The Alphabet Of Life: n-gram analysis of proteins

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Presentation plan

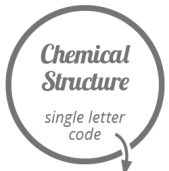
1. Aminoacids and proteins
2. n-grams and simplified alphabets
3. Amyloid prediction
4. Other applications

Aminoacids and proteins

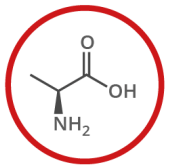
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

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.

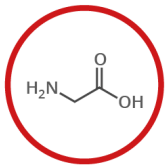
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL



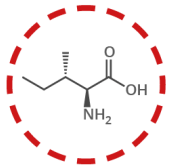
NAME A
three letter code
DNA codons



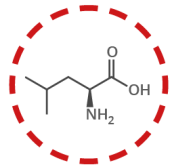
ALANINE A
Ala
GCT, GCC, GCA, GCG



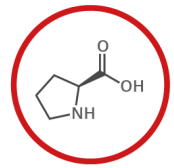
GLYCINE G
Gly
GGT, GGC, GGA, GGG



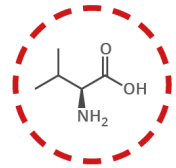
ISOLEUCINE I
Ile
ATT, ATC, ATA



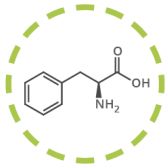
LEUCINE L
Leu
CTT, CTC, CTA, CTG, TTA, TTG



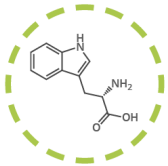
PROLINE P
Pro
CCT, CCC, CCA, CCG



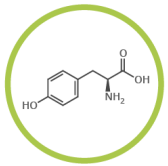
VALINE V
Val
GTT, GTC, GTA, GTG



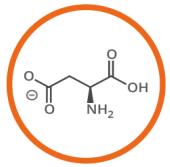
PHENYLALANINE F
Phe
TTT, TTC



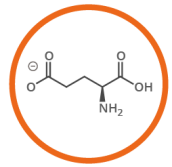
TRYPTOPHAN W
Trp
TGG



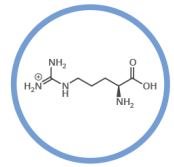
TYROSINE Y
Tyr
TAT, TAC



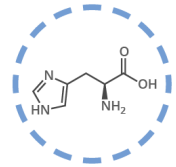
ASPARTIC ACID D
Asp
GAT, GAC



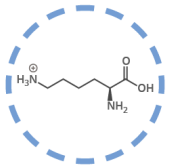
GLUTAMIC ACID E
Glu
GAA, GAG



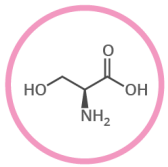
ARGININE R
Arg
CGT, CGC, CGA, CGG, AGA, AGG



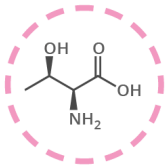
HISTIDINE H
His
CAT, CAC



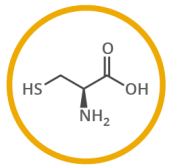
LYSINE K
Lys
AAA, AAG



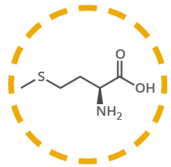
SERINE S
Ser
TCT, TCC, TCA, TCG, AGT, AGC



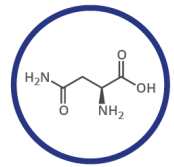
THREONINE T
Thr
ACT, ACC, ACA, ACG



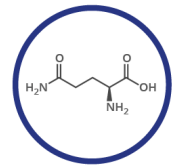
CYSTEINE C
Cys
TGT, TGC



METHIONINE M
Met
ATG



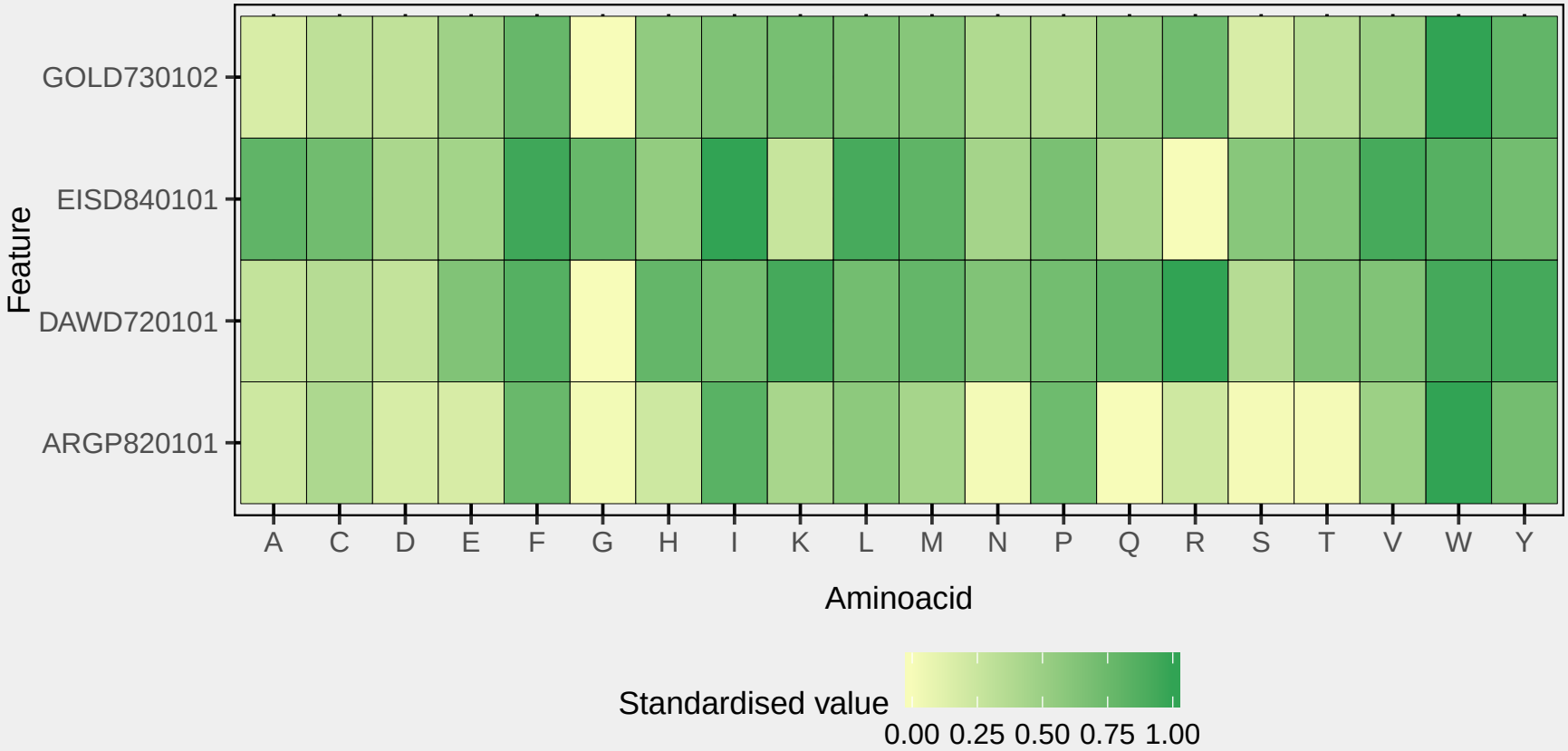
ASPARAGINE N
Asn
AAT, AAC



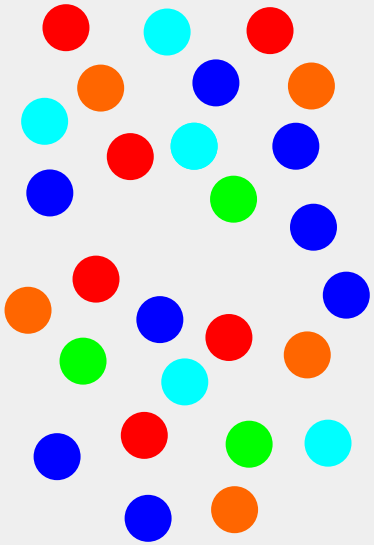
GLUTAMINE Q
Gln
CAA, CAG

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.

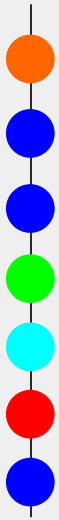
Aminoacids


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
Aminoacid
Standardised value
0.00 0.25 0.50 0.75 1.00

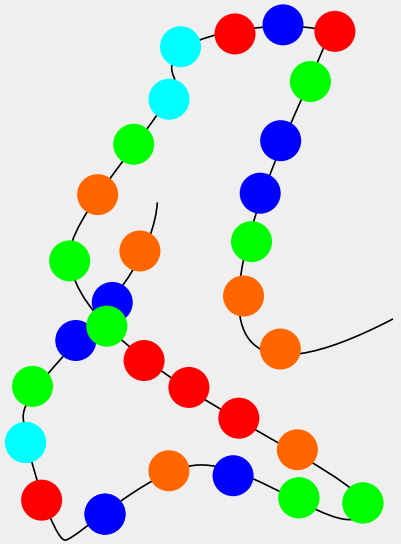
Proteins



Aminoacids

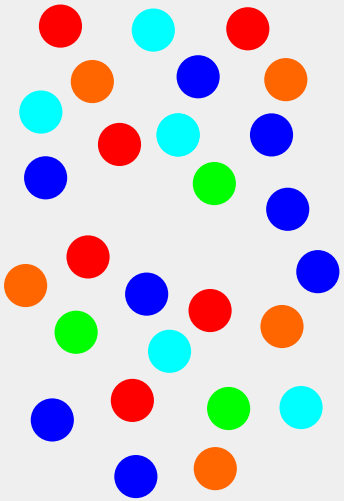


Protein (primary structure)

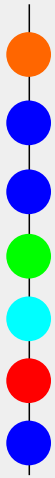


Protein (higher tier structures)

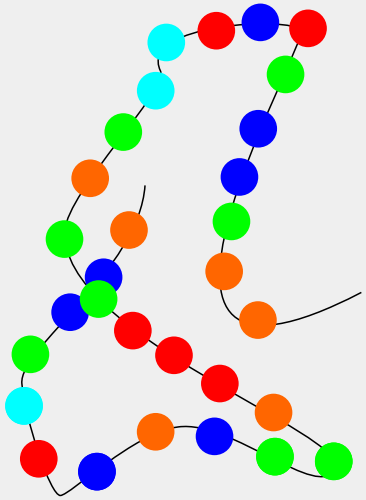
Proteins



Aminoacids



Protein (primary structure)

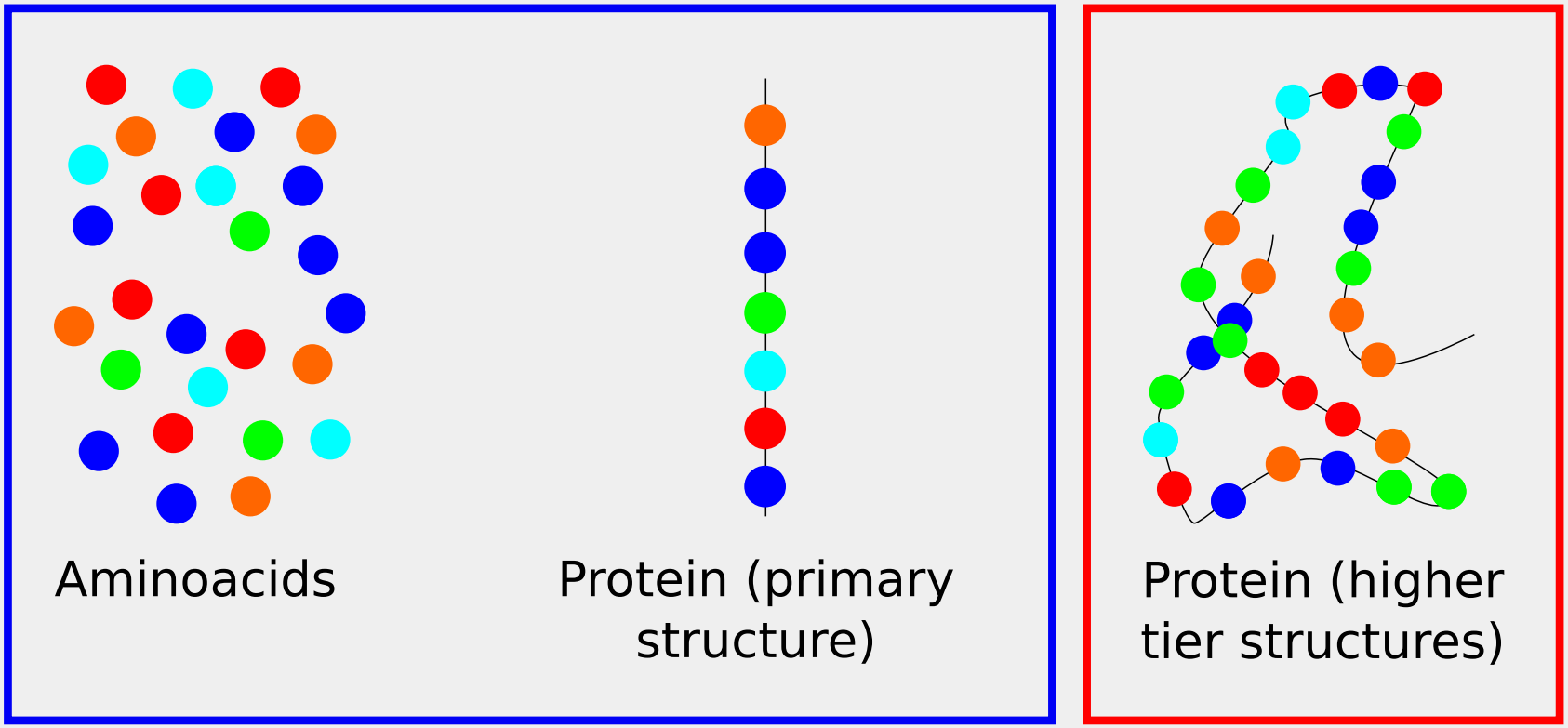


Protein (higher tier structures)

