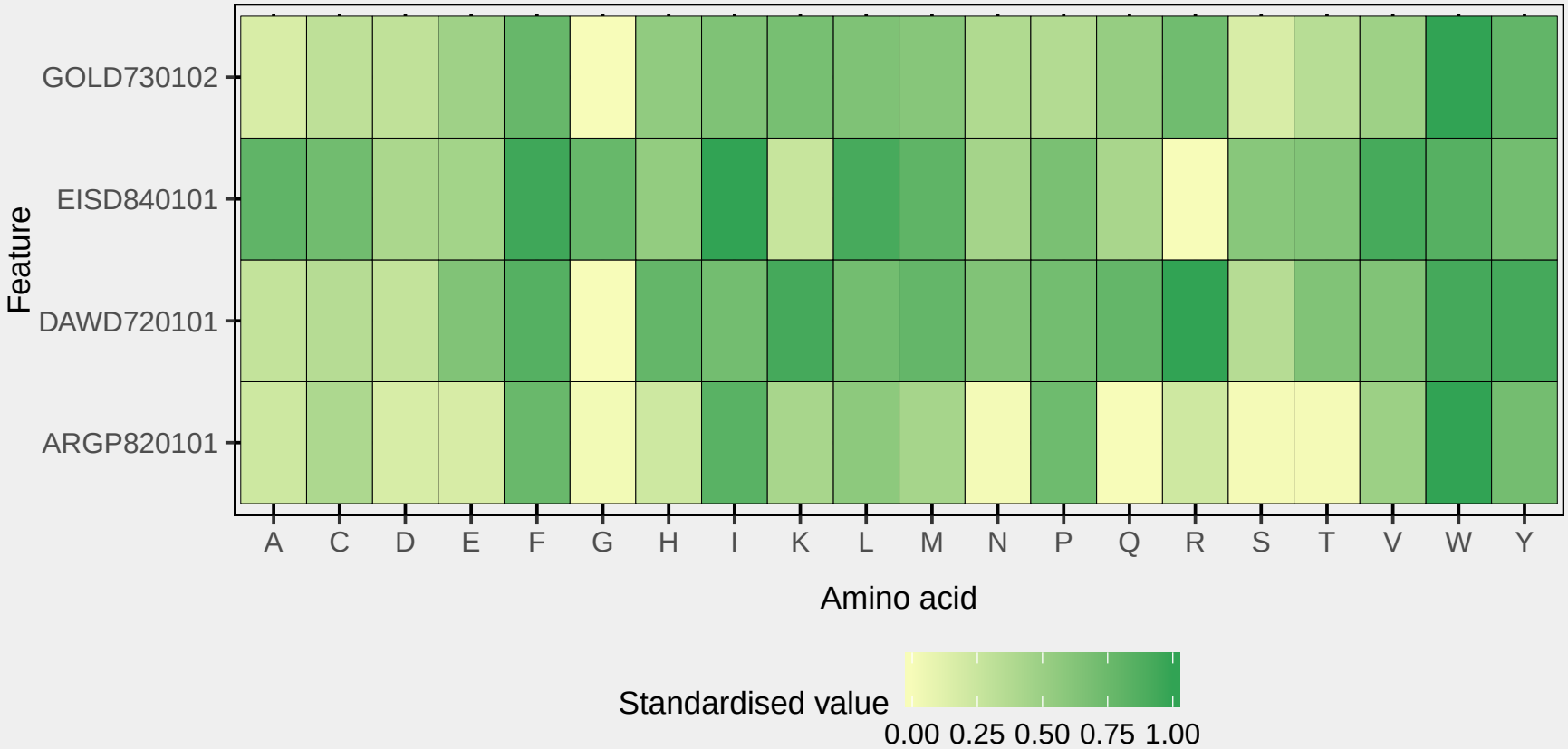
ASPARAGINE (N)
Asn
AAT, AAC



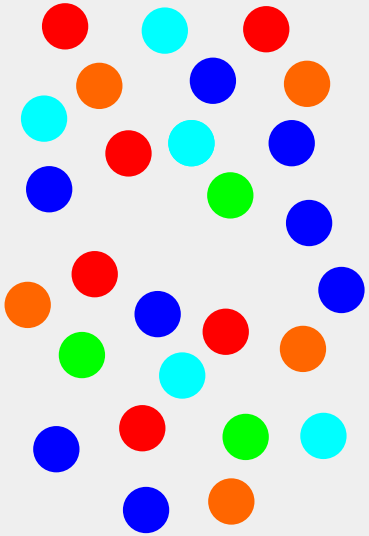
GLUTAMINE (Q)
Gln
CAA, CAG

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

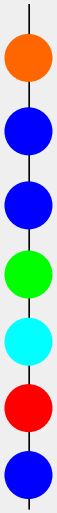
Amino acids


GOLD730102
EISD840101
DAWD720101
ARGP820101
Feature
A C D E F G H I K L M N P Q R S T V W Y
Amino acid
Standardised value
0.00 0.25 0.50 0.75 1.00

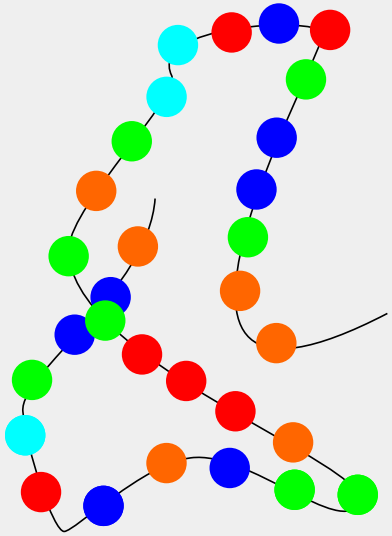
Proteins



Aminoacids

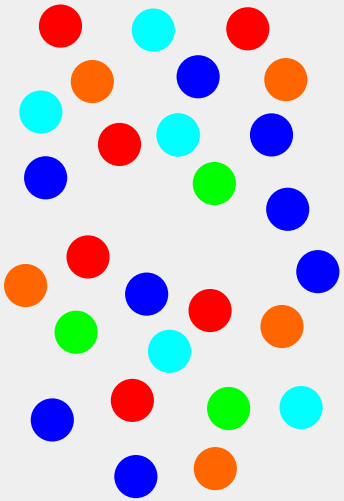


Protein (primary structure)

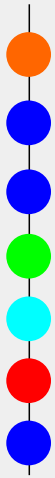


Protein (higher order structures)

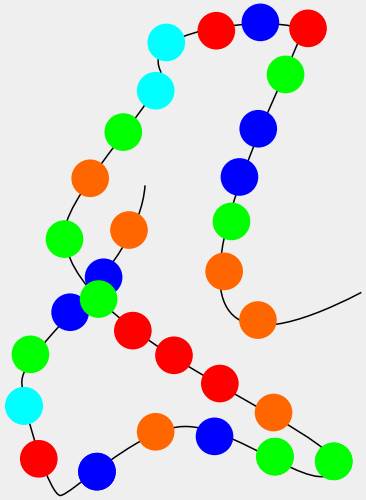
Proteins



Aminoacids



Protein (primary structure)

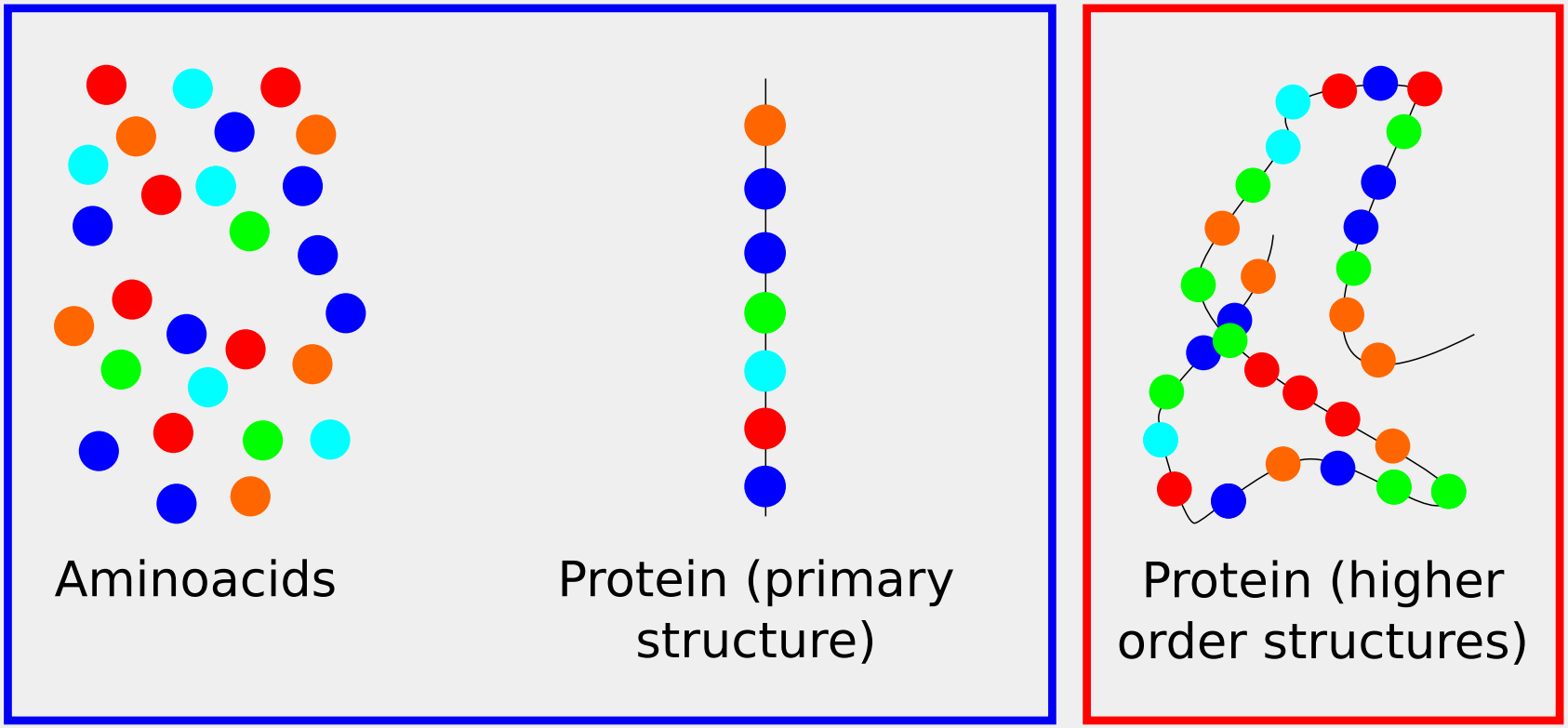


Protein (higher order structures)

KNOWN

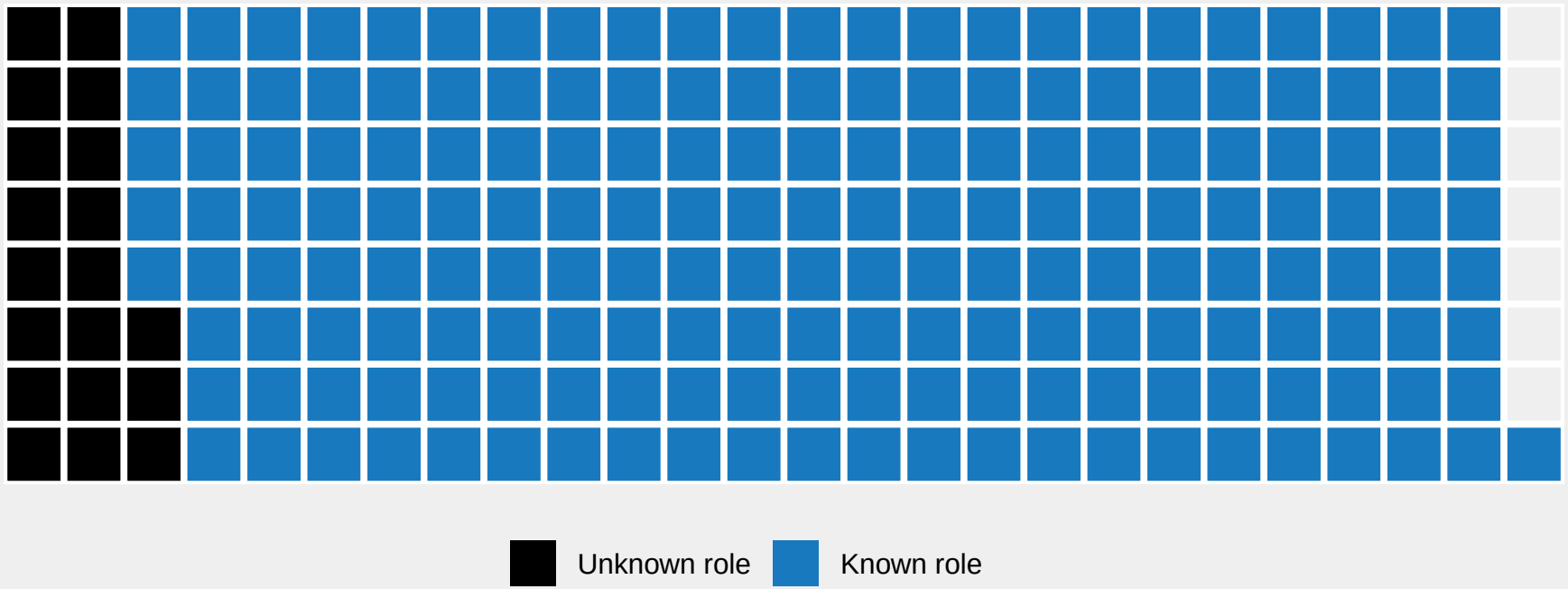
UNKNOWN

Proteins



Protein higher order structures determines its function.

Human proteom



1937 human proteins have unknown role (dark proteome) (Young-Ki Paik et al., 2018).

Goal

Development of methods for predicting protein properties on the basis of their primary structure in a way that is understandable for biologists and experimentally validated.

n-grams and reduced alphabets

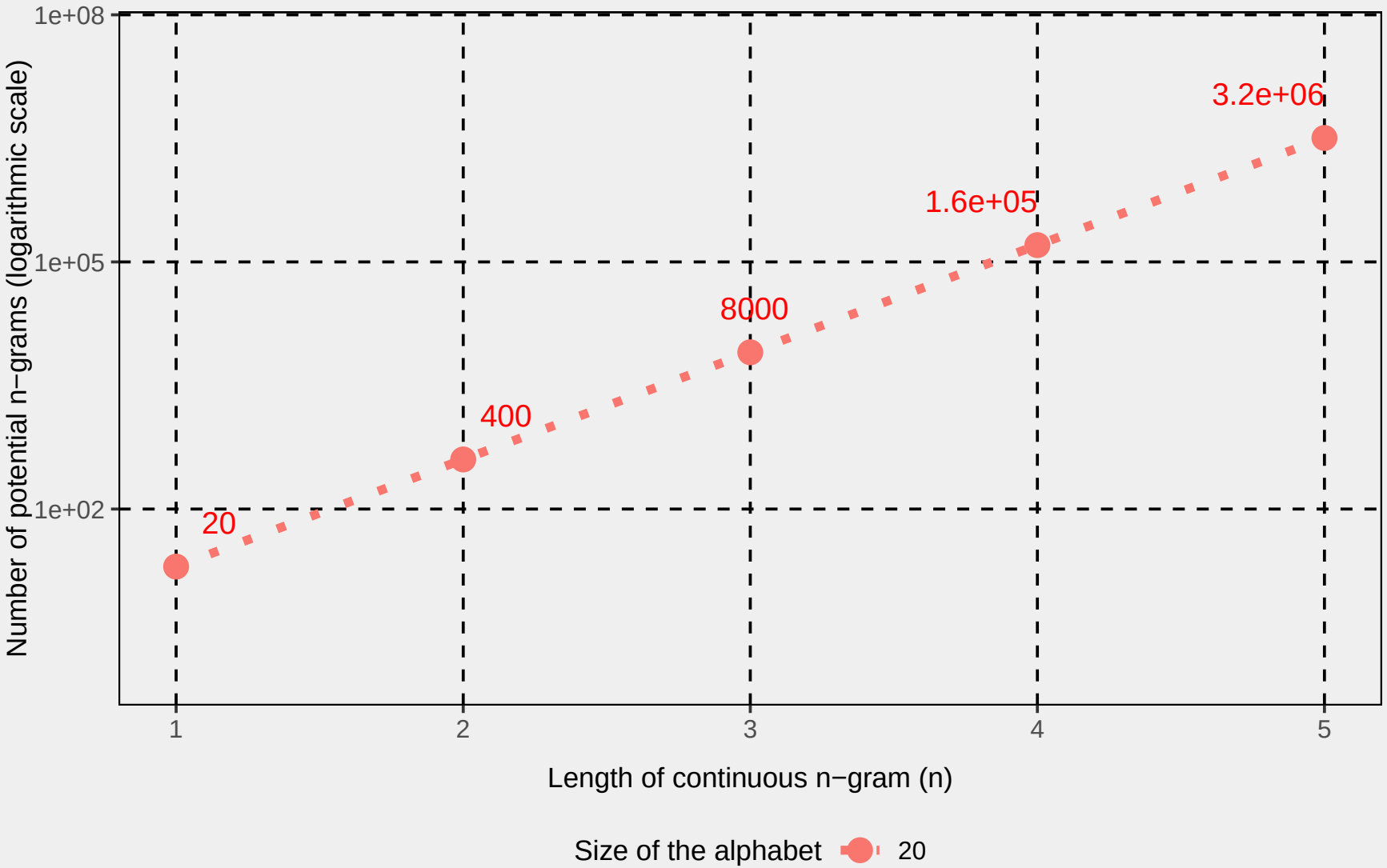
n-grams (k-tuple, k-mers):

- subsequences (continuous or discontinuous) *n* amino acid or nucleotide residues,
- more informative than the individual residues.