KNOWN

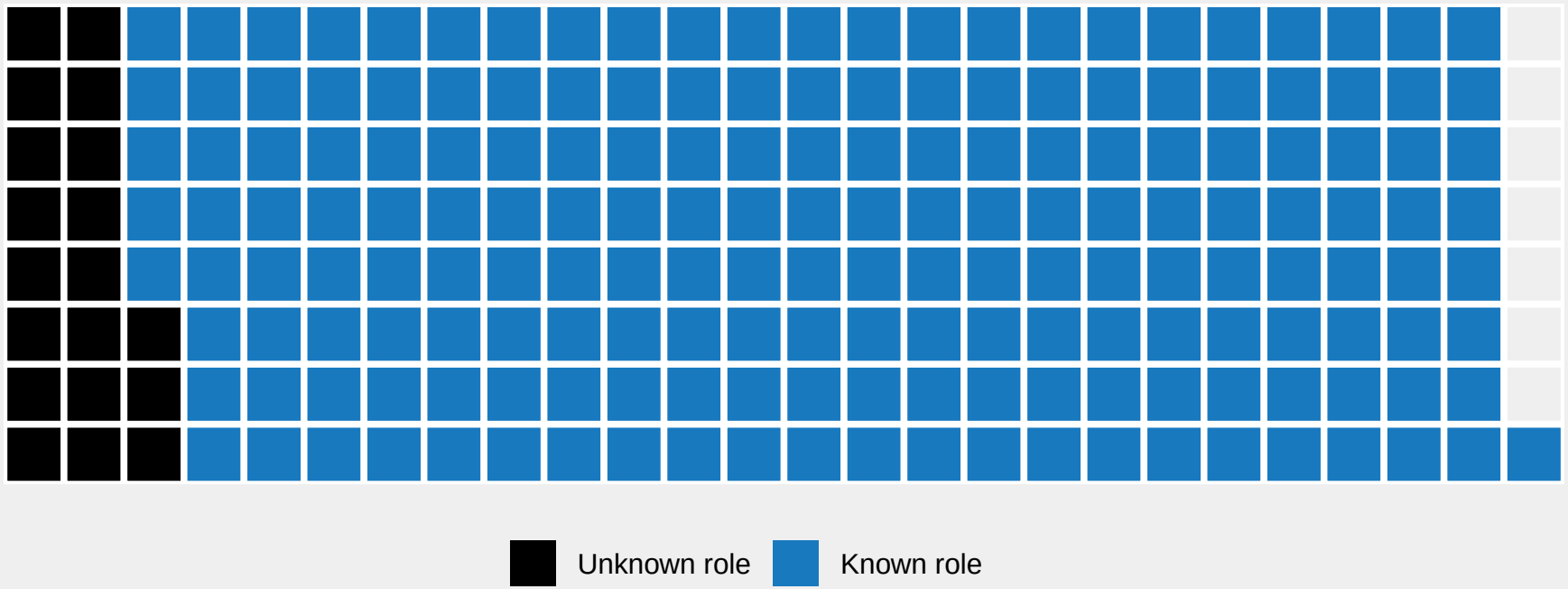
UNKNOWN

Proteins



Protein quaternary structure 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 simplified alphabets

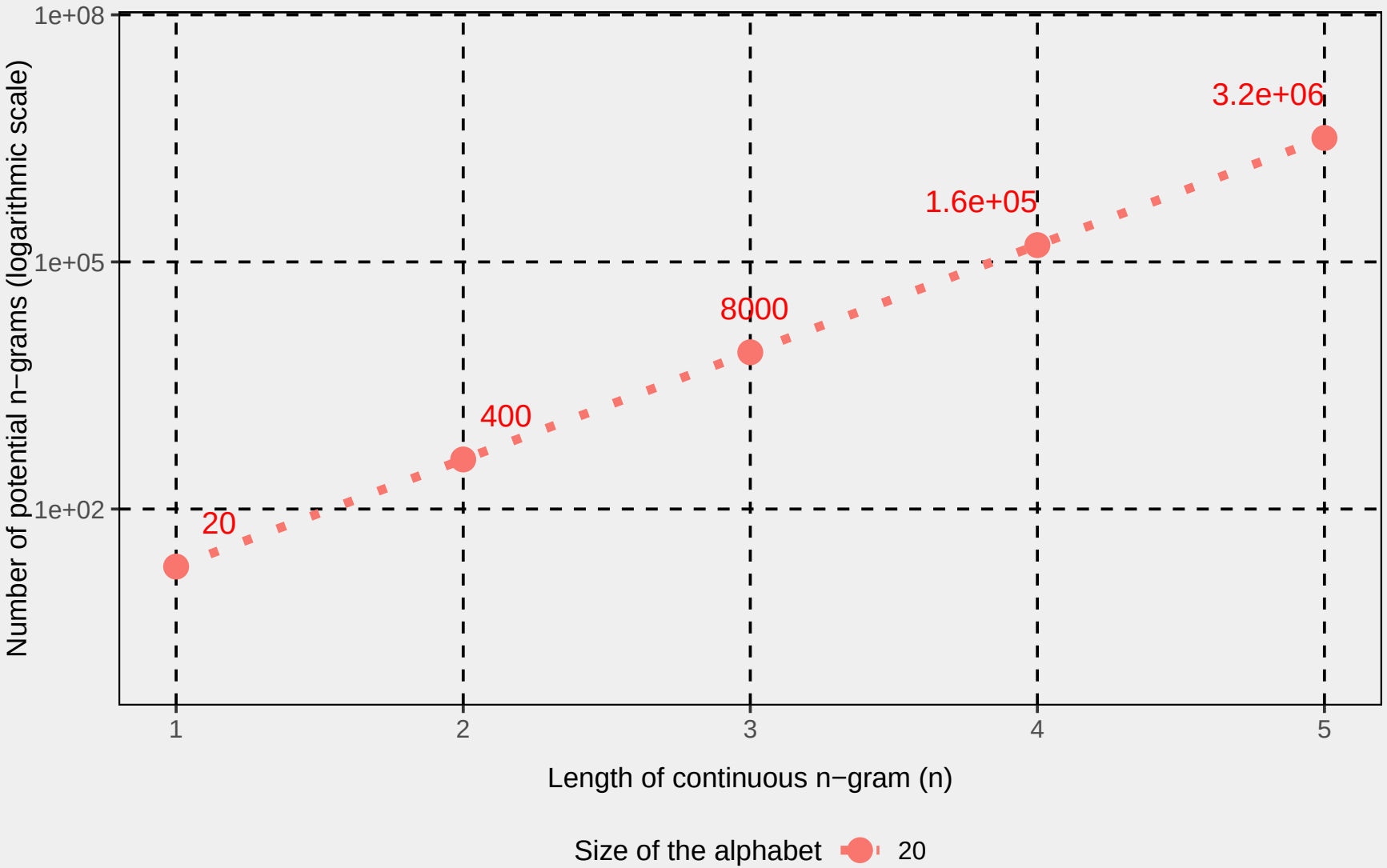
n-grams (k-tuple, k-mers):

- subsequences (continuous or discontinuous) *n* aminoacid 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.

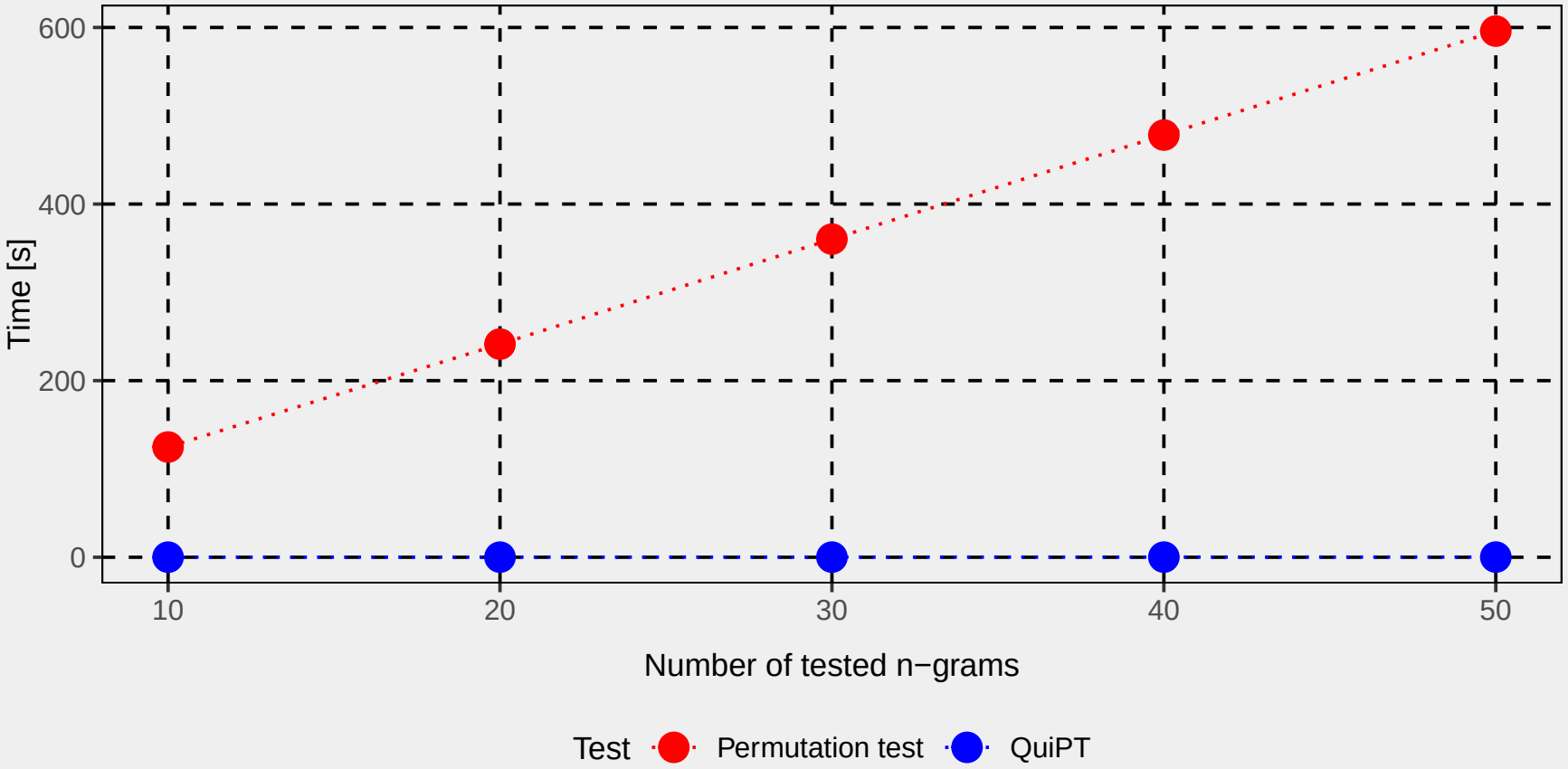


Longer n-games are more informative, but create larger attribute spaces that are more difficult to analyze.

Slam - package for rare matrices

Counting n-grams creates rare matrices, that are causing dimensional problems.
To manage them, we use a slam package that can store such large amounts of data.
It is a package designed for rare matrices.

QuiPT



QuiPT (avaible as function in biogram package) is faster than classic permutation tests.

Simplified alphabets

Simplified 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.