Peptide I: FKVWPDHGSG
Peptide II: YMCIYRAQTN

n-gram examples from peptide I and II:

1. 1-gram: F, Y, K, M,
2. 2-gram: FK, YM, KV, MC,
3. 2-gram (discontinuous): F-V, Y-C, K-W, M-I,
4. 3-gram (discontinuous): F–WP, Y–IY, K–PD, M–YR.

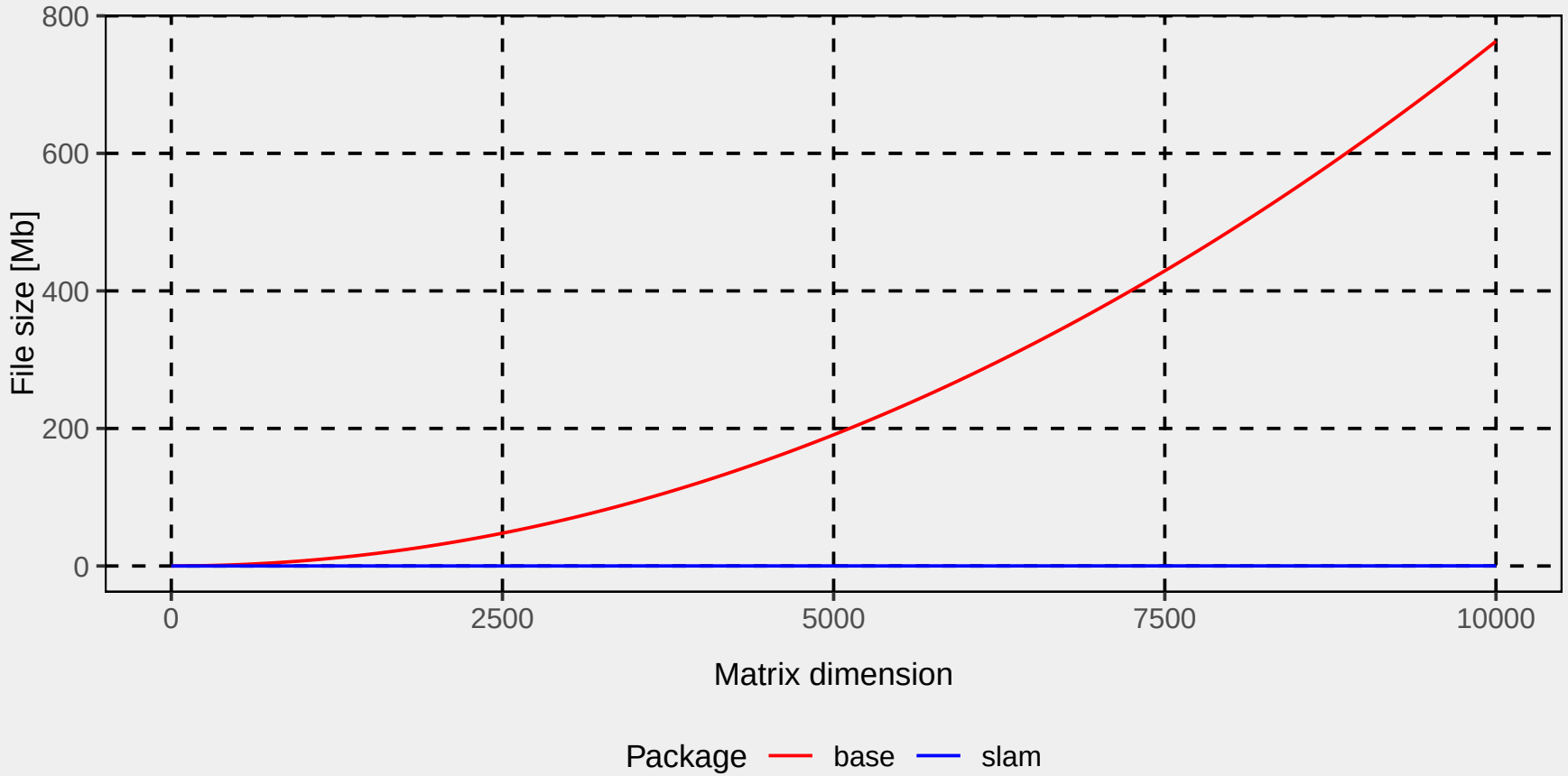


Longer n-games are more informative, but create larger attribute spaces that are more difficult to analyze.

Sparse matrices

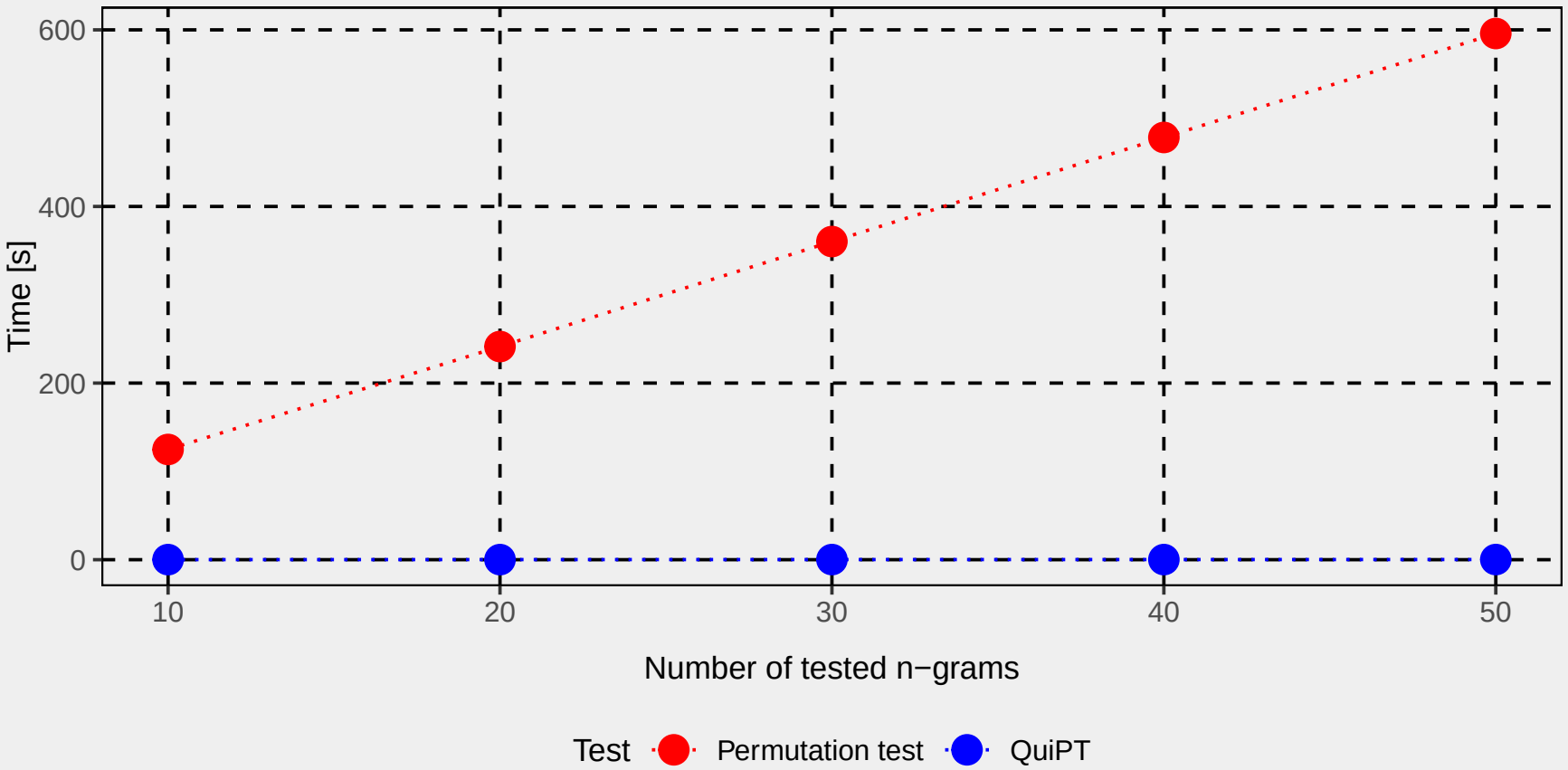
n-gram counting yields to sparse matrices (curse of dimensionality).

slam: Sparse Lightweight Arrays and Matrices



slam package: easy operations on sparse matrices in R.

QuiPT



Quick Permutation Test is a fast alternative to permutation tests for n-gram data. It also allows precise estimation of p-value. QuiPT is avaible as part of the biogram R package.

Reduced alphabets

Reduced alphabets:

- amino acids are grouped into larger yields on the basis of specific criteria,
- easier anticipation of structures (Murphy et al., 2000),
- creation of more generalised models.

Reduced alphabets

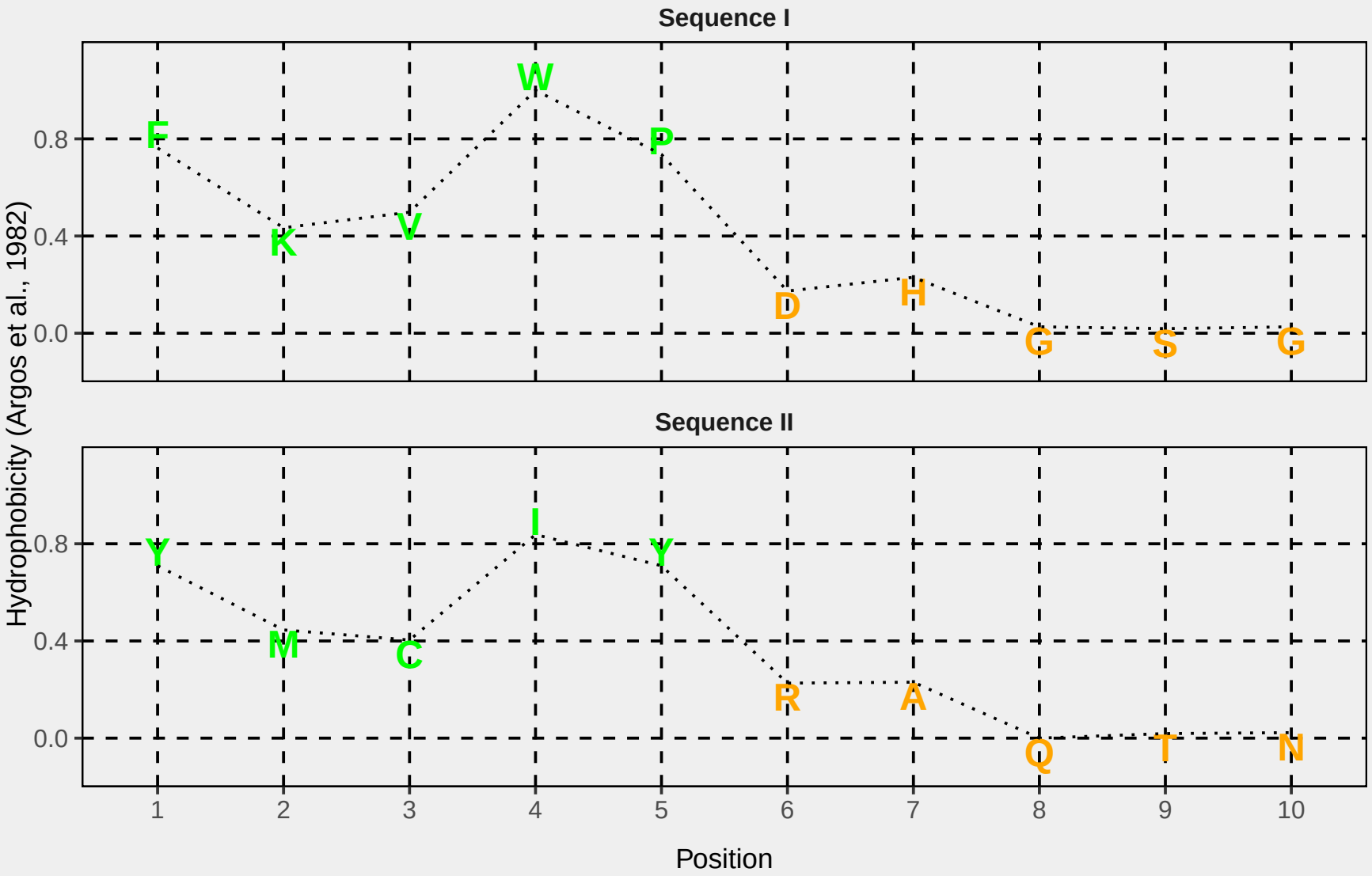
Following peptides appear to be completely different in terms of amino acid composition.

Peptide I:

FKVWPDHGSG

Peptide 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

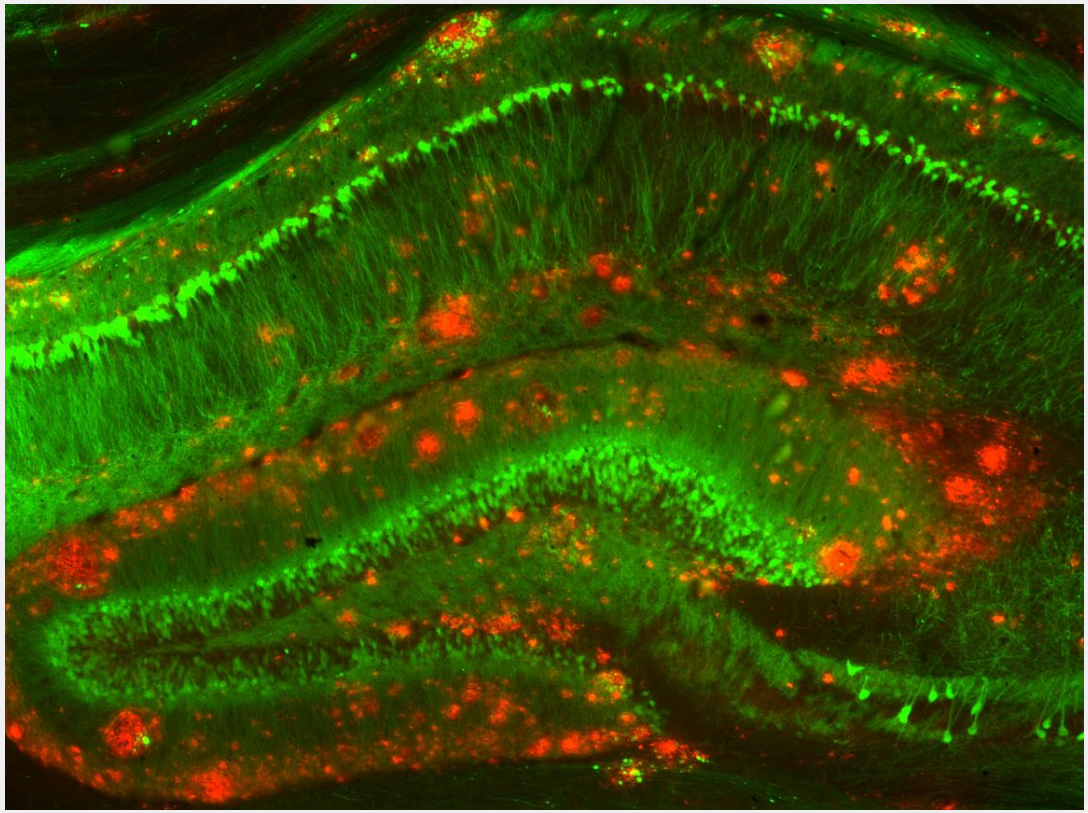
Group	Amino acids
1	C, I, L, K, M, F, P, W, Y, V
2	A, D, E, G, H, N, Q, R, S, T