Simplified alphabets

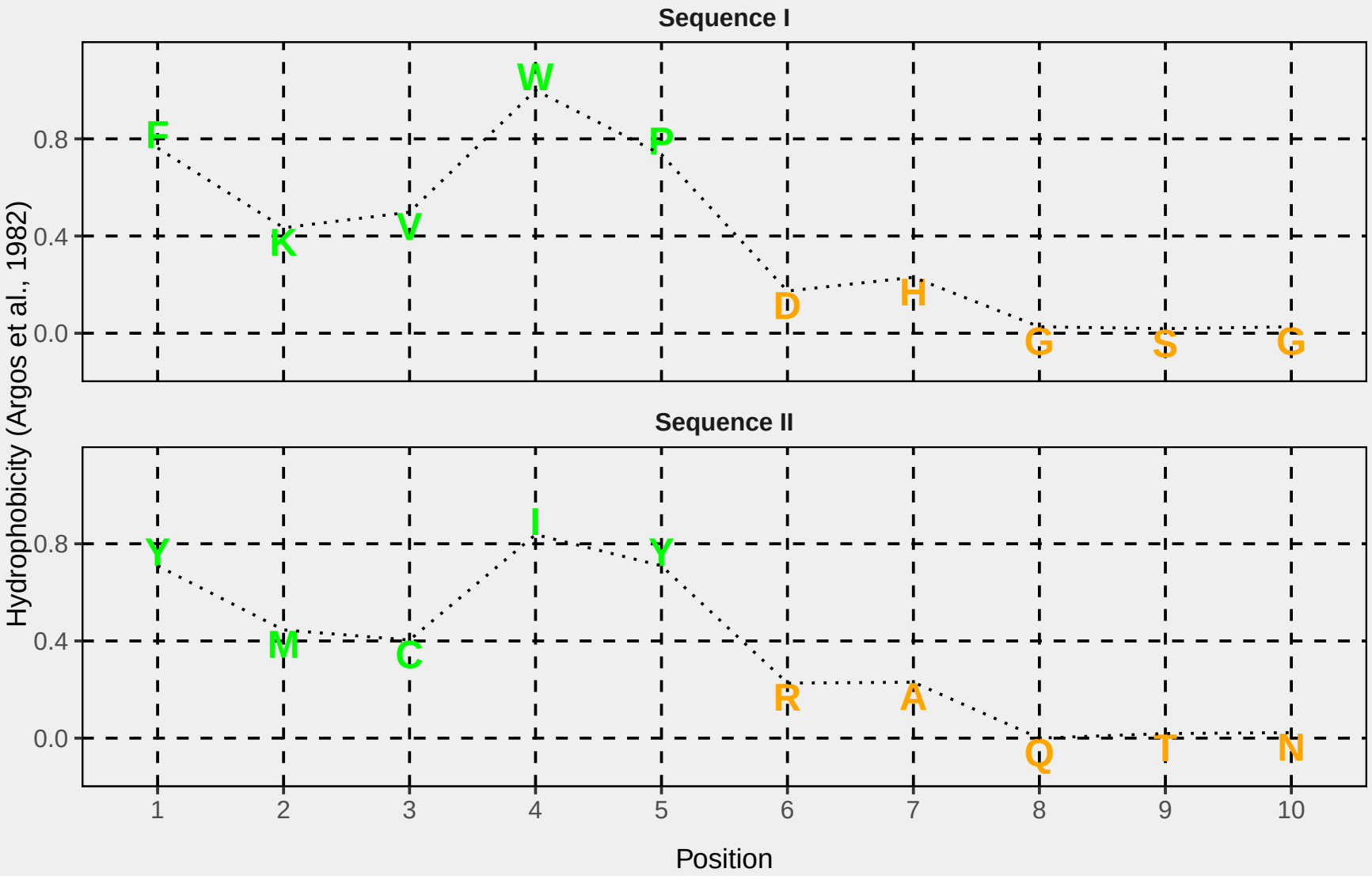
Following peptides appear to be completely different in terms of amino acid composition.

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

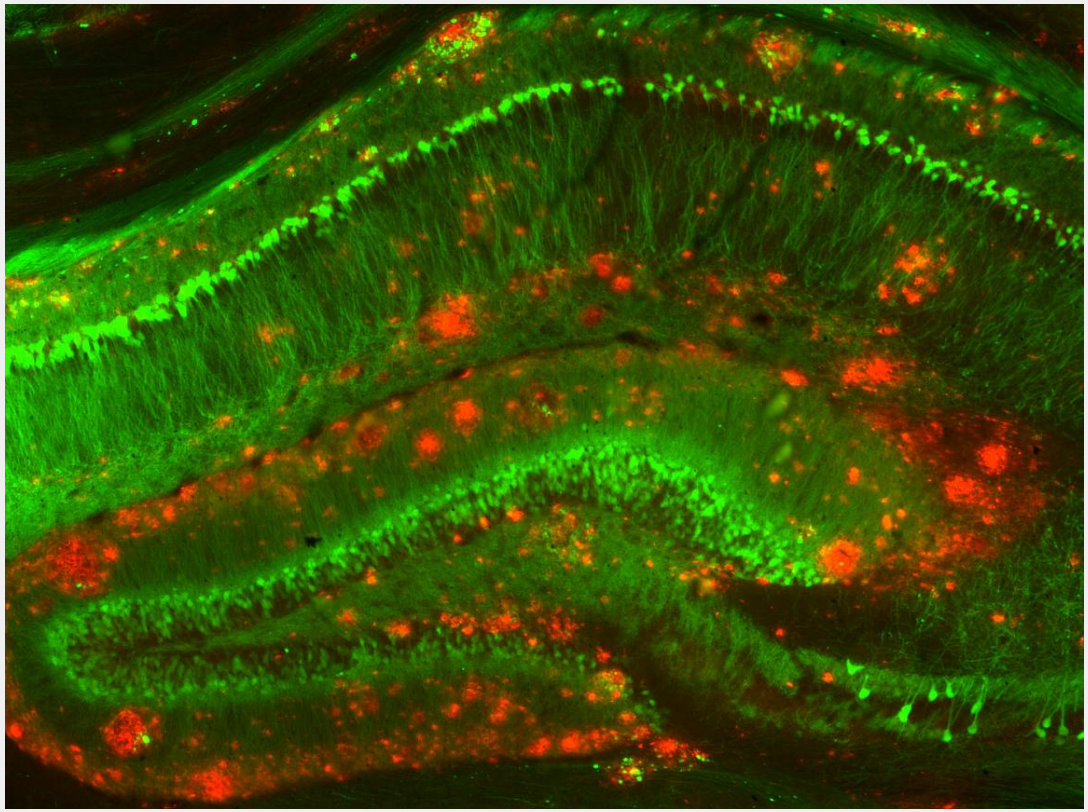
Peptide I: FKVWPDHGSG → 1111122222

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.

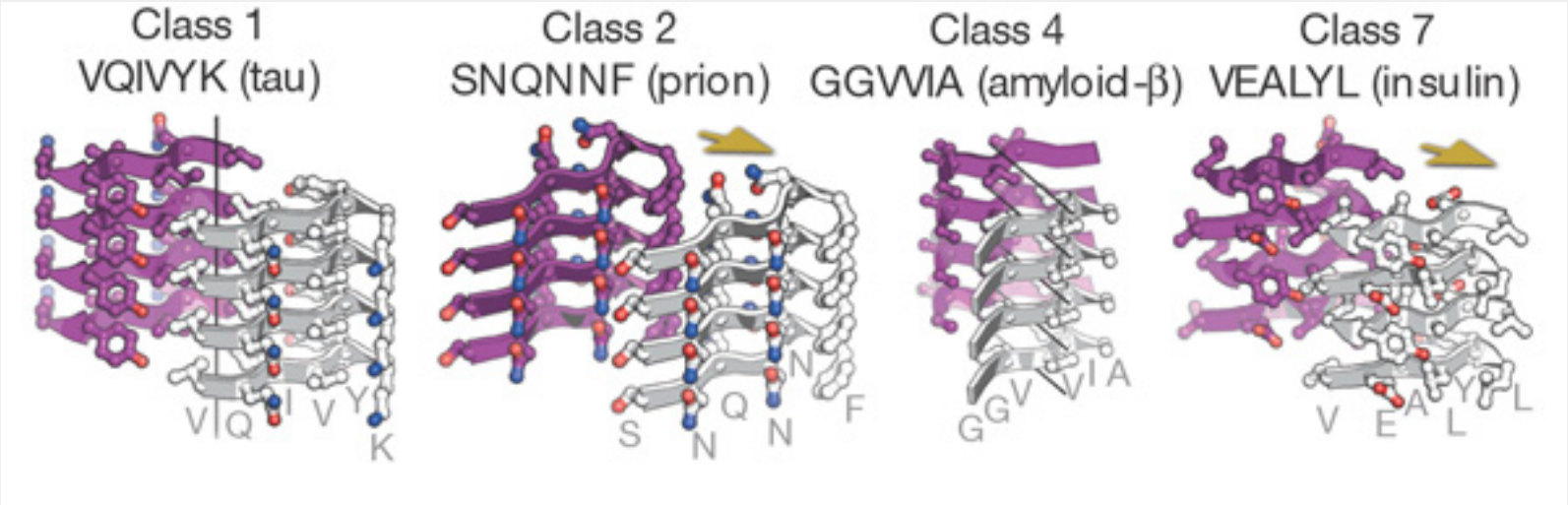


Amyloid aggregates (red) around neurons (green). Strittmatter Laboratory, Yale University.

Amyloid proteins

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

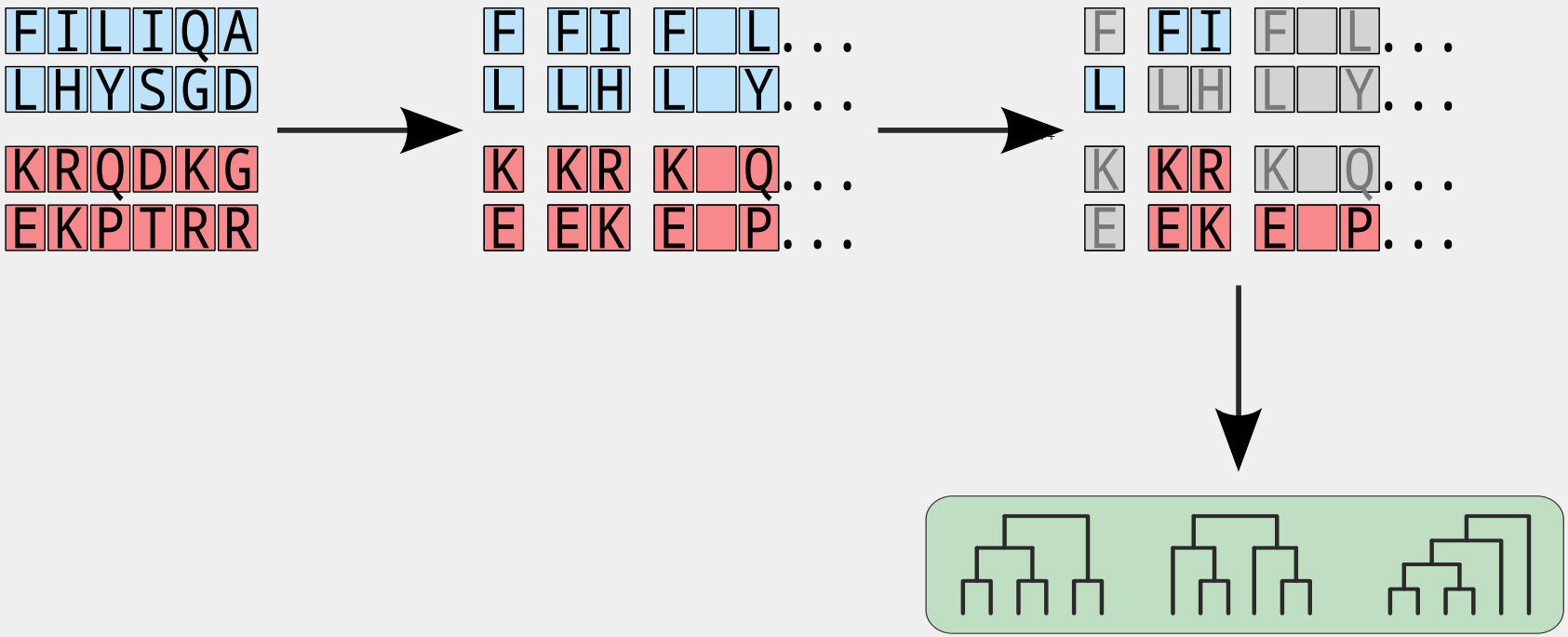
- short (6-15 aminoacids),
- very variable, usually hydrophobic, aminoacid composition,
- create unique β-structures.