Peptide I: FKVWPDHGSG → 1111122222
Peptide II: YMCIYRAQTN → 1111122222

Amyloid prediction

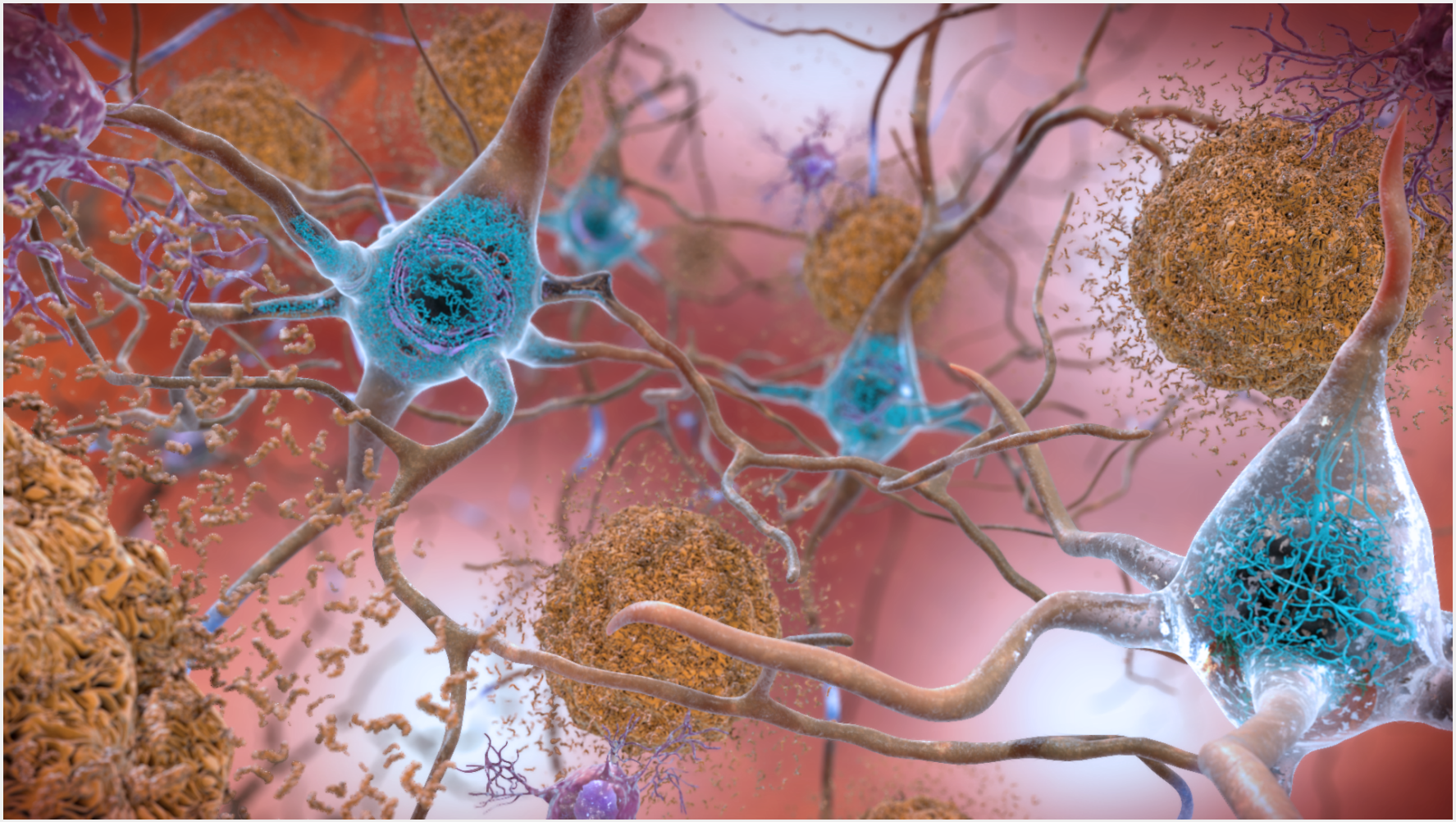
Amyloids

Amyloid aggregates are found in tissues of people suffering from neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and many other diseases.



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

Amyloids

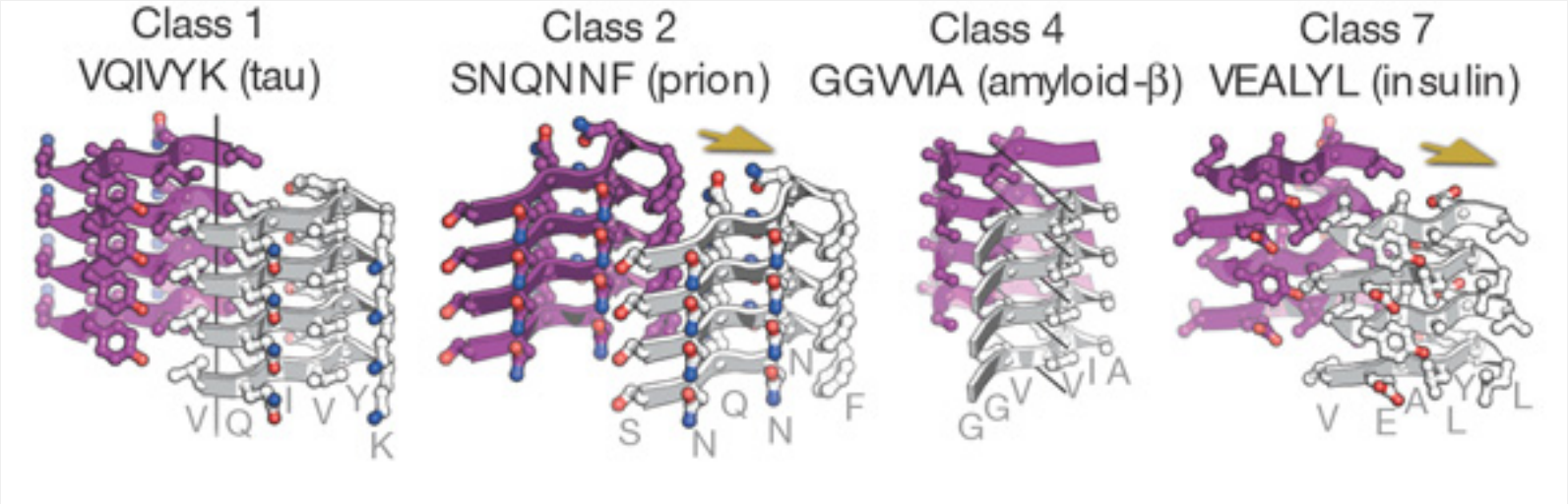


Source: National Institute on Aging (NIA) | National Institutes of Health (NIH)

Amyloid proteins

Peptide sequences with amyloidogenic properties are responsible for the aggregation of amyloidogenic proteins (hot spots):

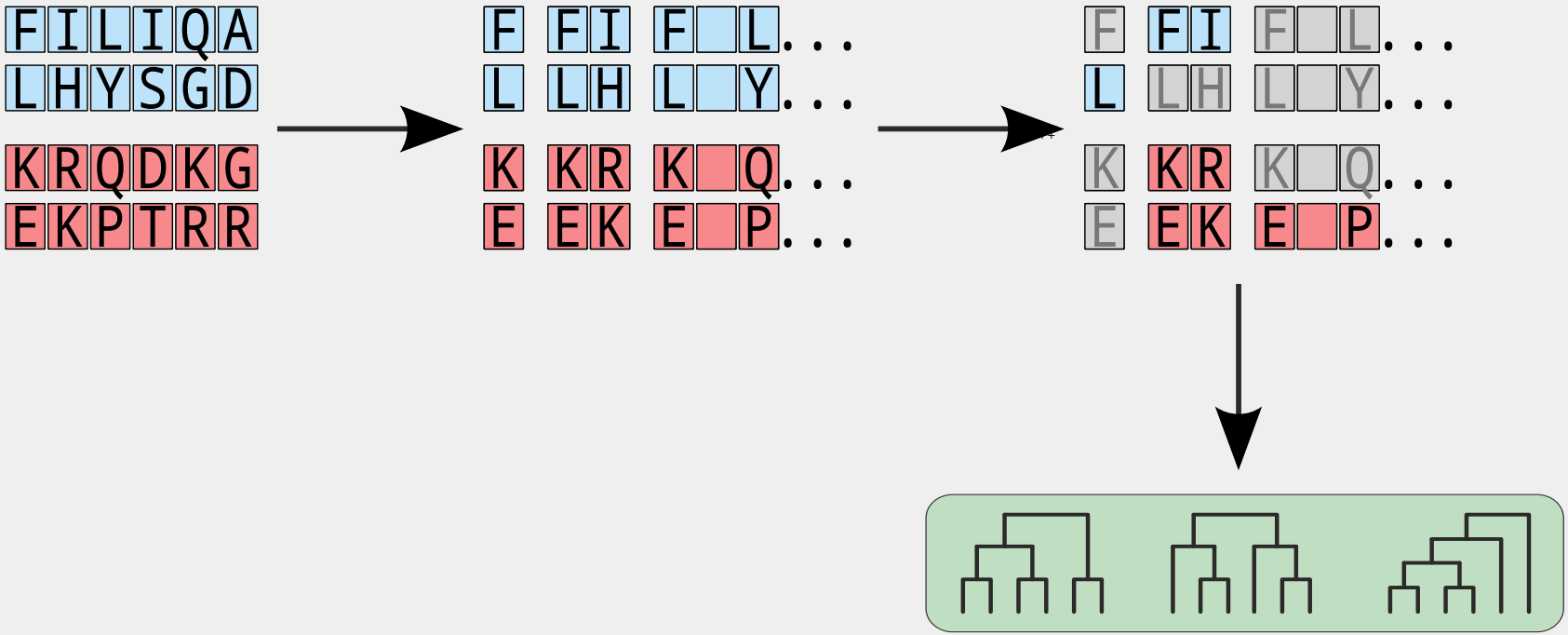
- short (6-15 amino acids),
- very variable, usually hydrophobic amino acid composition,
- create unique β-structures.



Sawaya et al. (2007)

AmyloGram

AmyloGram: n-gram-based amyloid prediction tool (Burdukiewicz et al., 2016, 2017).



Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

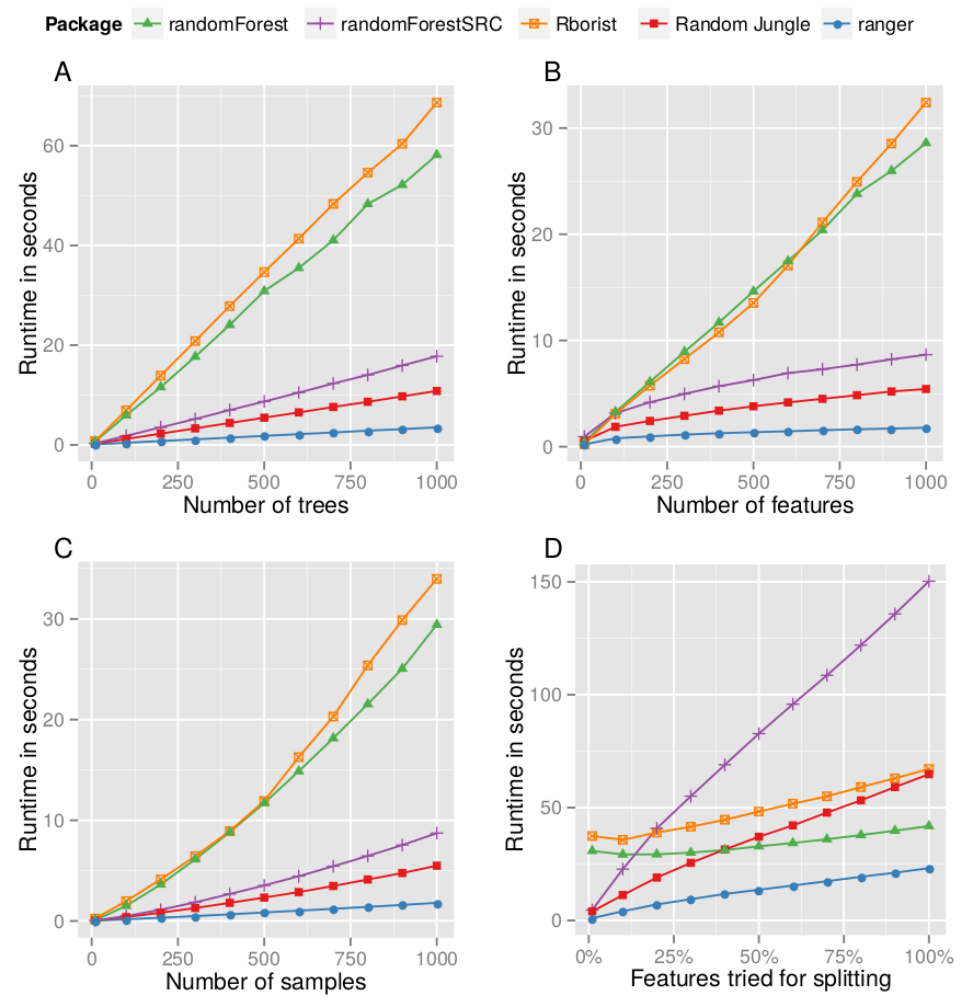
ranger: A Fast Implementation of Random Forests

Package	Runtime [h]			Memory usage [GB]
	mtry=			
	5000	15,000	135,000	
randomForest	101.24	116.15	248.60	39.05
randomForest (MC)	32.10	53.84	110.85	105.77
bigrf	NA	NA	NA	NA
randomForestSRC	1.27	3.16	14.55	46.82
Random Jungle	1.51	3.60	12.83	0.40
Rborist	NA	NA	NA	>128
ranger	0.56	1.05	4.58	11.26
ranger (save.memory)	0.93	2.39	11.15	0.24
ranger (GWAS mode)	0.23	0.51	2.32	0.23

Runtime and memory usage for the analysis of a simulated dataset mimicking a genome-wide association study (GWAS). NA values indicate unsuccessful analyses:
without disk caching failed because of memory shortage for all mtry values and number of CPU cores.
With disk caching, we stopped bigrf after 16 days of computation.

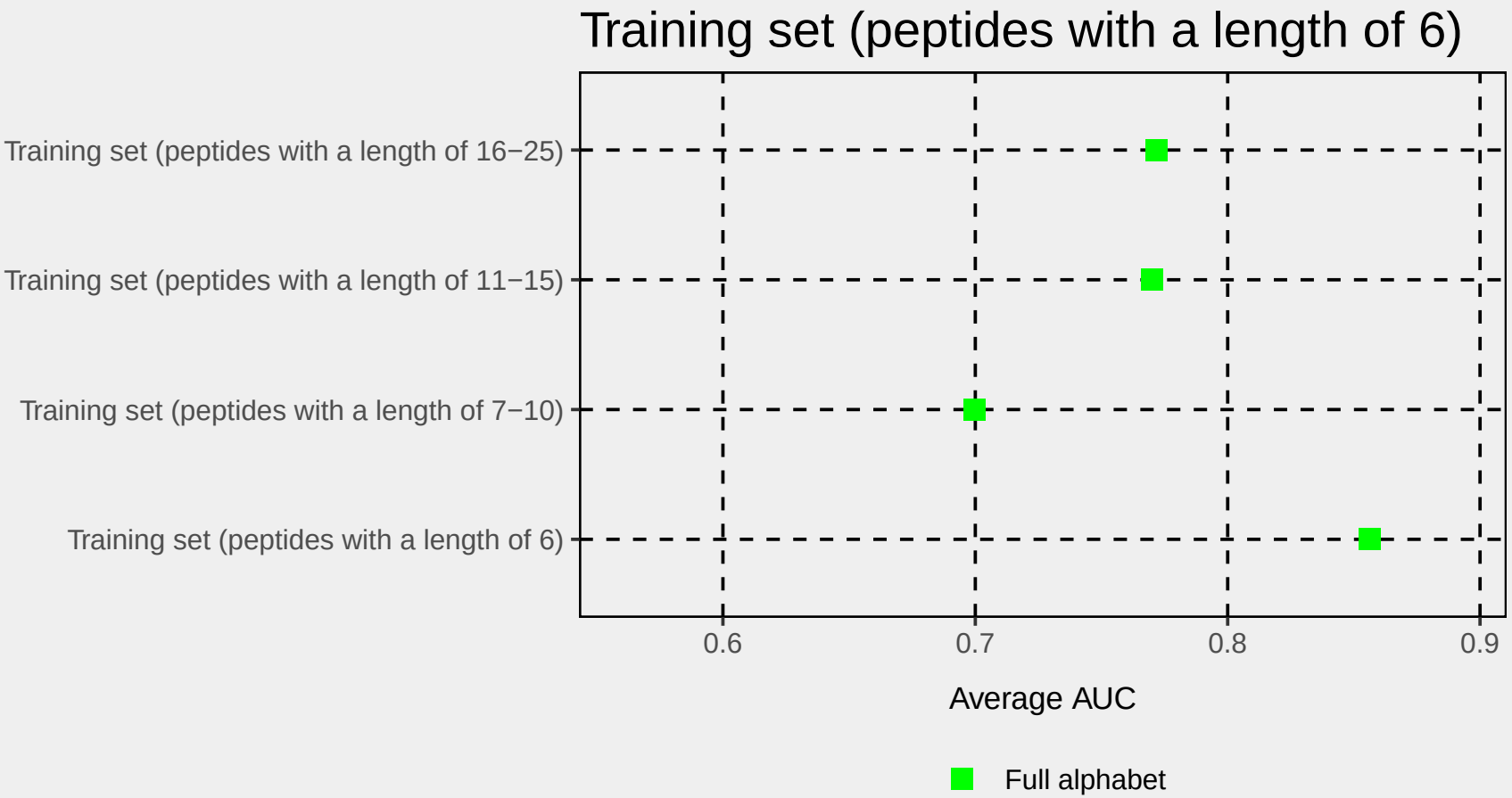
Marvin N. Wright and Andreas Ziegler. (2017). ranger: A Fast Implementation of Random Forests for High Dimensional Data in C++ and R. Journal of Statistical Software 1, 77

ranger: A Fast Implementation of Random Forests



Marvin N. Wright and Andreas Ziegler. (2017). ranger: A Fast Implementation of Random Forests for High Dimensional Data in C++ and R. Journal of Statistical Software 1, 77

Cross-validation

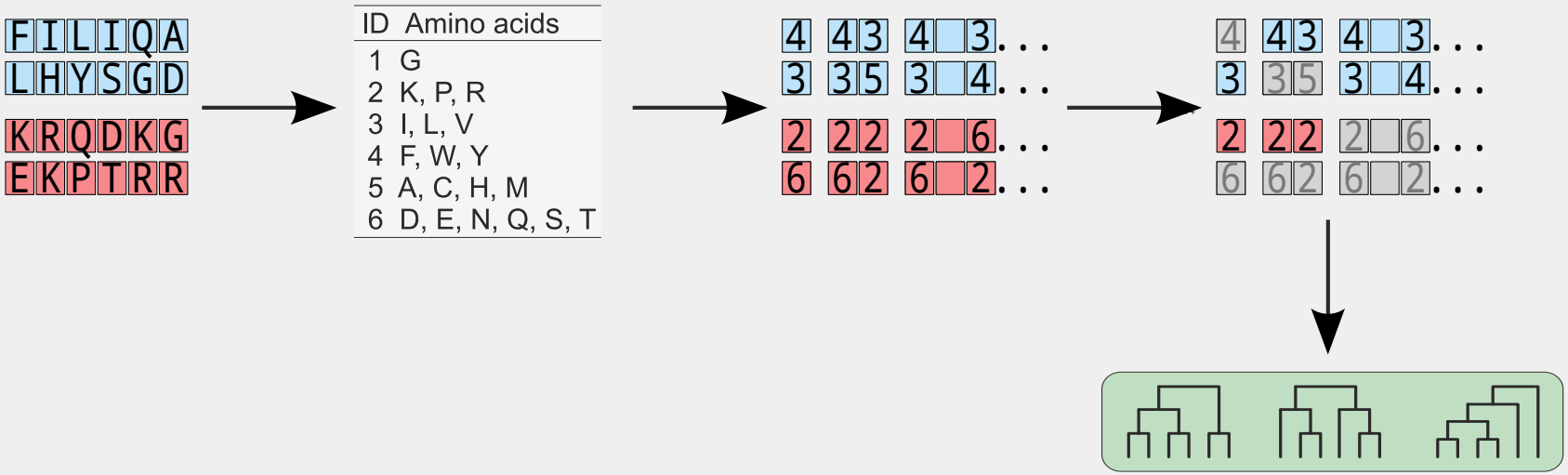


Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Standard reduced alphabets

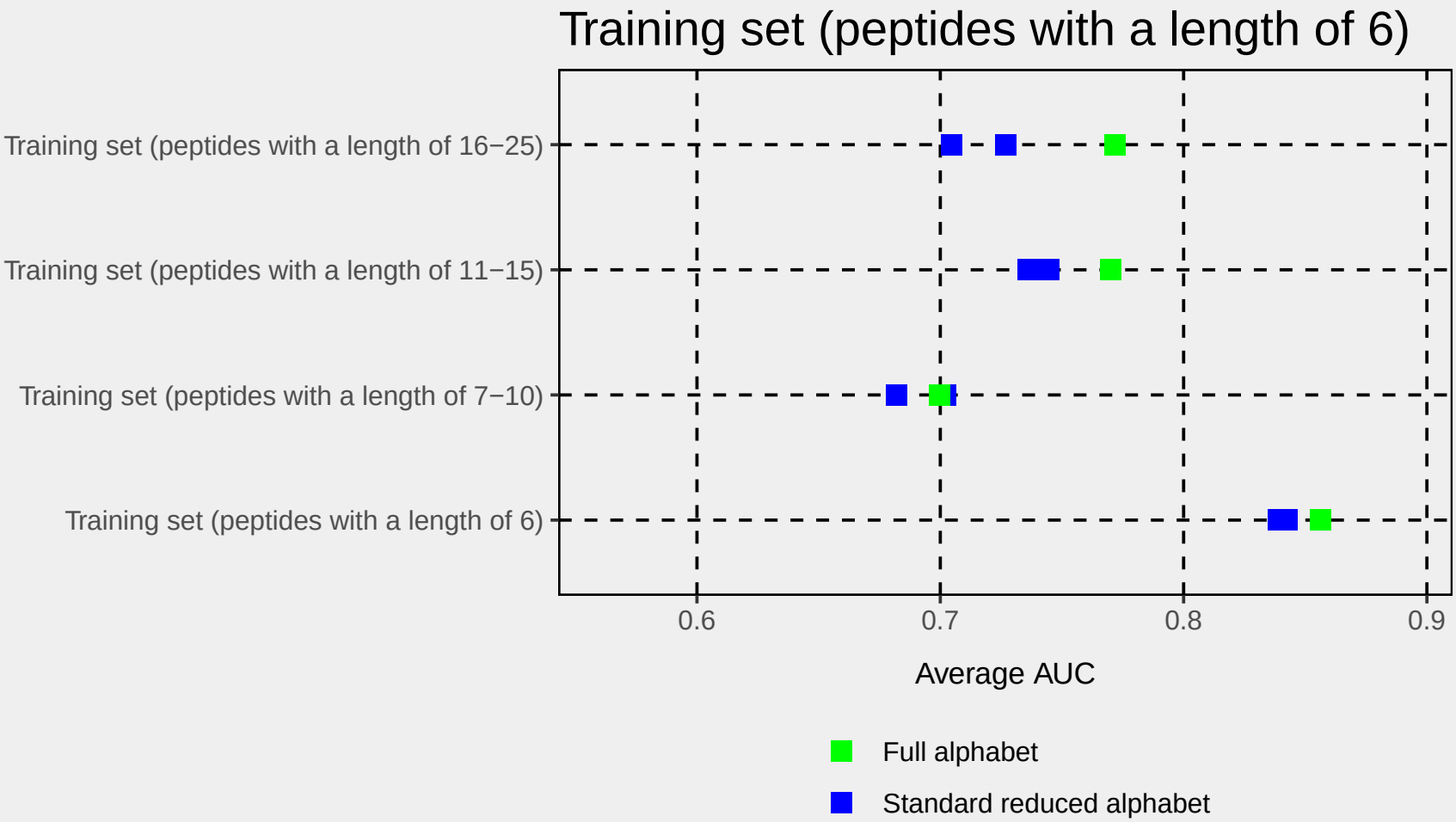
Do standard reduced alphabets developed for different biological issues help to improve amyloid prediction?

Standard reduced alphabets



Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Standard reduced alphabet



Standard amino acid alphabets do not improve the quality of amyloid prediction.

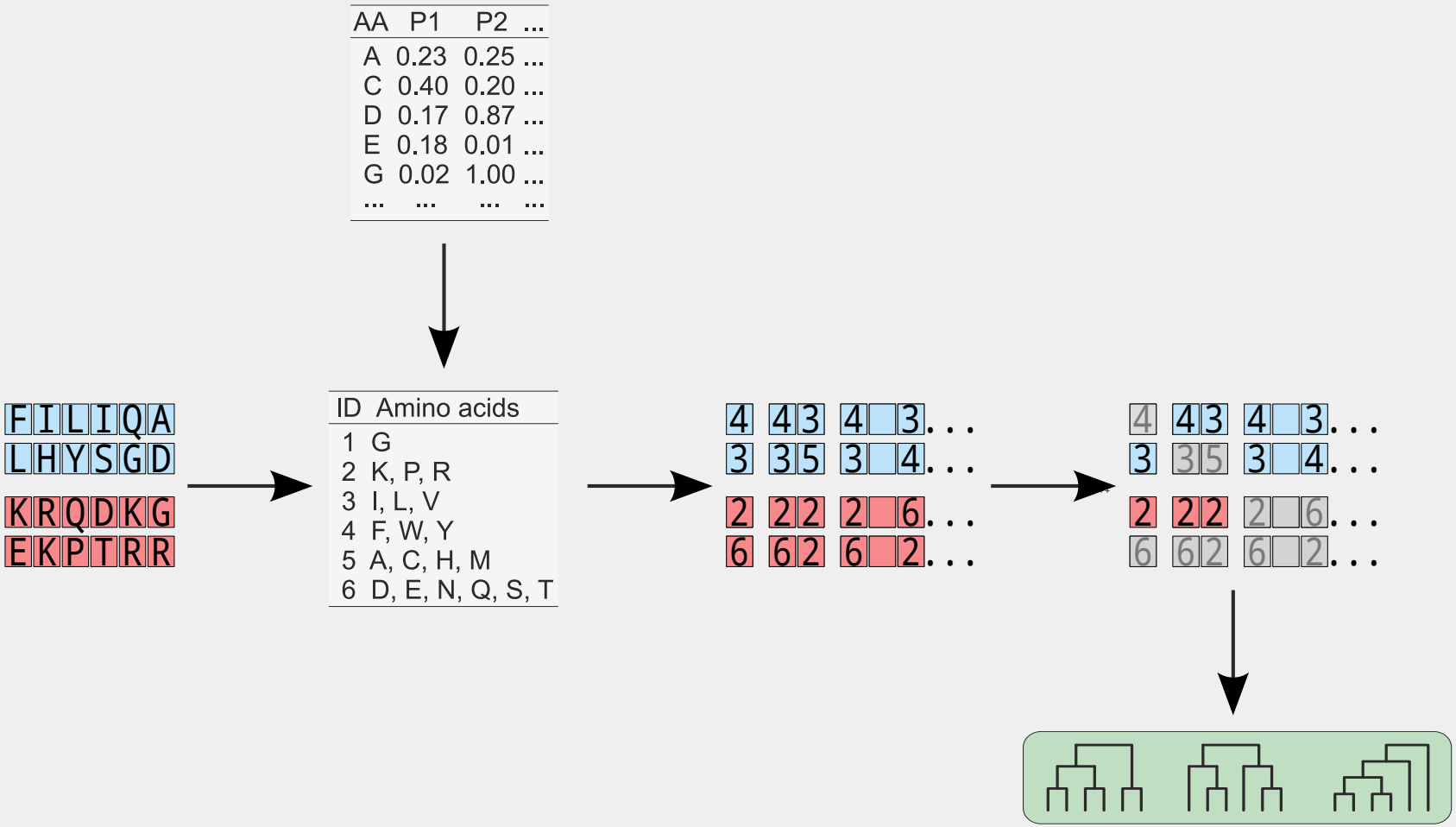
Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

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).

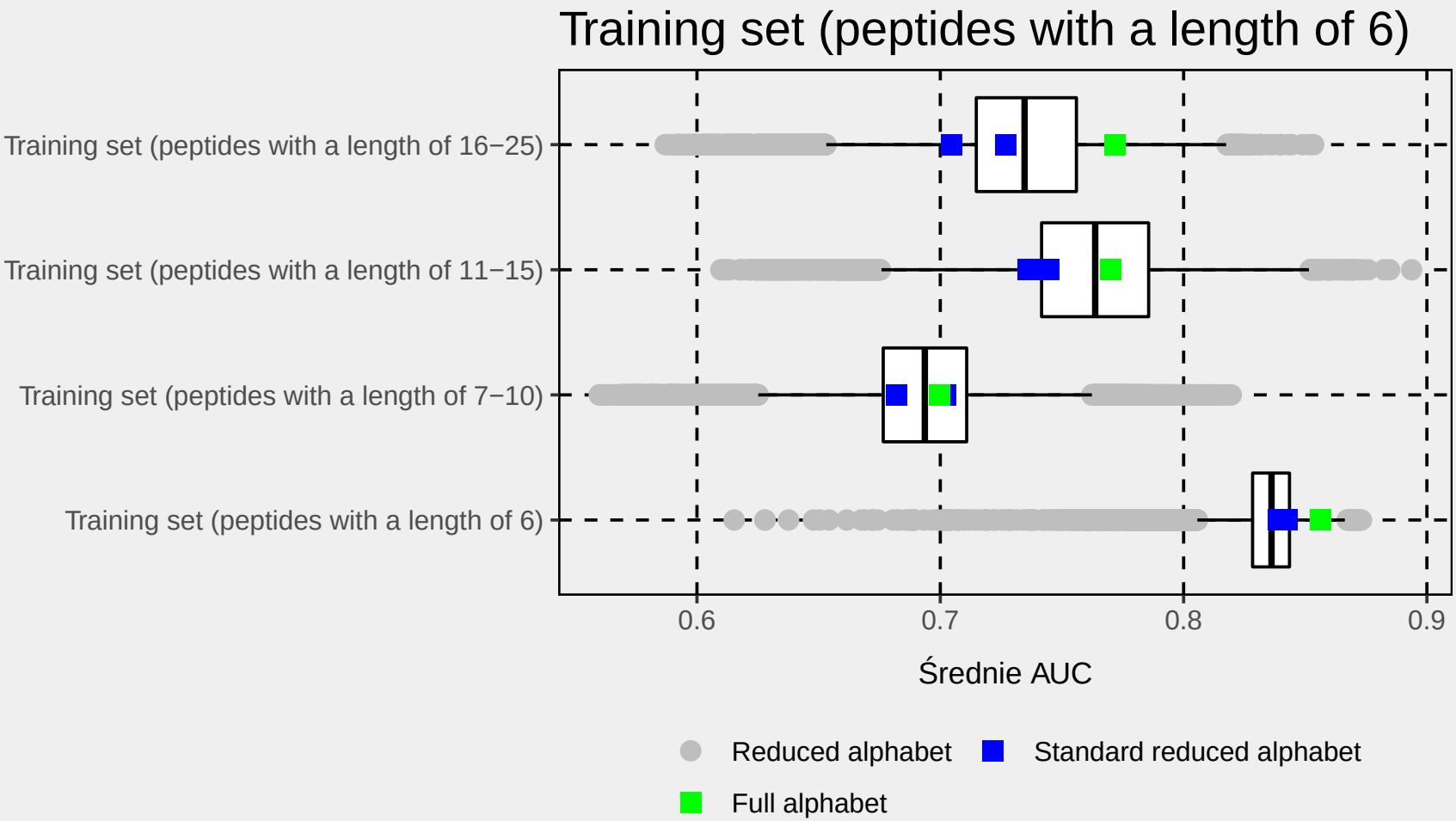
Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Novel reduced amino acid alphabets