Sawaya et al. (2007)

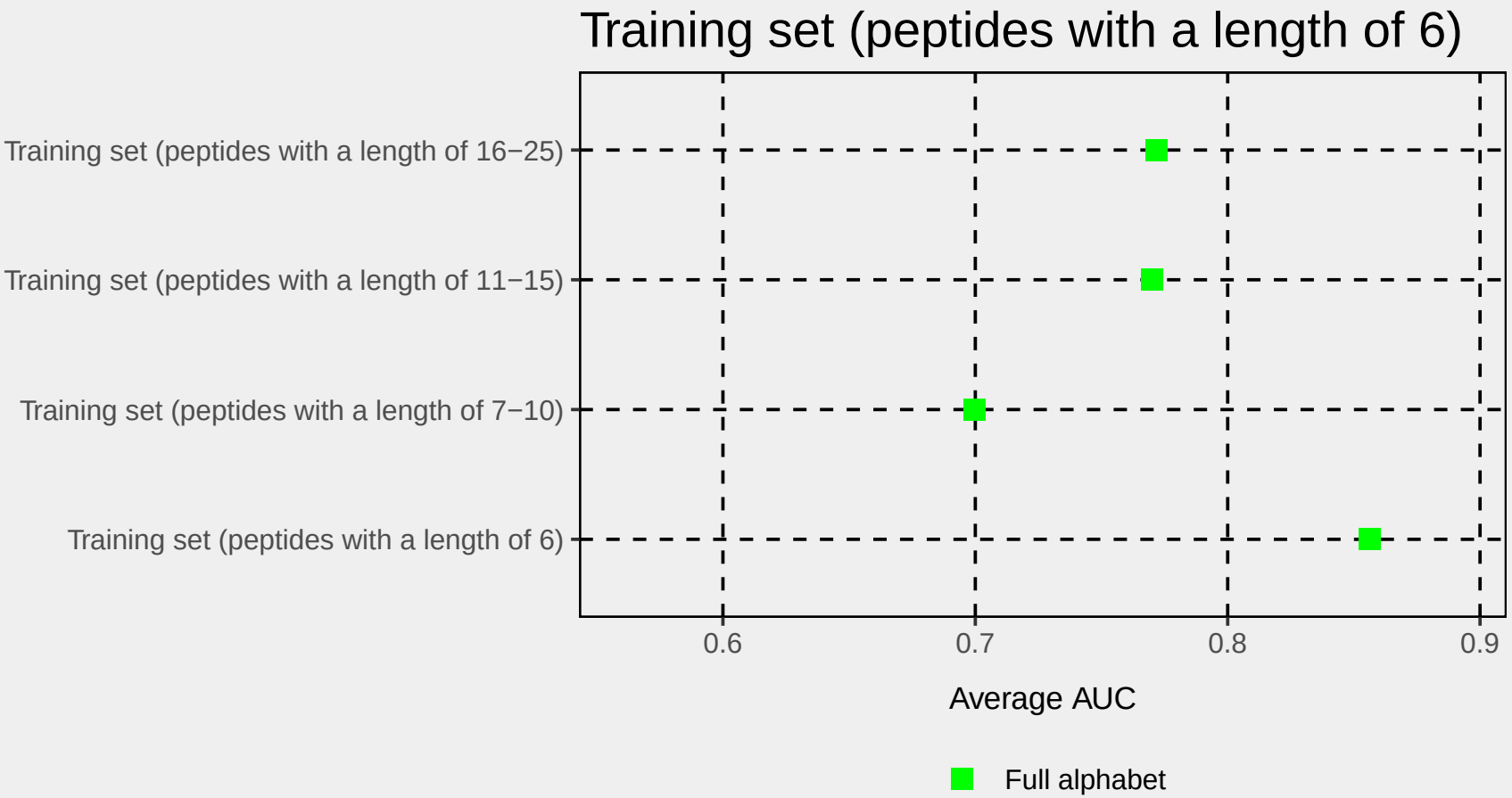
AmyloGram

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

Cross-validation



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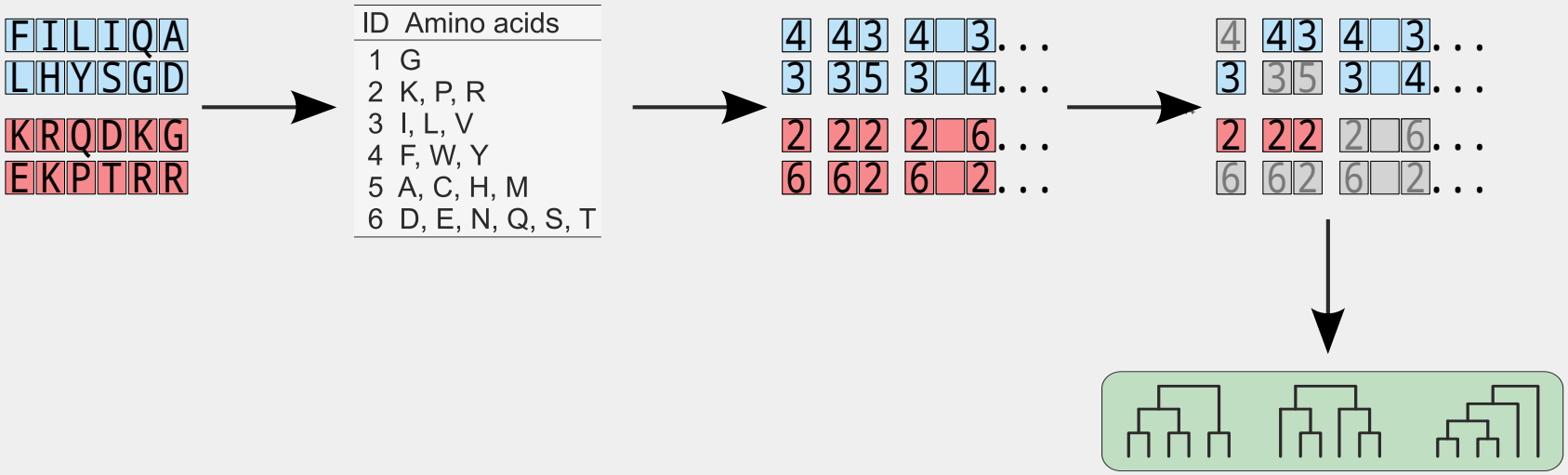
Standardowe uproszczone alfabety

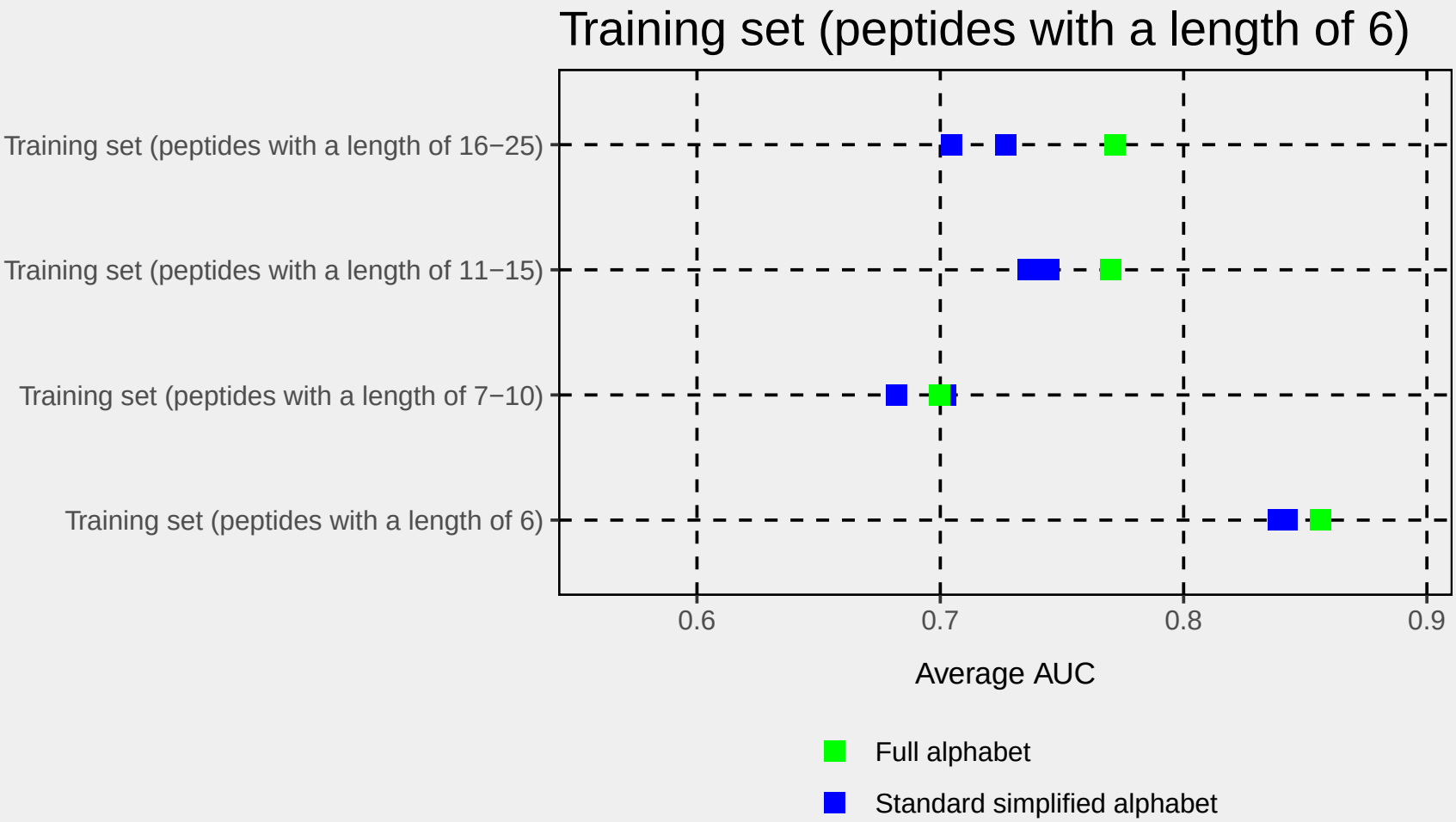
Czy standardowe uproszczone alfabety opracowane dla różnych zagadnień biologicznych pomagają lepiej przewidywać amyloidy?

Standardowe uproszczone alfabety



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 simplified alphabet



Standard aminoacid alphabets do not improve the quality of amyloid prediction.

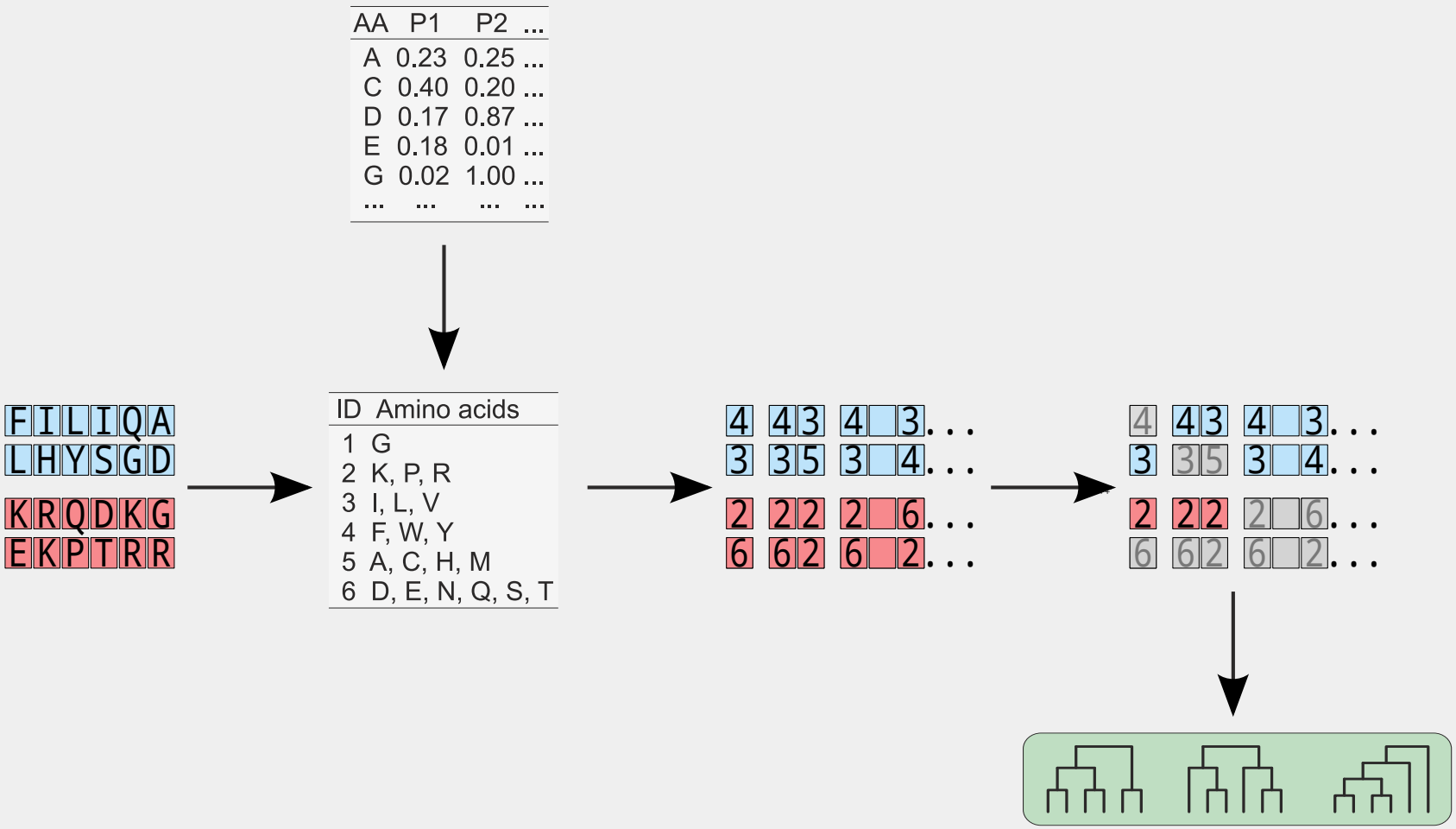
Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