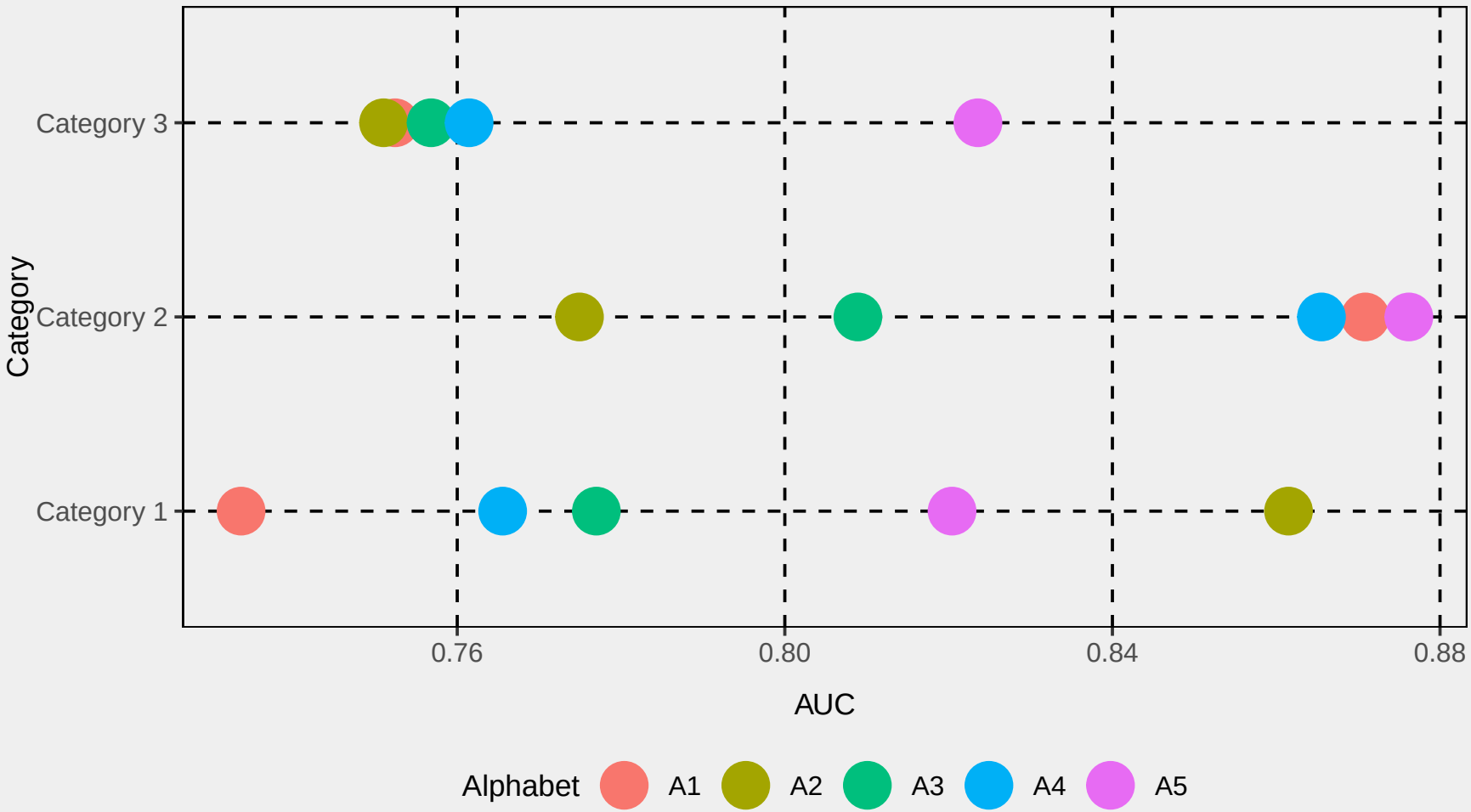
Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Standard reduced alphabet

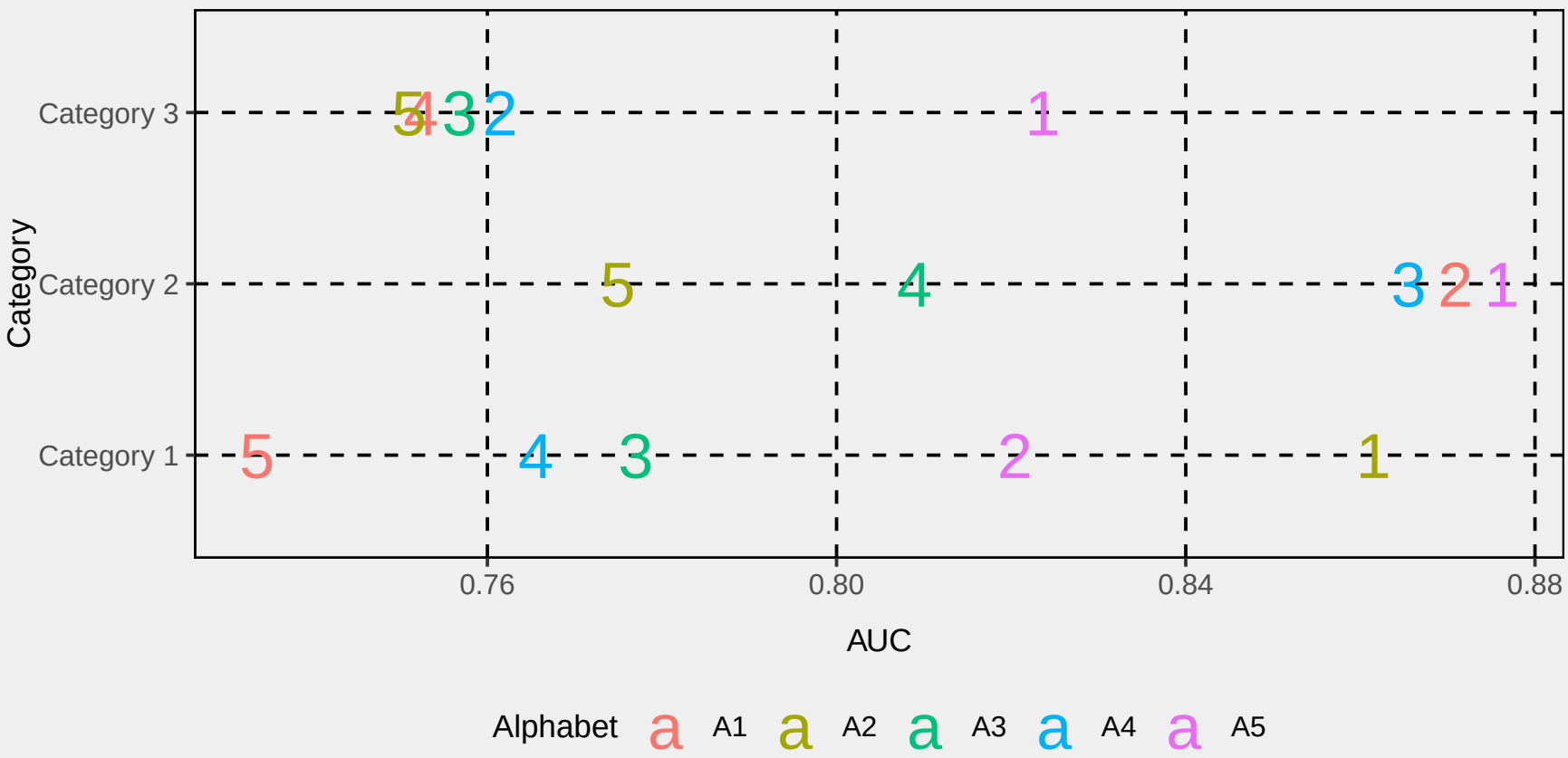


Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Selection of best-performing reduced alphabet

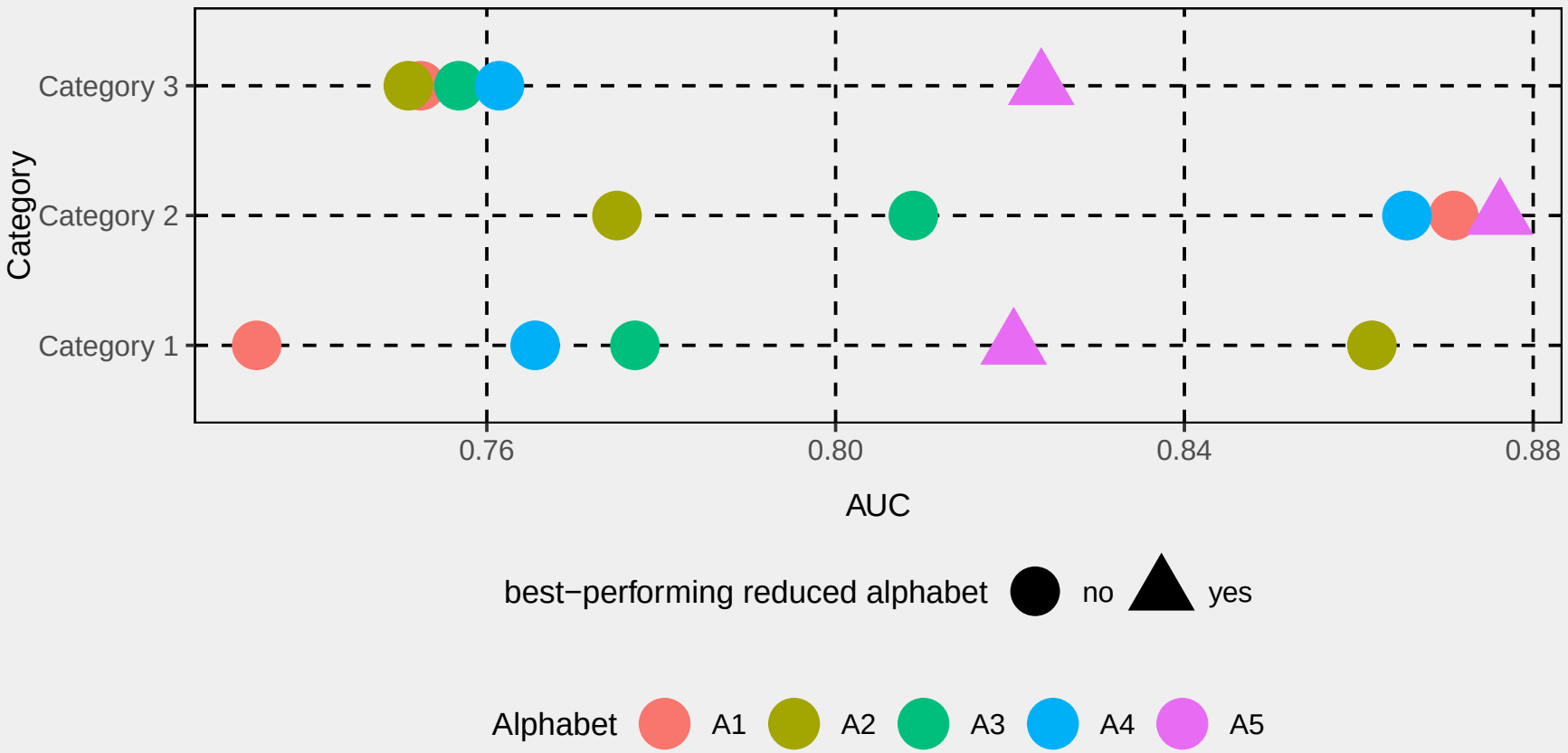


Selection of best-performing reduced alphabet



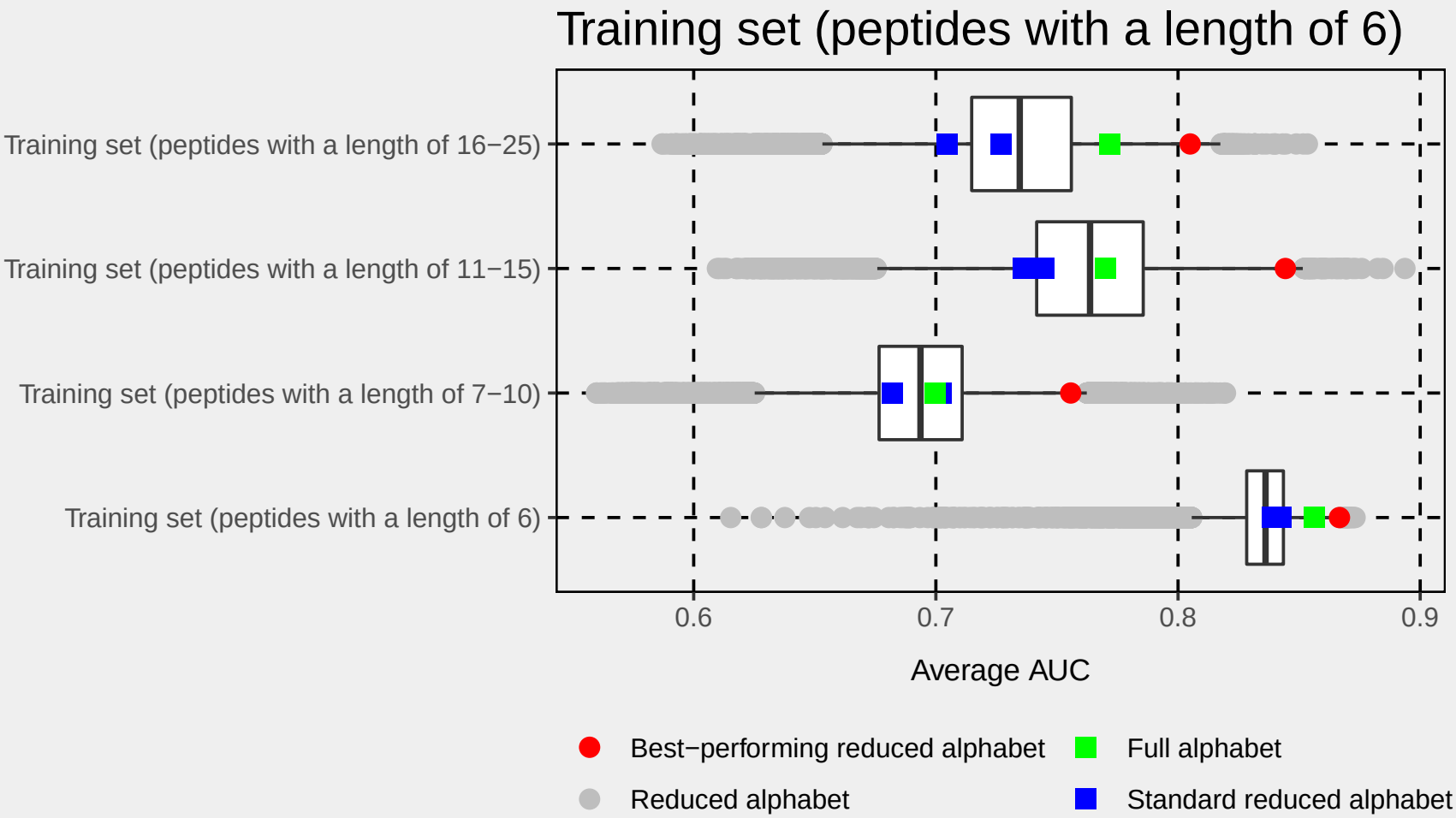
For each category the alphabets have been ranked (rank 1 for the best AUC, etc.).

Selection of best-performing reduced alphabet



The best alphabet was the one with the lowest rank sum.

Best-performing reduced alphabet



Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Best-performing reduced alphabet

Group	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

Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Best-performing reduced alphabet

Group	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

Grupy 3 i 4 - hydrophobic amino acids.

Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Best-performing reduced alphabet

Group	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

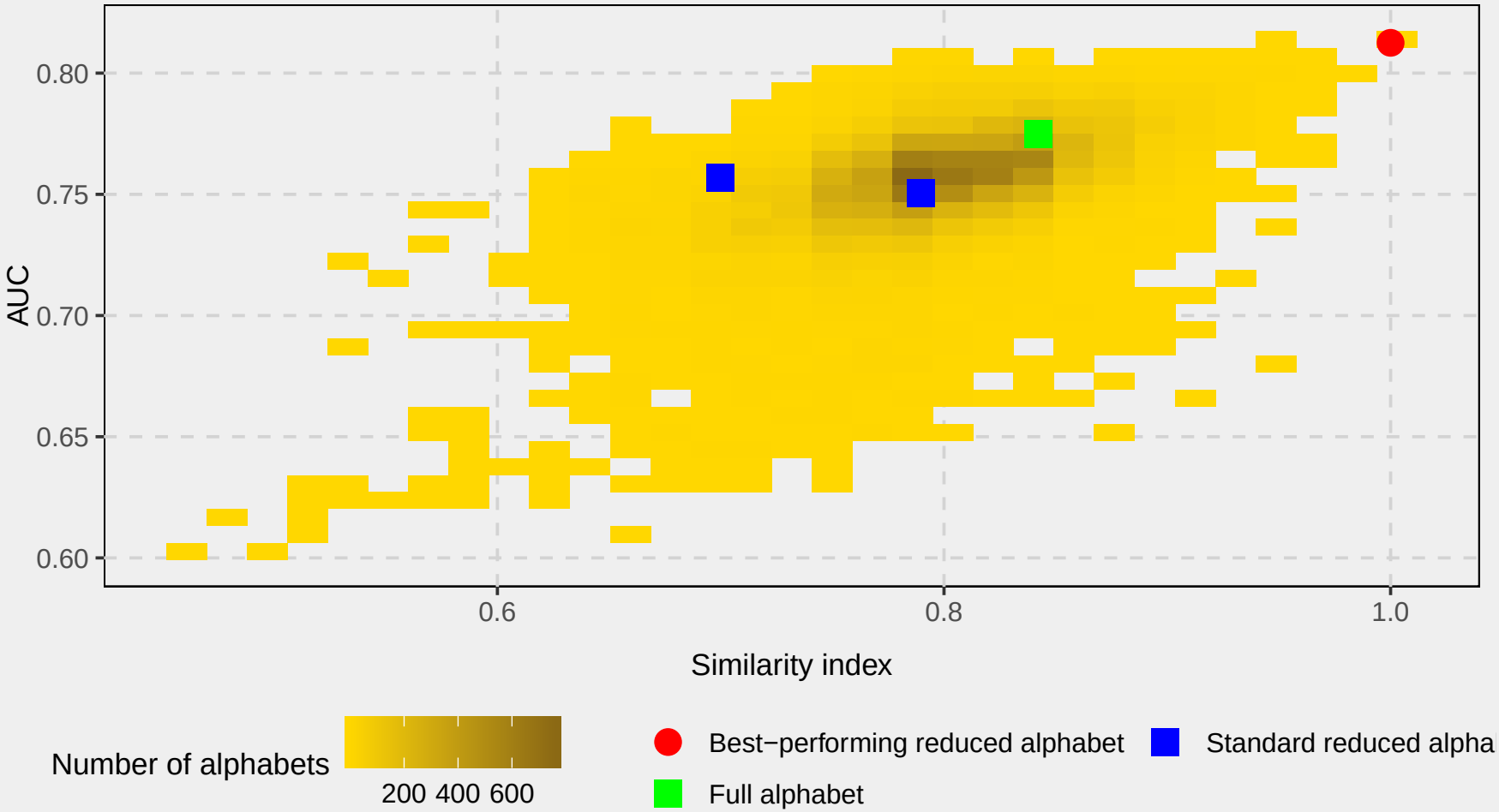
Group 2 - amino acids disrupting the β-structure (β-breakers).

Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Alphabet similarity and quality of prediction

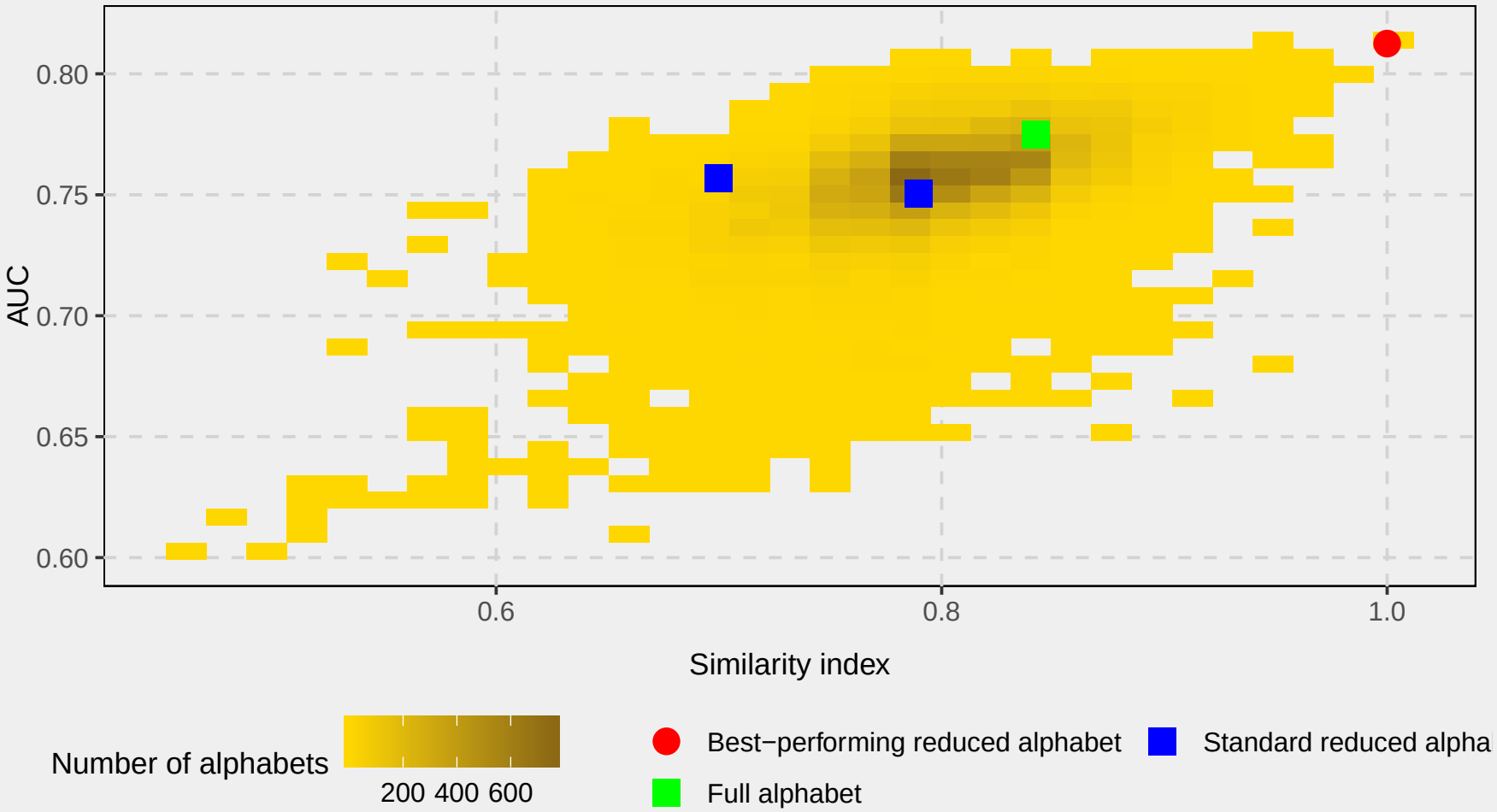
Is the best-performing reduced amino acid alphabet associated with amyloidogenicity?

Similarity index



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

Similarity index

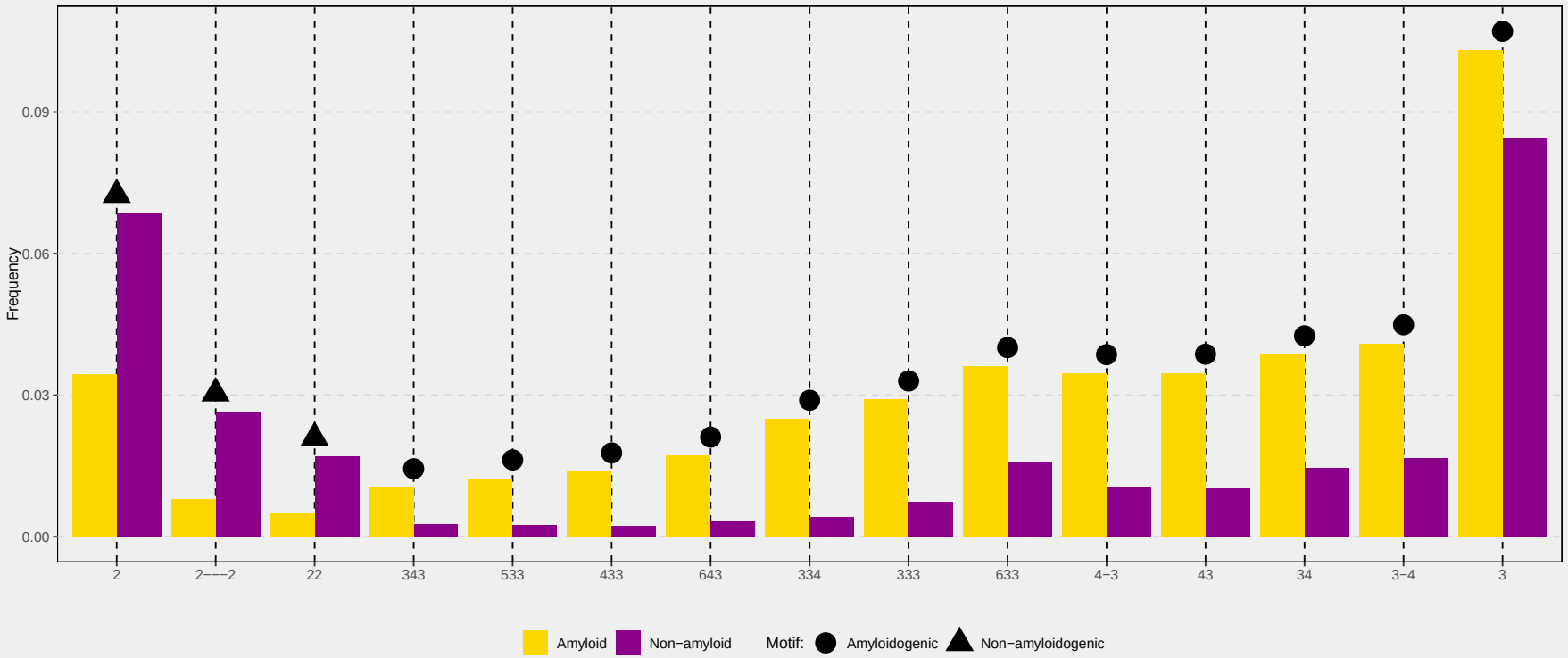


The correlation between the similarity index and the average AUC is important ($\text{p-value} \leq 2.2^{-16}$; $\rho = 0.51$).

Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

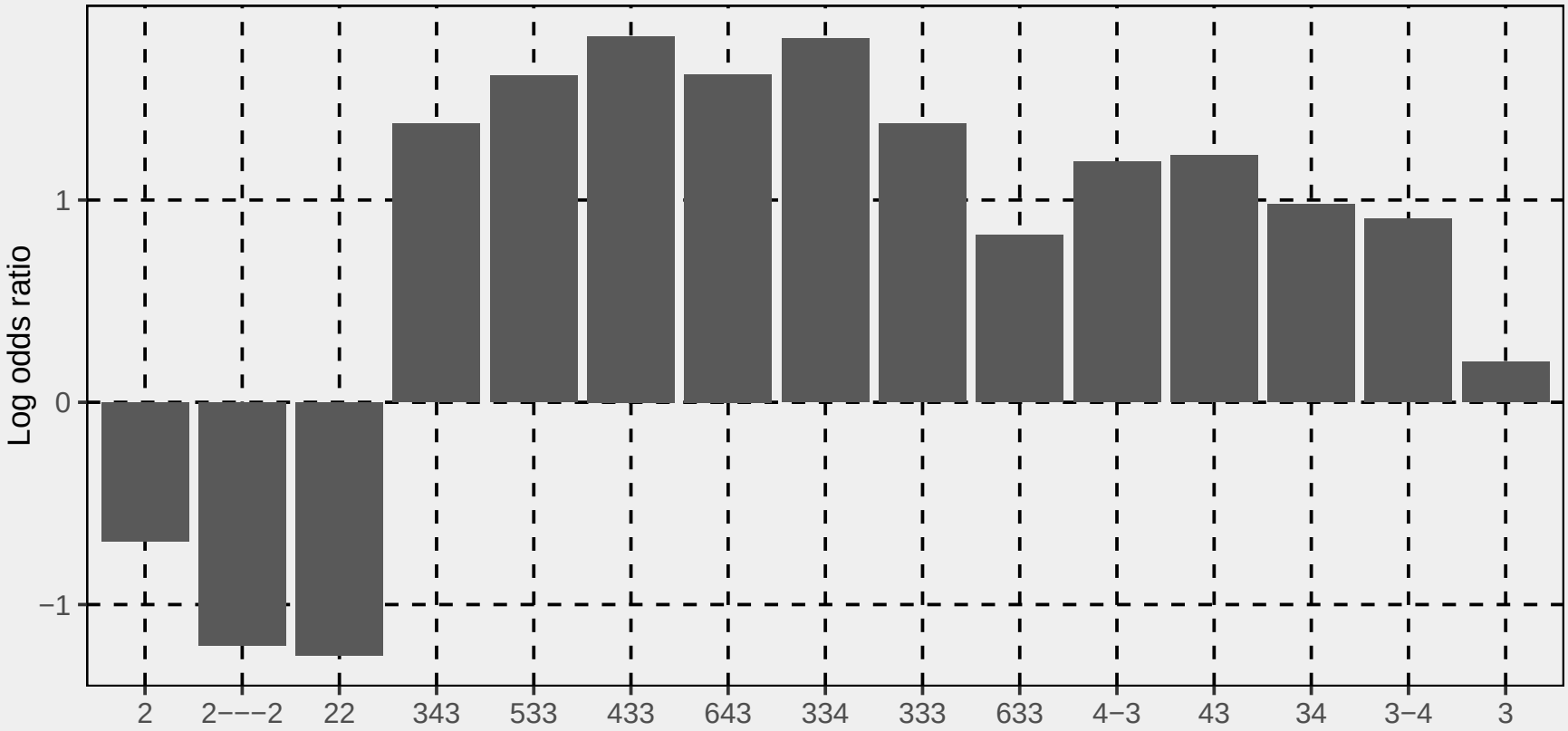
Are informative n-grams found by QuiPT associated with amyloidogenicity?

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).

Informative n-grams



Of the 65 most informative n-grams, 15 (23%) are also present in amino acid motifs found experimentally (Paz and Serrano, 2004).

Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

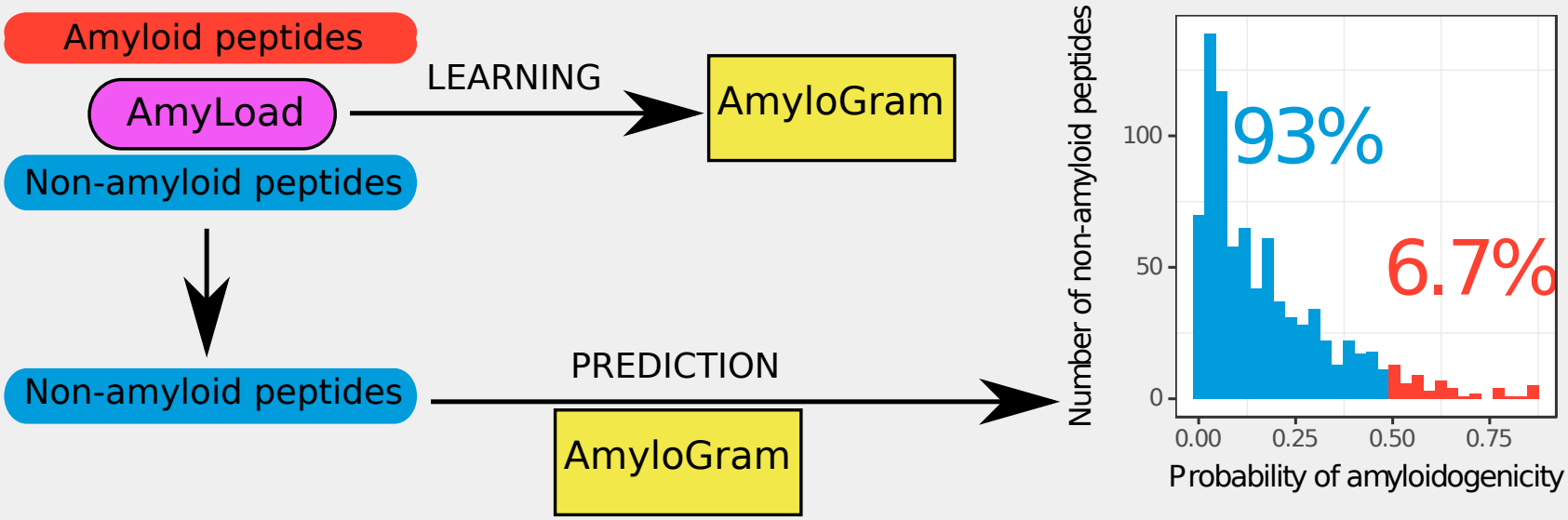
Benchmark results

Program	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

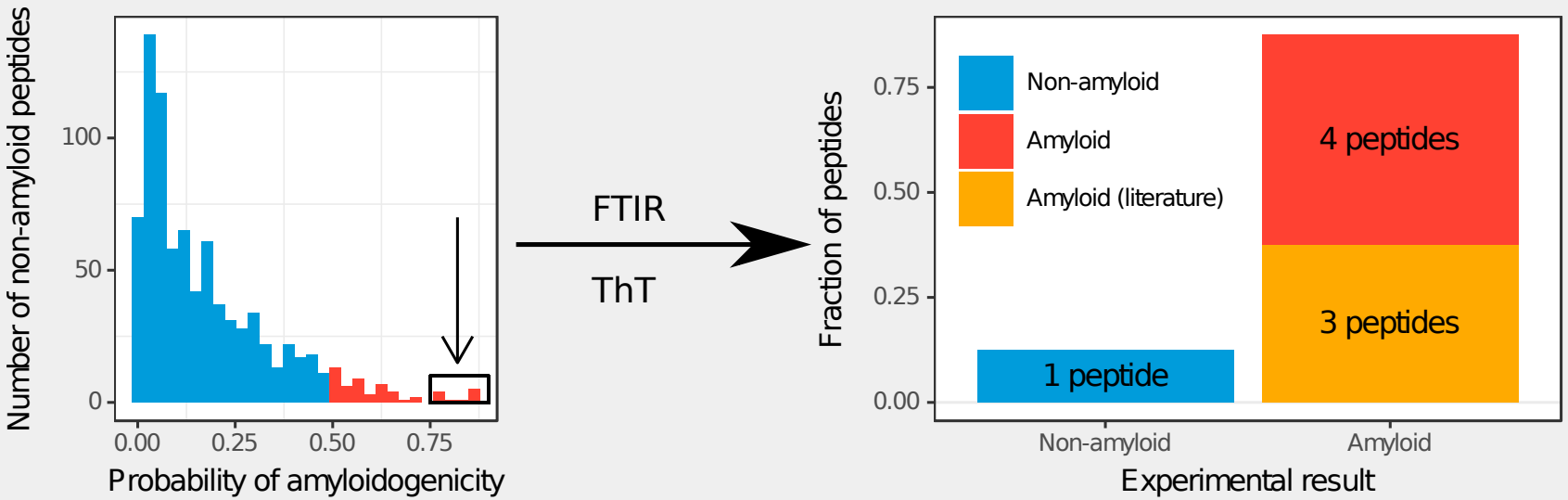
The classifier trained using the best reduced alphabet, AmyloGram, has been compared with other amyloid prediction tools using an external dataset pep424.

Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

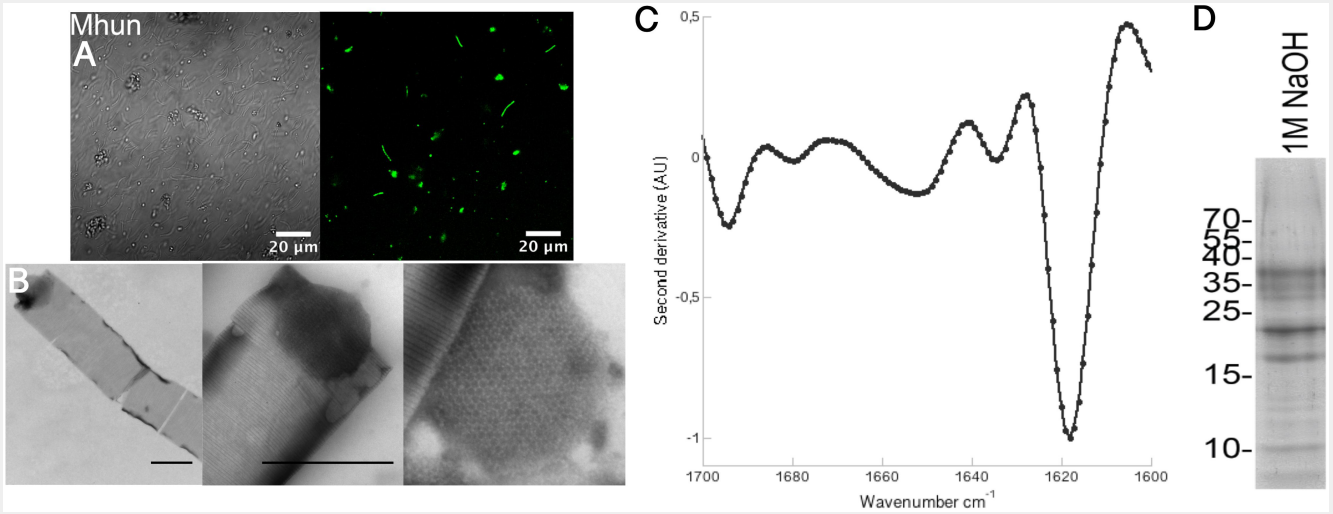
Experimental validation



Experimental validation



New amyloid



A new functional amyloid produced by Methanospirillum sp. (Christensen et al., 2018) was selected for in vitro analysis by AmyloGram.

Shiny web server

AmyloGram web server

AmyloGram

AmyloGram predicts amyloidogenic sequences (hot spots) in eukaryotic proteins using random forests and n-gram analysis.

Restrictions:

- Be patient - calculations can take up to few minutes.
- Up to 50 sequences may be analyzed at the same time using web server. If you need larger query, please use the AmyloGram package for **R**.

Authors: Michal Burdukiewicz, Piotr Sobczyk.

Citation: Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961.

Exemplary sequences

```
>AMY133|Alpha 1|N-terminal domain of Ribosomal prot
GYANNFLFKQG
>Ada-2h
VPSNEEQIKNLLQLEAQEHLQY
>AMY138|Alpha 6|Glutathione S Transeferase P domain
QISFADYNLLDLLRIHQVLN
>AMY143|M8|Spectrin SH3
DILTLLNSTNKDWWKVEVND
>CsgA|region 1
SELNIYQYGGGNSALALQTDARN
```

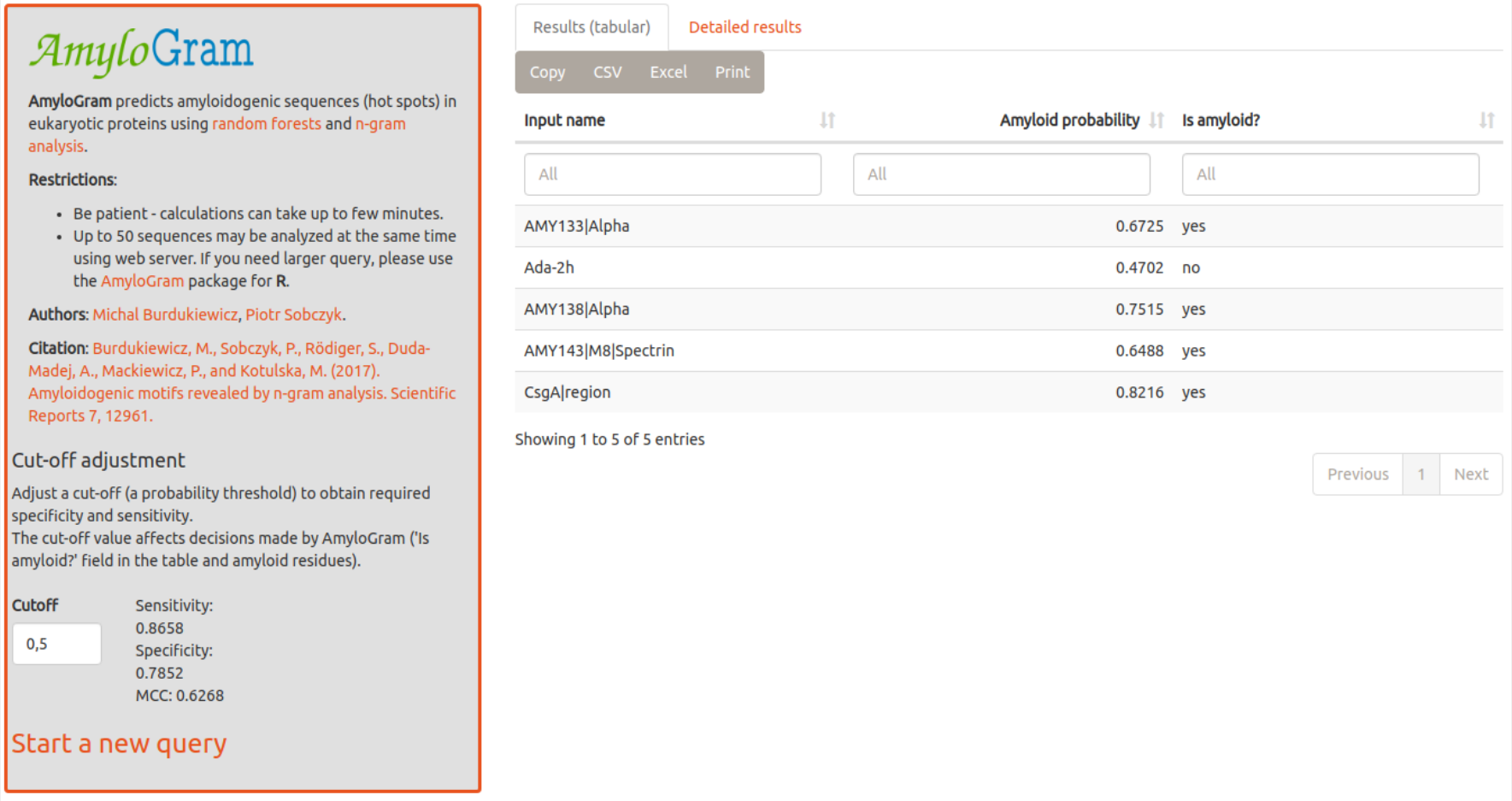
Paste sequences (FASTA format required) here...

Submit data from field above

Submit .fasta or .txt file:

Browse... No file selected

AmyloGram web server



AmyloGram web server

AmyloGram

AmyloGram predicts amyloidogenic sequences (hot spots) in eukaryotic proteins using random forests and n-gram analysis.

Restrictions:

- Be patient - calculations can take up to few minutes.
- Up to 50 sequences may be analyzed at the same time using web server. If you need larger query, please use the AmyloGram package for **R**.

Authors: Michal Burdukiewicz, Piotr Sobczyk.

Citation: Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961.

Cut-off adjustment

Adjust a cut-off (a probability threshold) to obtain required specificity and sensitivity.
The cut-off value affects decisions made by AmyloGram ('Is amyloid?' field in the table and amyloid residues).

Cutoff 0,5

Sensitivity: 0.8658
Specificity: 0.7852
MCC: 0.6268

Start a new query

Results (tabular) | Detailed results

Amyloid residues

Residues are defined as belonging to the amyloid part of a protein, if their amyloid probability is higher than the cut-off

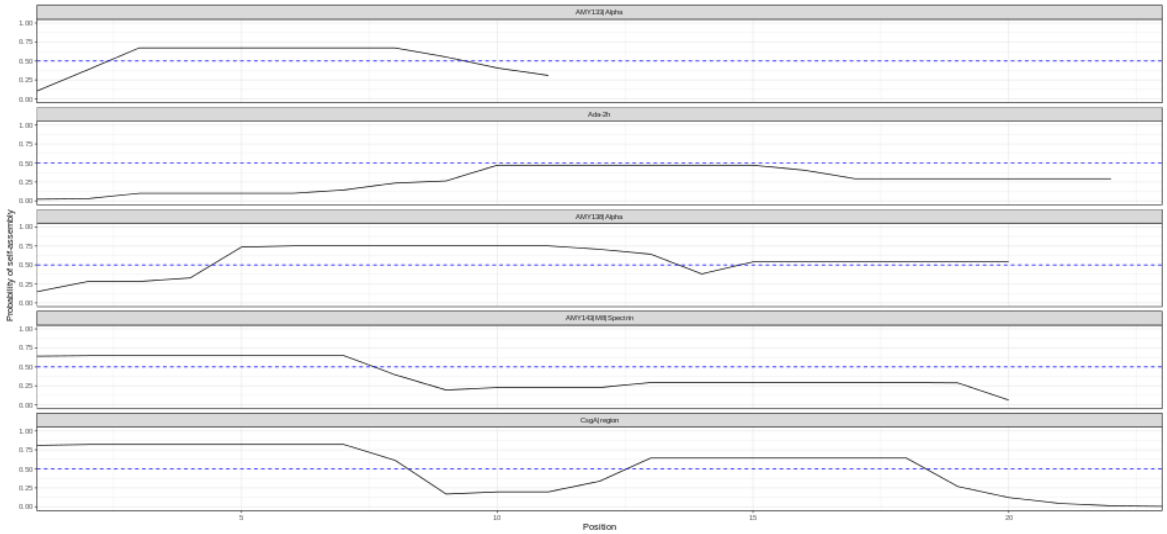
Copy CSV Excel Print

Protein	Fraction of amyloid residues
AMY133|Alpha	0.6364
Ada-2h	0.0000
AMY138|Alpha	0.7500
AMY143|M8|Spectrin	0.3500
CsgA|region	0.6087

Showing 1 to 5 of 5 entries

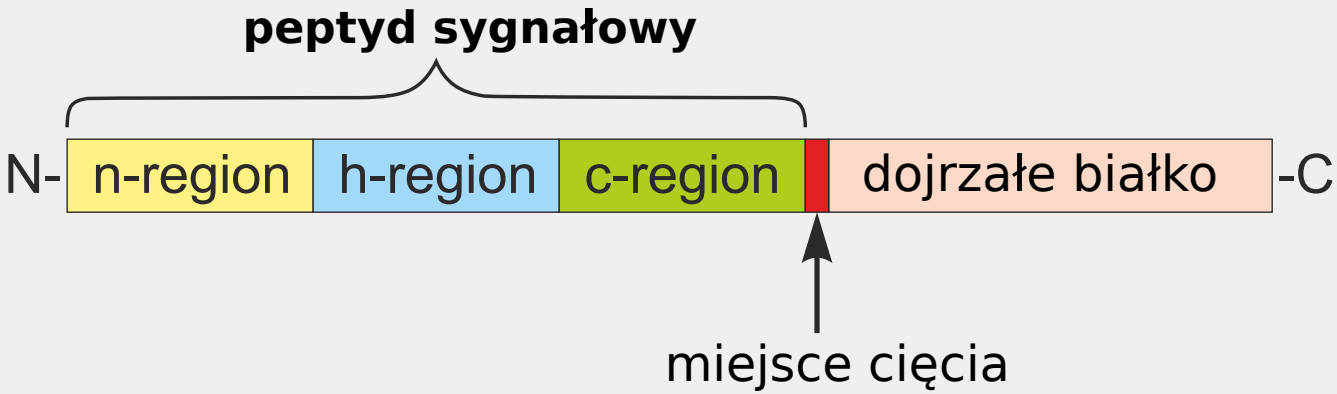
Previous 1 Next

Amyloid regions



Other applications

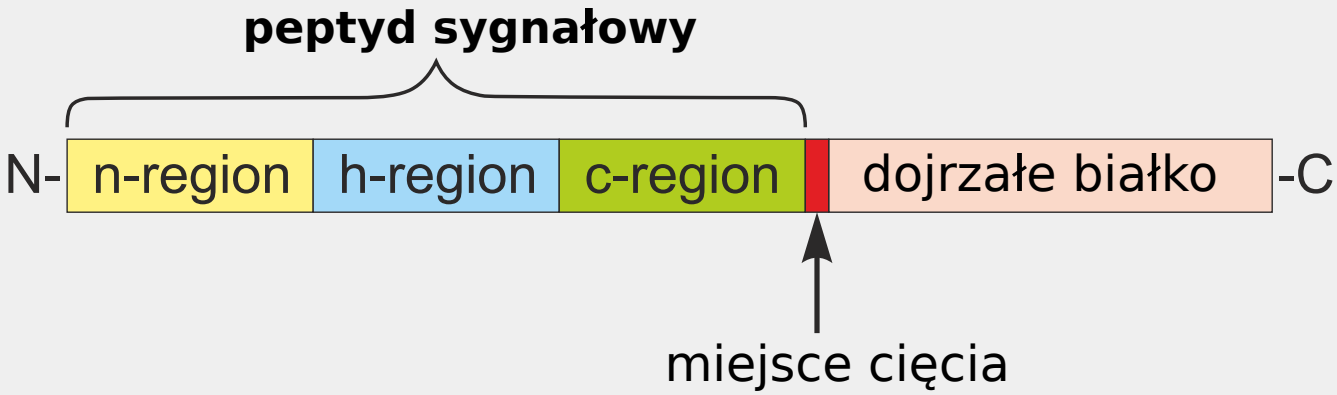
Signal peptides



Signal peptides (Hegde and Bernstein, 2006):