New simplified alphabets

- 17 physicochemical parameters selected from AAindex database:
  - size,
  - hydrophobicity,
  - frequency in β-sheets,
  - ability to make contact.
- 524 284 simplified aminoacid alphabets of various sizes (from 3 to 6 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

New simplified alphabets



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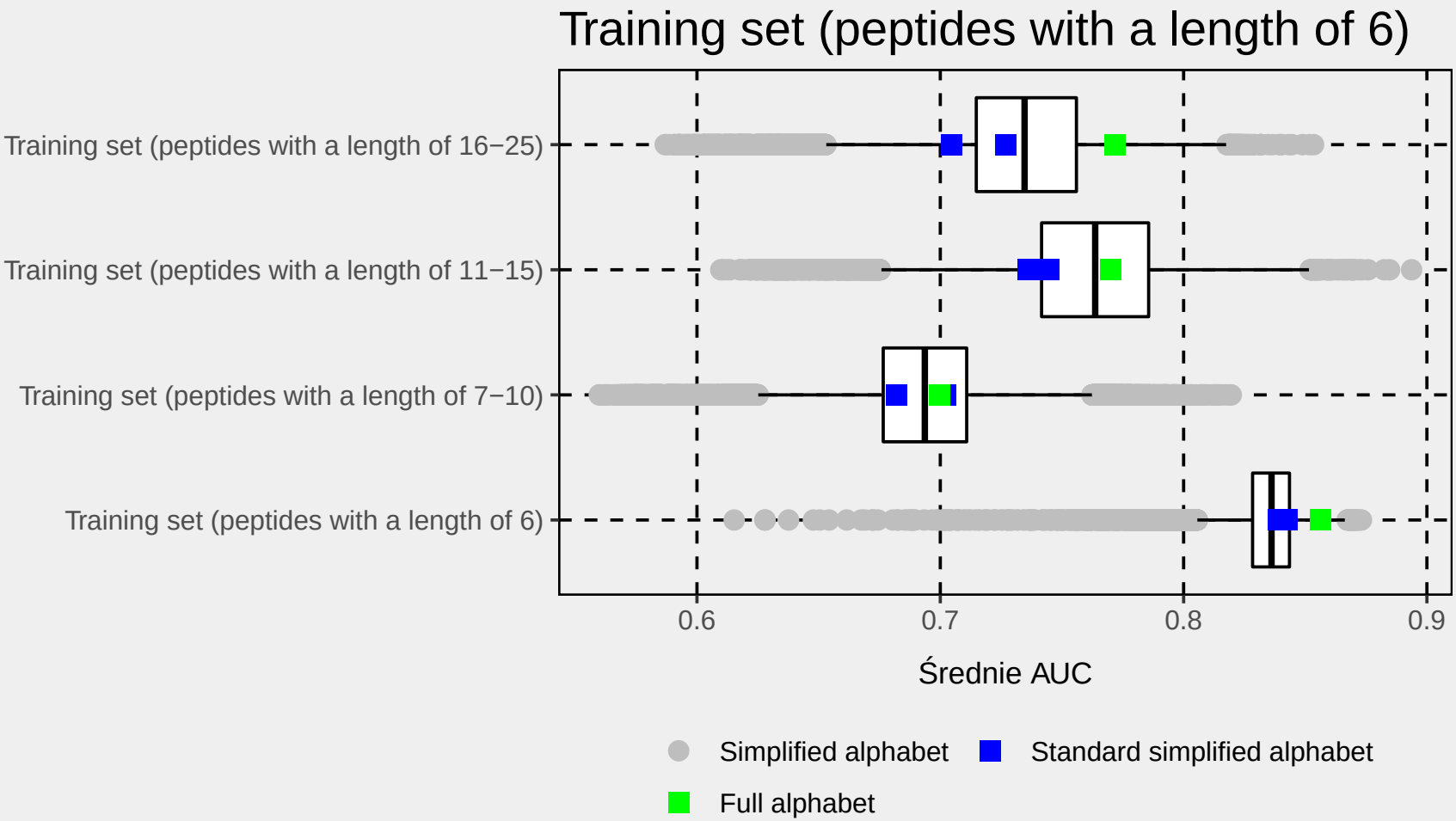
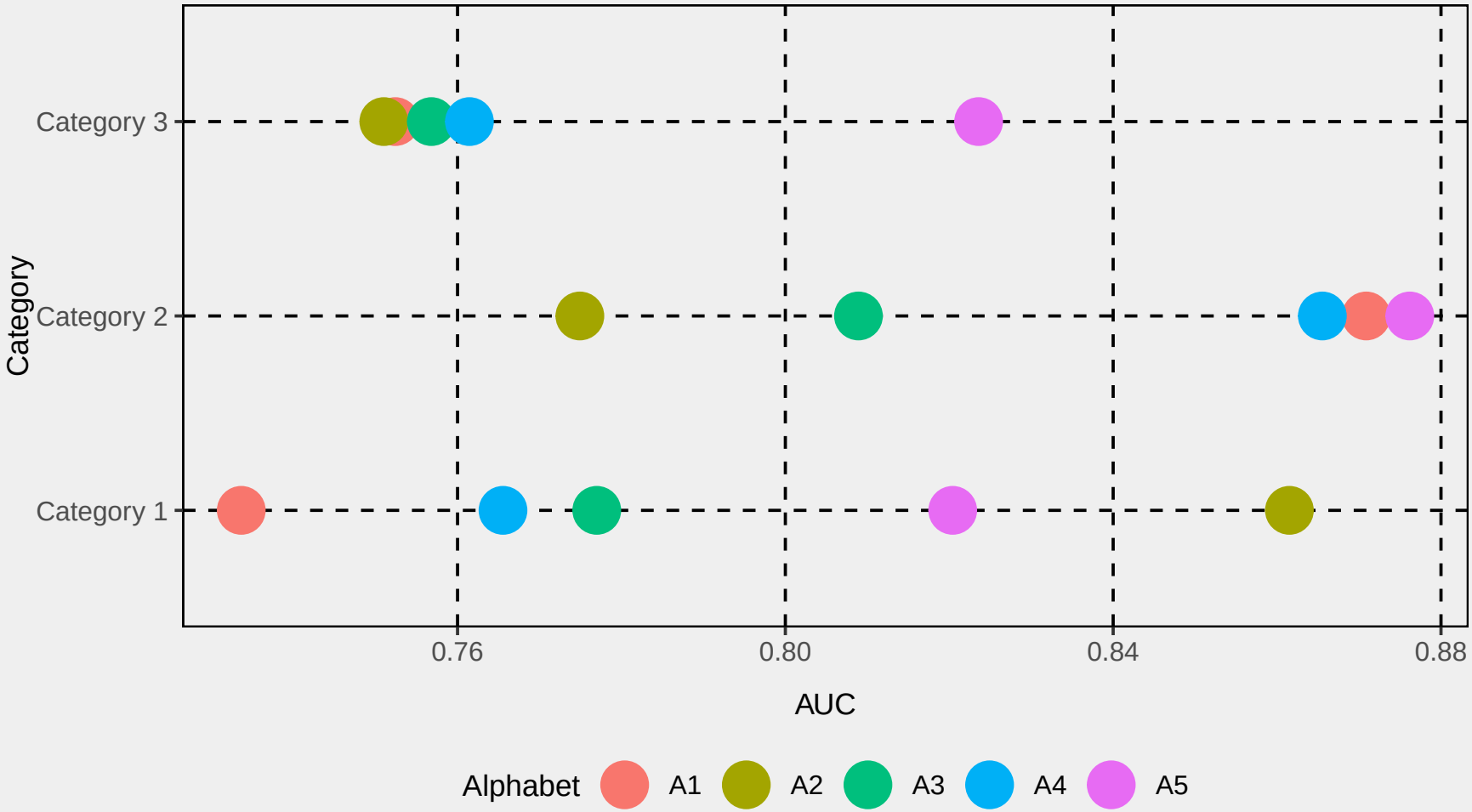
Standard simplified alphabet

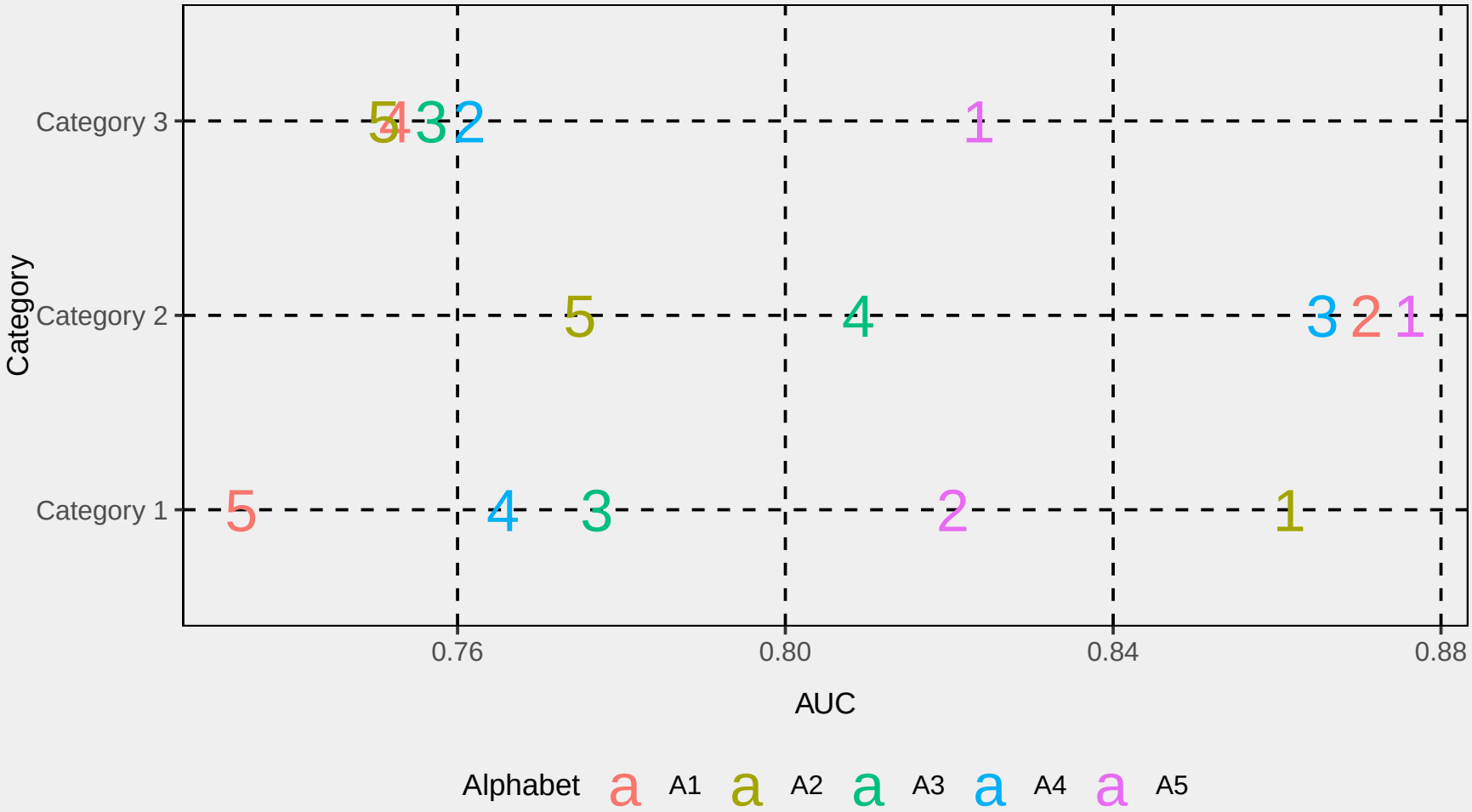


Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. Scientific Reports 7, 12961