- short (20-30 residuals) N-terminal protein fragments forming α-helices,

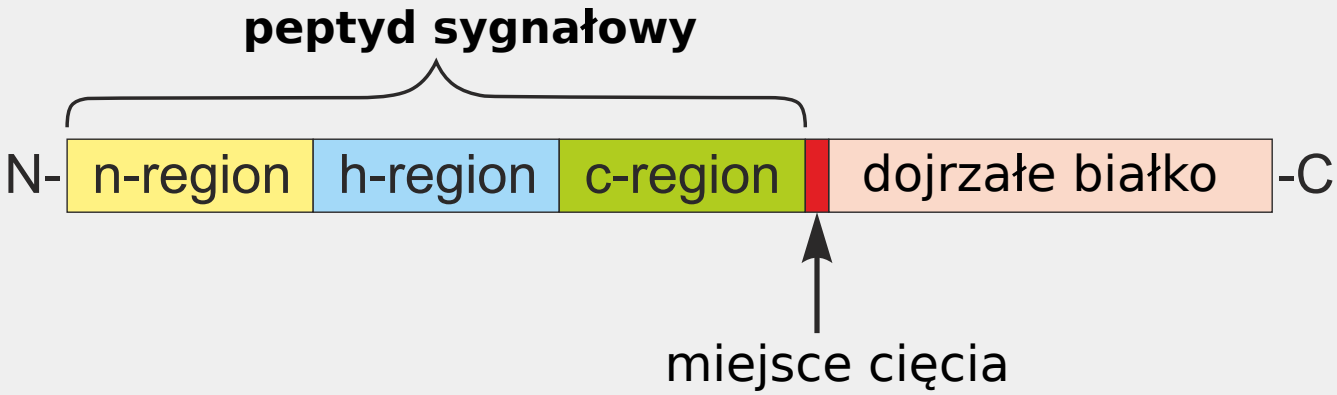
Signal peptides



Signal peptides (Hegde and Bernstein, 2006):

- short (20-30 residuals) N-terminal protein fragments forming α-helices,
- they direct proteins to the intracellular matrix and then for secretion or cell compartiments,

Signal peptides

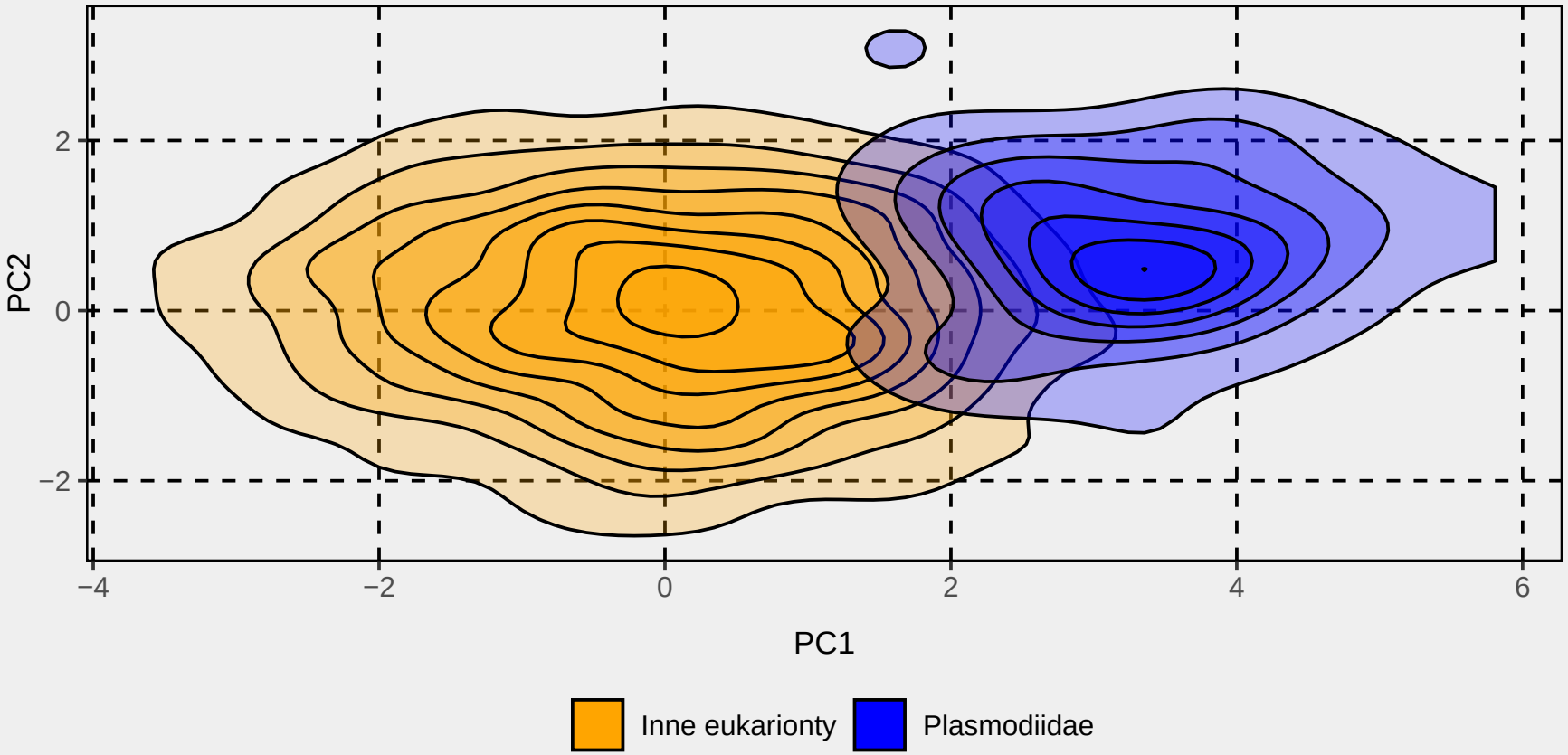


Signal peptides (Hegde and Bernstein, 2006):

- short (20-30 residuals) N-terminal protein fragments forming α-helices,
- they direct proteins to the intracellular matrix and then for secretion or cell compartiments,
- very variable, but always containing three characteristic domains.

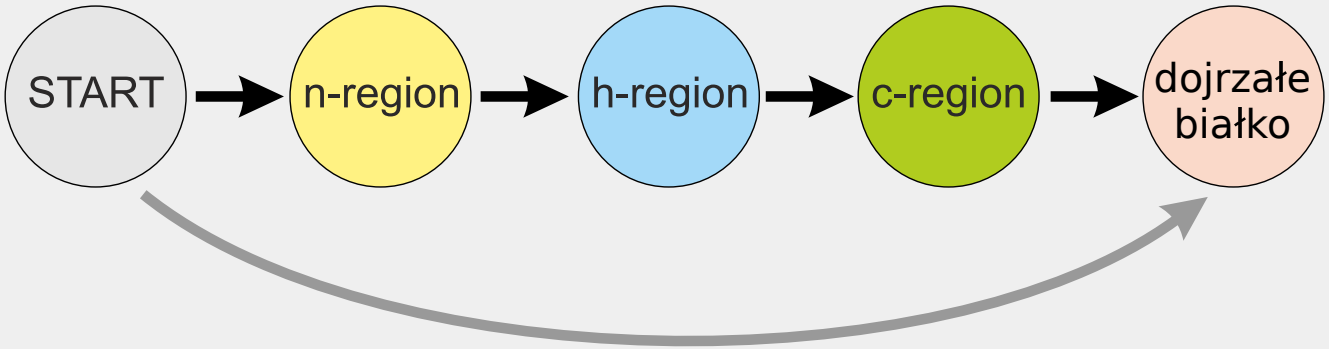
Signal peptides

The amino acid composition of signal peptides in Plasmodium sp. (ex. Plasmodium malariae, wchich causes malaria) is different from that of the signal peptides of well known eukaryotes.



PCA amino acid frequency.

signalHsmm (Burdukiewicz et al., 2018): use of hidden semi-Mark models and reduced amino acid alphabets to predict signal peptides in Plasmodium sp. proteins.



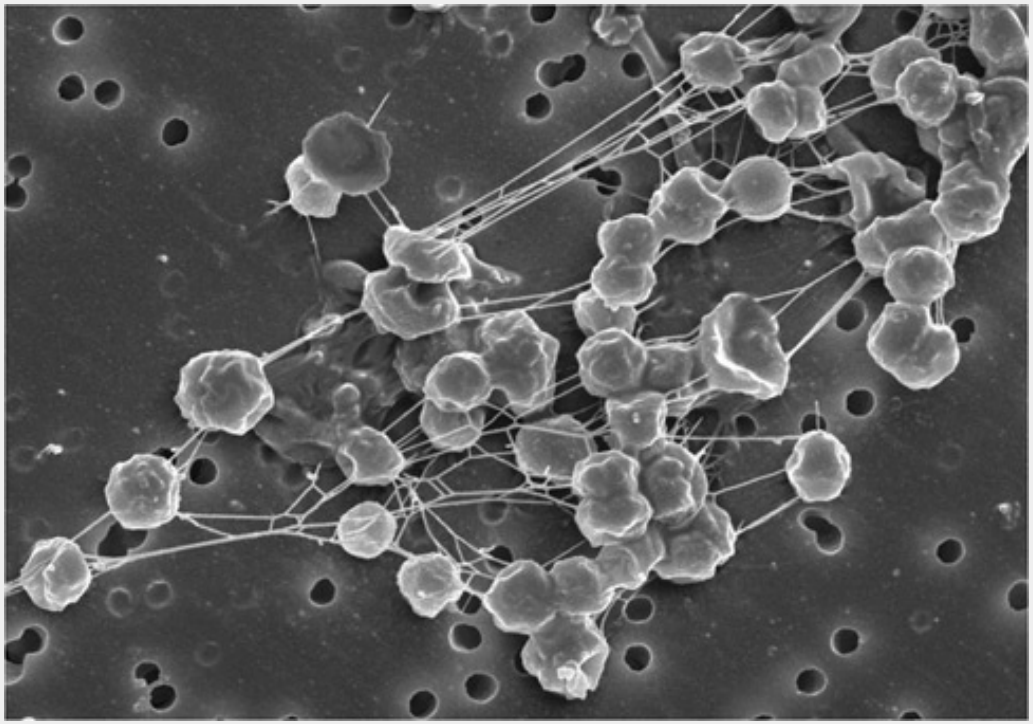
Benchmark with other predictors

Algorithm	Sensitivity	Specificity	MCC	AUC
signalP 4.1 (no tm)	0.8235	0.9100	0.6872	0.8667
signalP 4.1 (tm)	0.6471	0.9431	0.6196	0.7951
signalP 3.0 (NN)	0.8824	0.9052	0.7220	0.8938
signalP 3.0 (HMM)	0.6275	0.9194	0.5553	0.7734
PrediSi	0.3333	0.9573	0.3849	0.6453
Philius	0.6078	0.9336	0.5684	0.7707
Phobius	0.6471	0.9289	0.5895	0.7880
signalHsmm-2010	0.9804	0.8720	0.7409	0.9262

Burdukiewicz, M., Sobczyk, P., Chilimoniuk, J., Gagat, P., and Mackiewicz, P. (2018). Prediction of Signal Peptides in Proteins from Malaria Parasites. International Journal of Molecular Sciences 19, 3709.

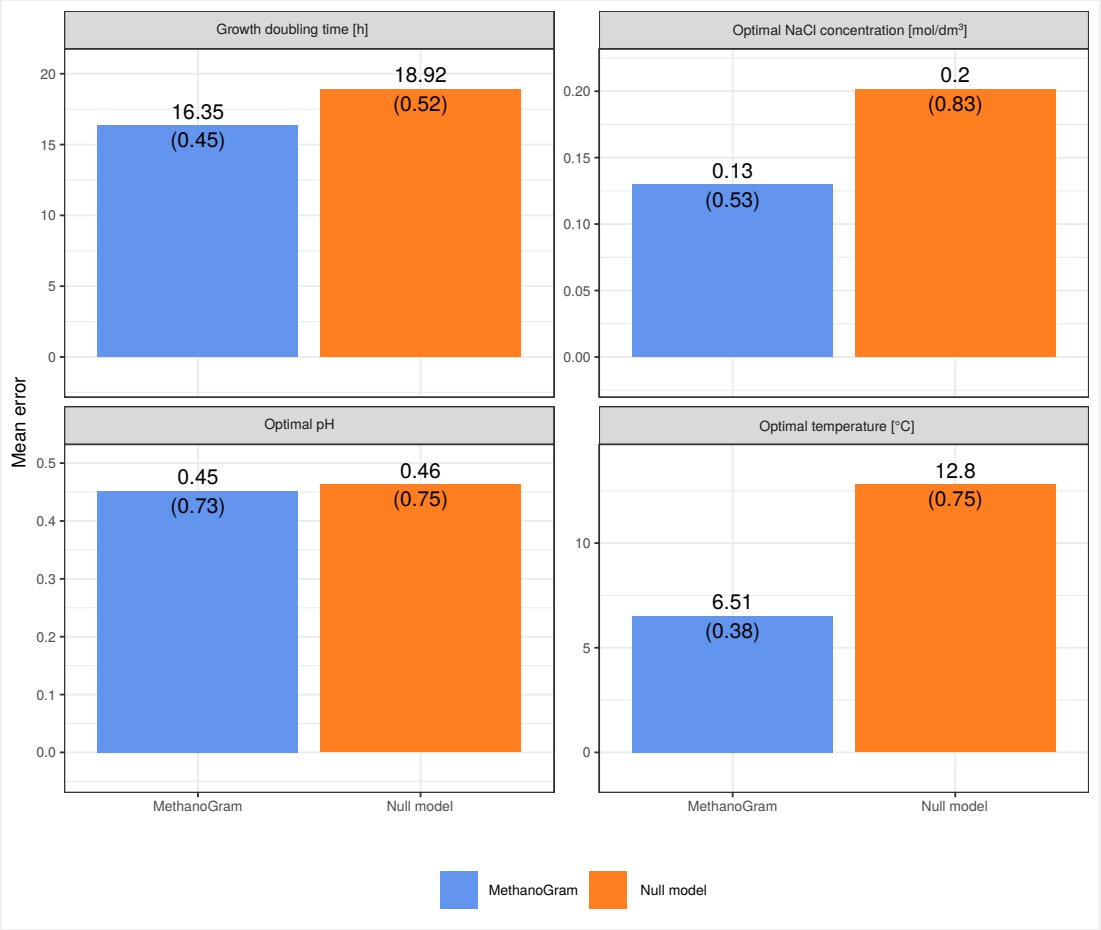
Methanogram: predicting methanogens culturing conditions

Methanogens are a complex group of archaeobacteria producing methane with different culturing requirements. A methanogram allows the prediction of the culturing conditions of a methanogens based on its genetic information.



Metanogeny. Maryland Astrobiology Consortium, NASA, and STScI.

Methanogram: predicting methanogens culturing conditions



Burdukiewicz, M., Gagat, P., Jabłoński, S., Chilimoniuk, J., Gaworski, M., Mackiewicz, P., and Łukaszewicz, M. (2018). PhyMet2: a database and toolkit for phylogenetic and metabolic analyses of methanogens. Environmental Microbiology Reports 10, 378–382.

Summary

- n-grams effectively characterize proteins and.
- Reduced alphabets allows us to discern amino acid motifs related to biological functions.

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/.

R packages:

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

Summary

Models predicting the properties of proteins may be based on precise rules that are understandable to biologists and experimentally verifiable without losing their effectiveness.

Acknowledgements

- Jarosław Chilimoniuk (University of Wrocław).
- Małgorzata Kotulska (Wroclaw University of Technology).
- Stefan Rödiger (Brandenburg University of Technology Cottbus-Senftenberg).
- Paweł Mackiewicz (University of Wrocław).
- Piotr Sobczyk (Wroclaw University of Technology).

Acknowledgements

Funding:

- Polish National Science Centre (2015/17/N/NZ2/01845 i 2017/24/T/NZ2/00003).
- COST ACTION CA15110 (Harmonising standardisation strategies to increase efficiency and competitiveness of European life-science research).
- KNOW Wrocław Center for Biotechnology.
- German Federal Ministry of Education and Research (InnoProfile-Transfer-Projekt 03IPT611X).

MI² Data Lab

MI² Data Lab (`https://mi2.mini.pw.edu.pl/`), Faculty of Mathematics and Computer Science.

Contact: `michalburdukiewicz@gmail.com`.

References I

Burdukiewicz, M., Sobczyk, P., Chilimoniuk, J., Gagat, P., and Mackiewicz, P. (2018). Prediction of Signal Peptides in Proteins from Malaria Parasites. International Journal of Molecular Sciences, 19(12):3709.

Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports, 7(1):12961.

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.

Christensen, L. F. B., Hansen, L. M., Finster, K., Christiansen, G., Nielsen, P. H., Otzen, D. E., and Dueholm, M. S. (2018). The sheaths of methanospirillum are made of a new type of amyloid protein. Frontiers in Microbiology, 9:2729.

References II

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.

Hegde, R. S. and Bernstein, H. D. (2006). The surprising complexity of signal sequences. Trends in Biochemical Sciences, 31(10):563–571.

Murphy, L. R., Wallqvist, A., and Levy, R. M. (2000). Simplified amino acid alphabets for protein fold recognition and implications for folding. Protein Engineering, 13(3):149–152.

References III

Paz, M. L. d. l. and Serrano, L. (2004). Sequence determinants of amyloid fibril formation. Proceedings of the National Academy of Sciences, 101(1):87–92.

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. , Riekel, 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, 42(W1):W301–W307.