Selection of best-performing simplified alphabet

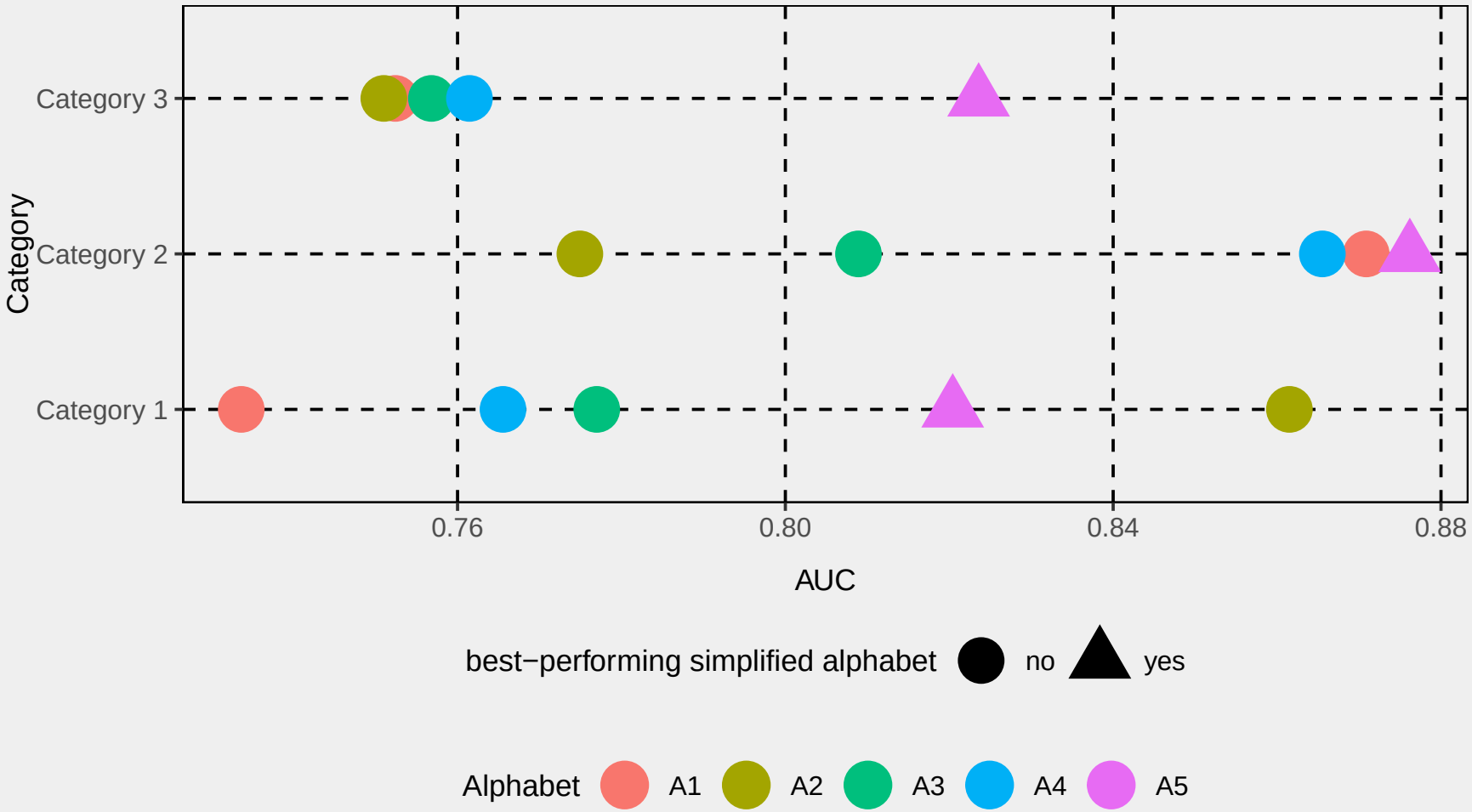


Selection of best-performing simplified alphabet



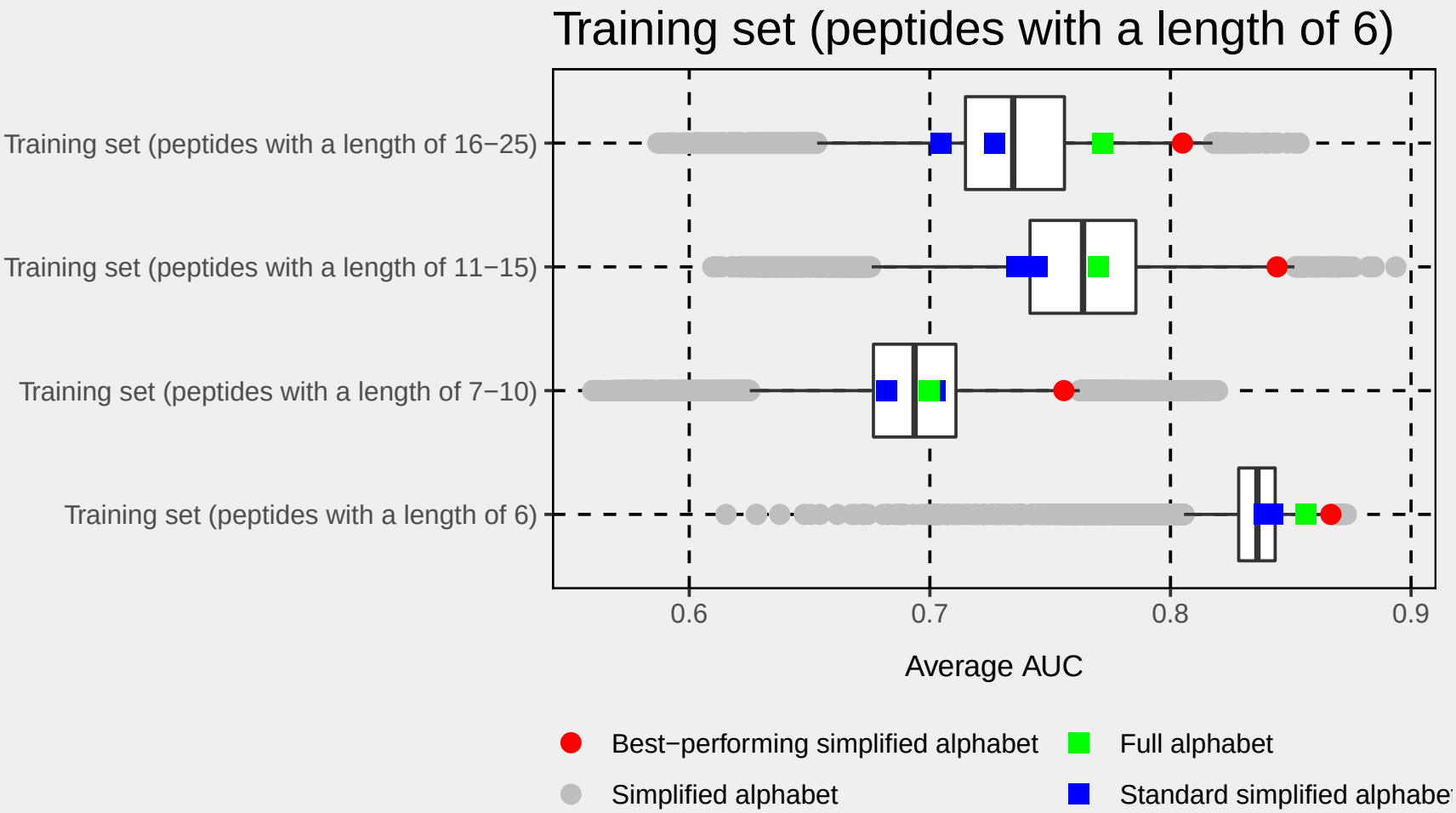
For each category the alphabets have been ranked (rank 1 for the best AUC, etc.)

Selection of best-performing simplified alphabet



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

Best-performing simplified 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 simplified alphabet

Group	Aminoacids
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

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1	G
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Grupy 3 i 4 - hydrophobic aminoacids.

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Best-performing simplified alphabet

Group	Aminoacids
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 - aminoacids disrupting the β-structure.

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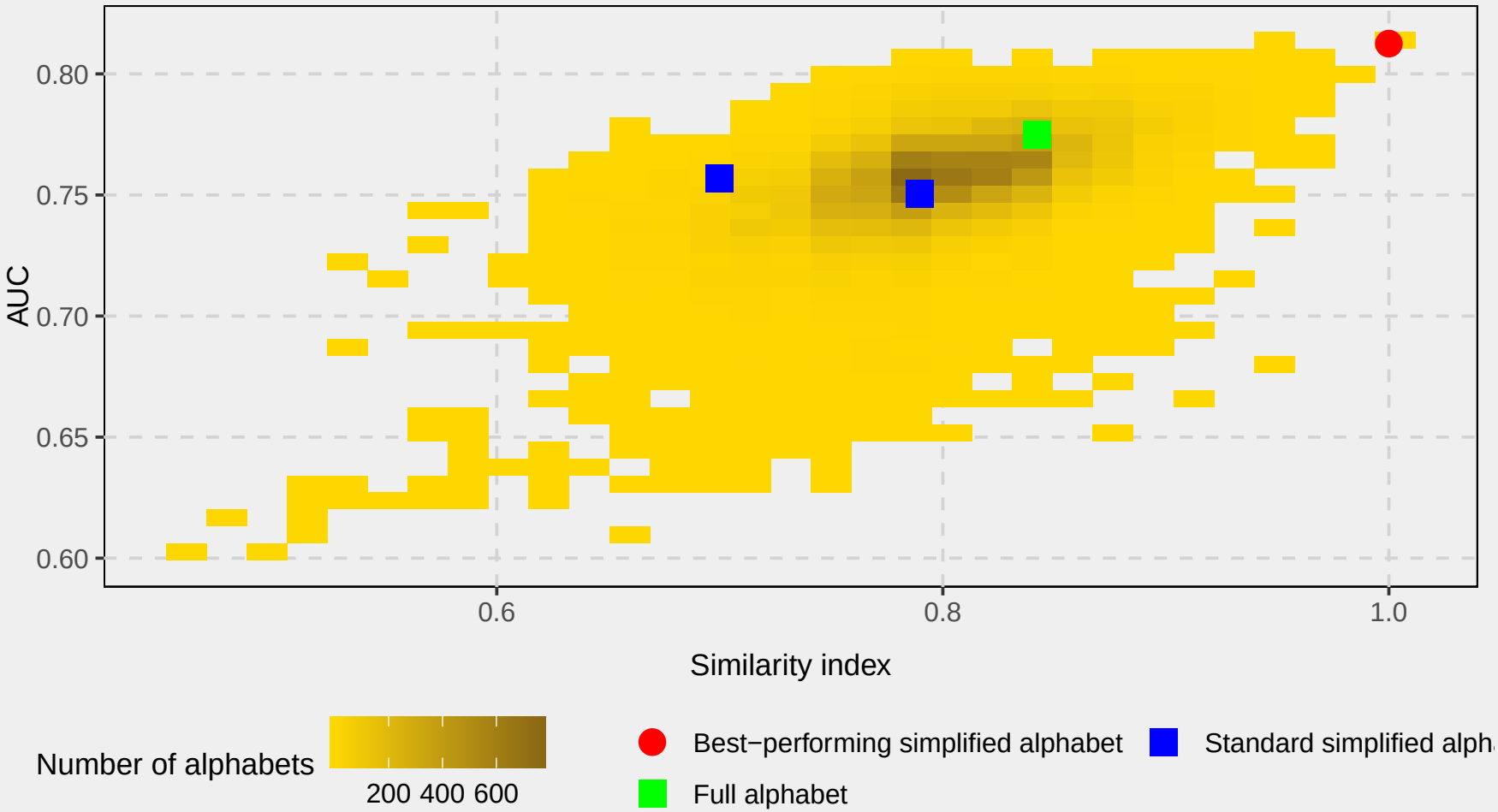
Alphabet similarity and quality of prediction

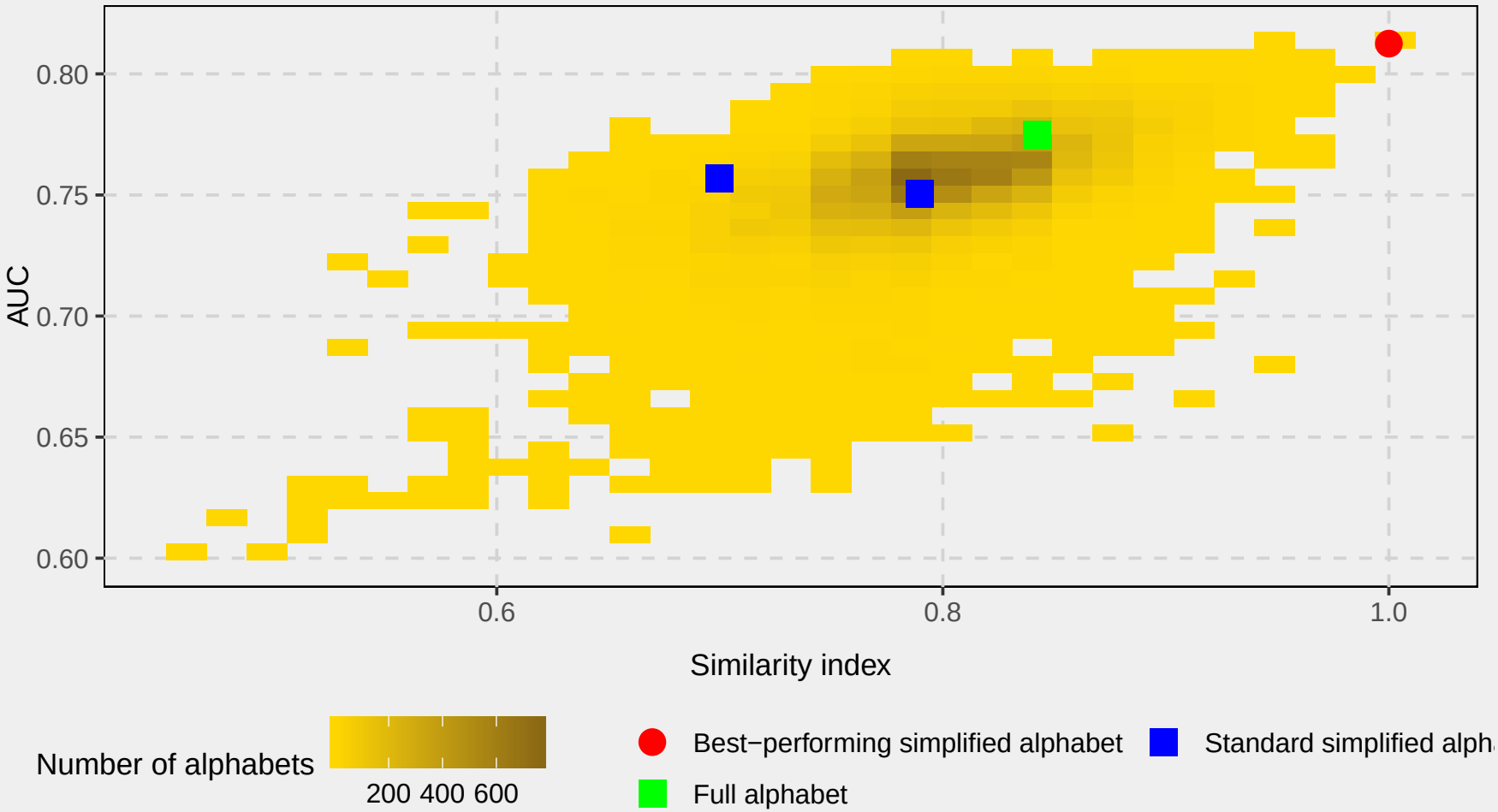
Do alphabets similar to the best simplified alphabet also support amyloid predictions?

Similarity index



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

Similarity index

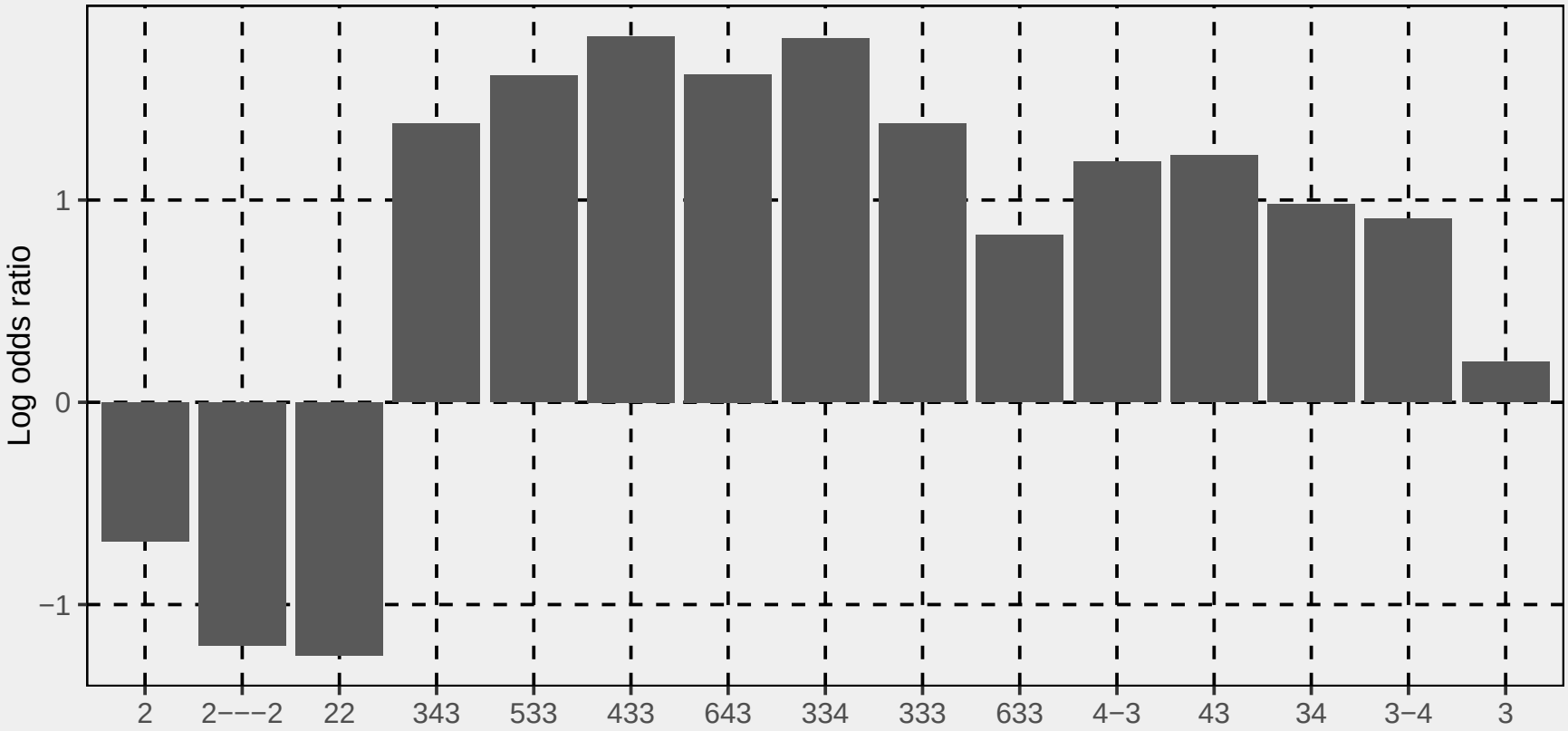


The correlation between the similarity index and the average AUC is important ($\text{p-value} \leq 2.2^{-16}$; $\rho = 0.51$).

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 the informative n-grams found by QuiPT are connected with amyloidogenicity?

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

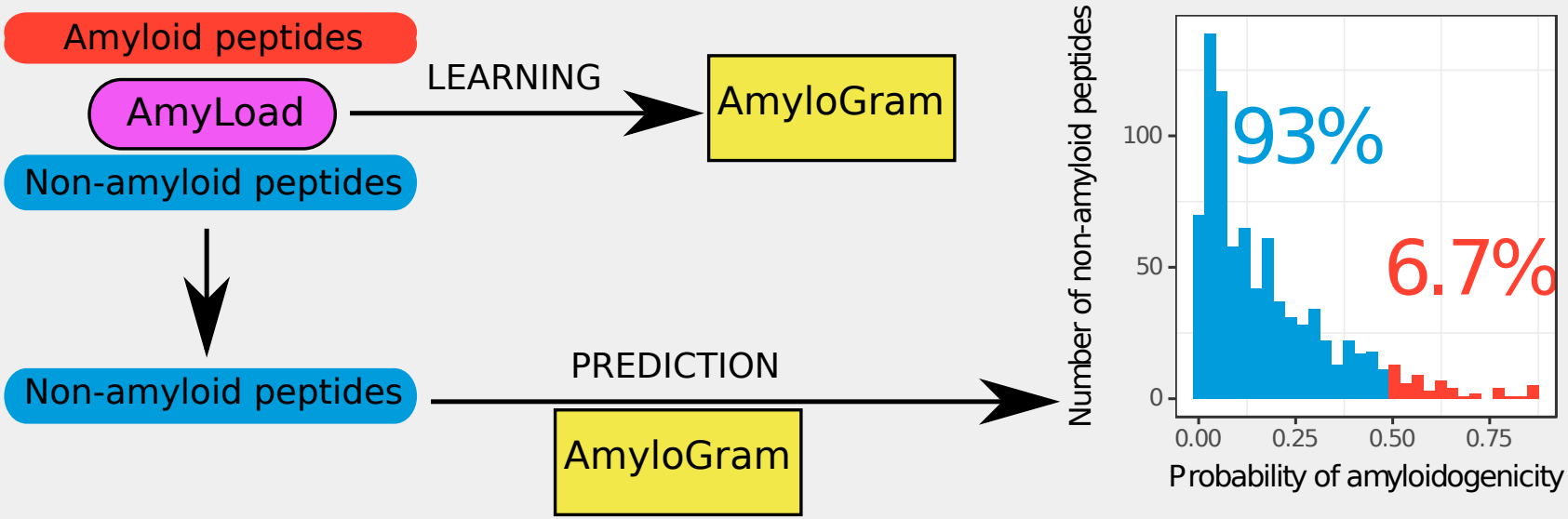
Benchmark with other software

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

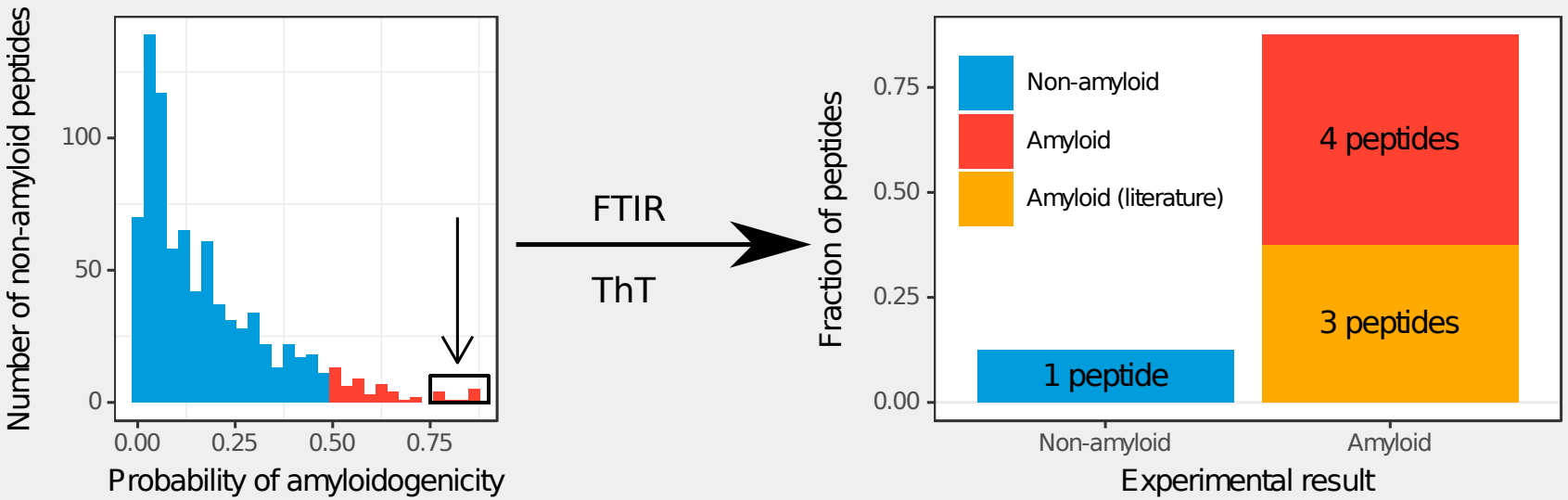
The classifier trained using the best simplified alphabet, AmyloGram, has been compared with other amyloid prediction tools using an external dataset pep424.

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

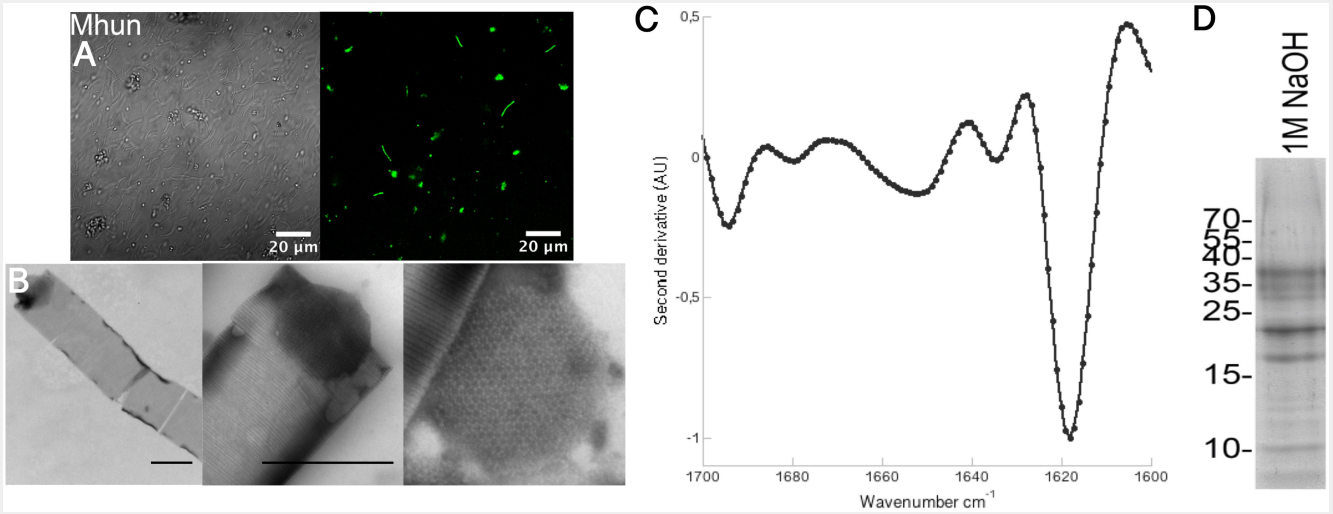
Experimental validation



Experimental validation



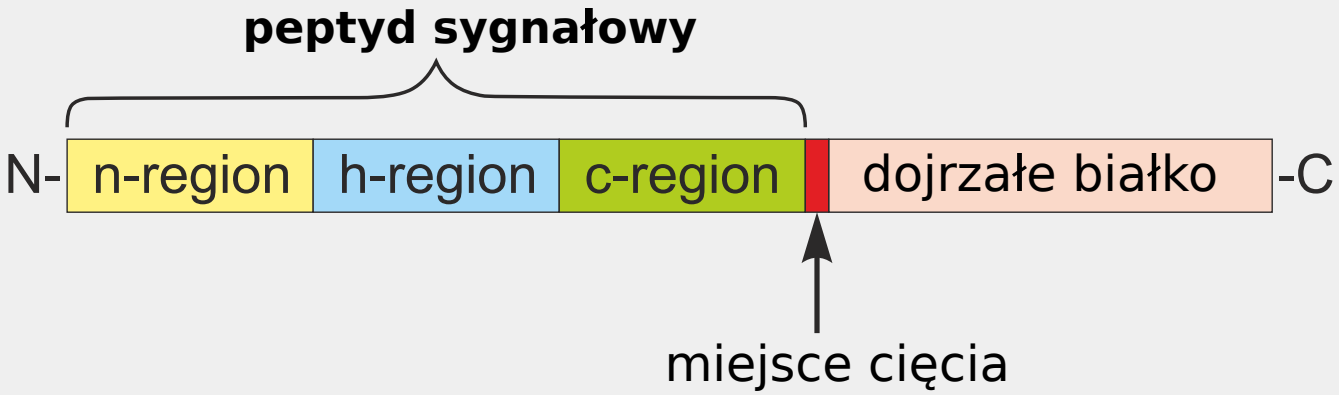
New amyloid



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

Other applications

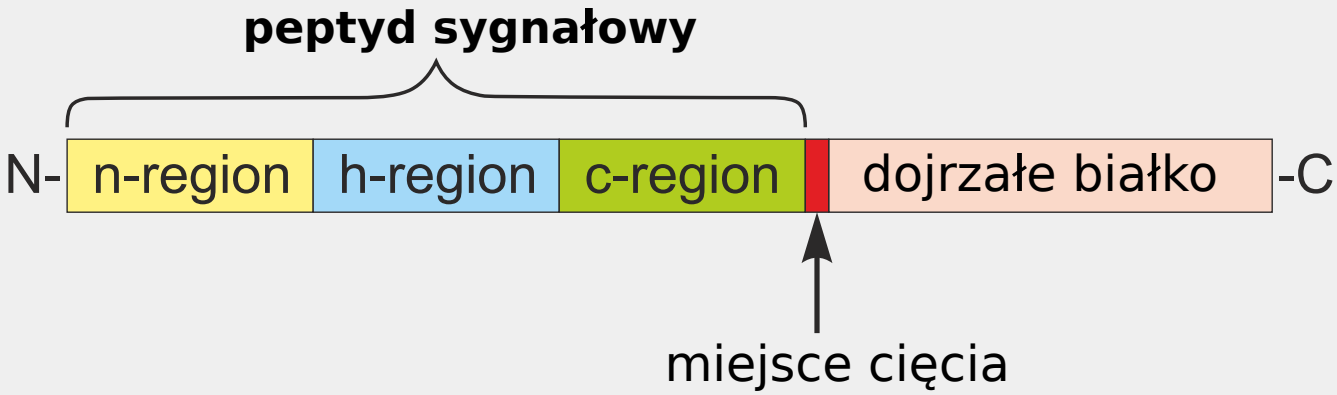
Signal peptides



Signal peptides (Hegde and Bernstein, 2006):

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

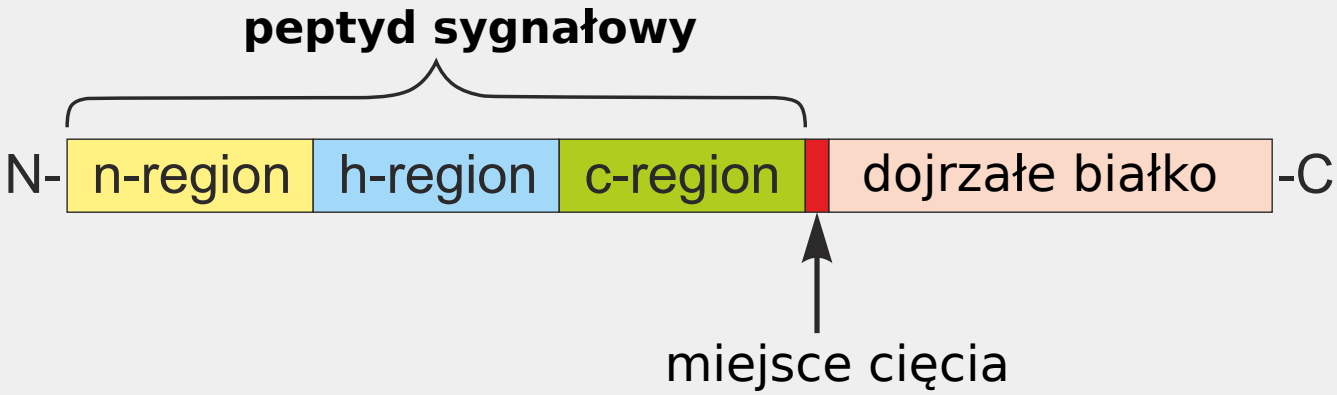
Signal peptides



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

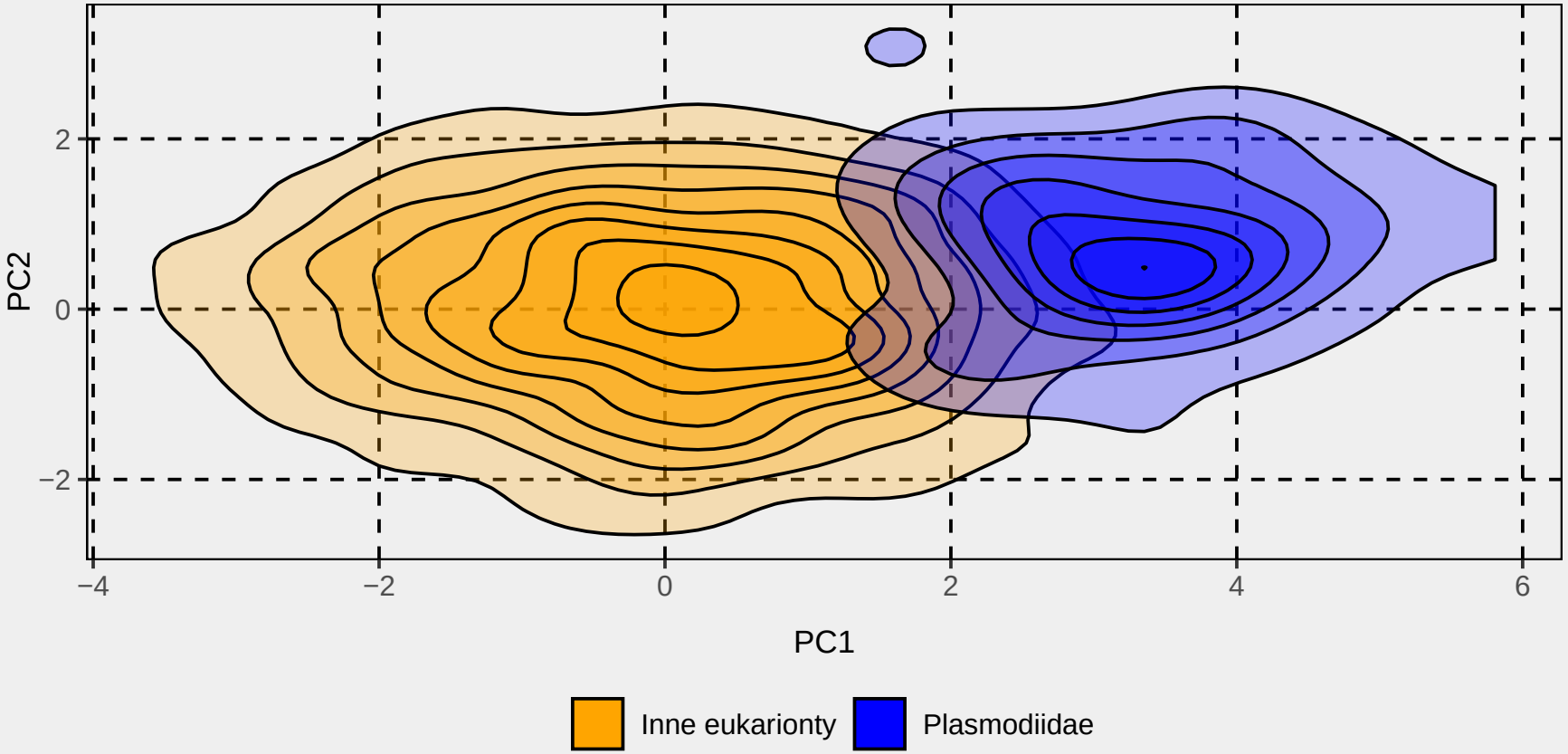


Signal peptides (Hegde and Bernstein, 2006):

- 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.

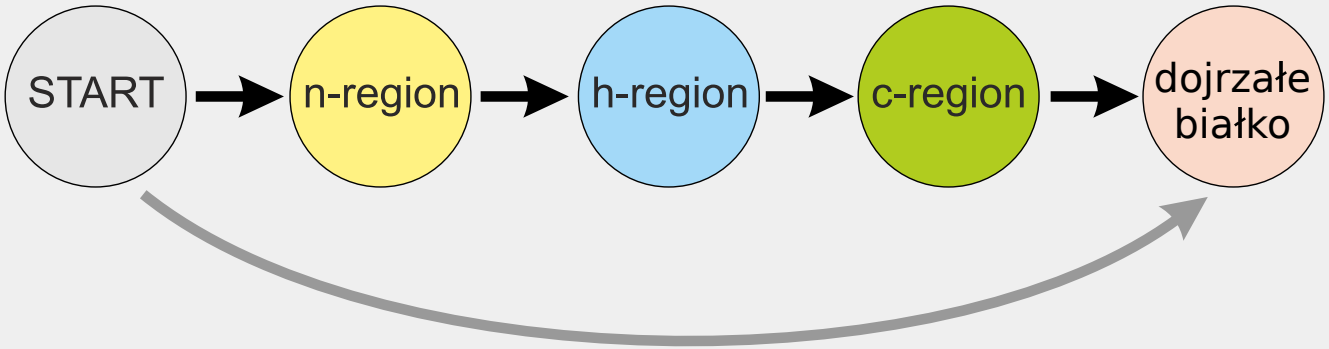
Signal peptides

The aminoacid 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 aminoacid frequency.

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



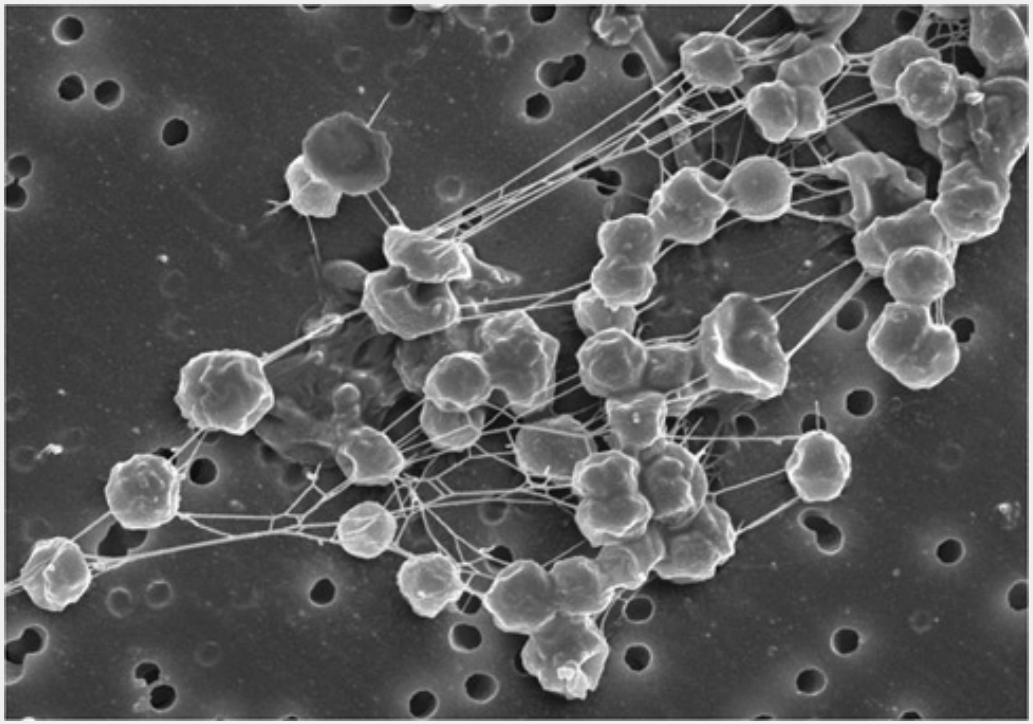
Benchmark with other predictors

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.

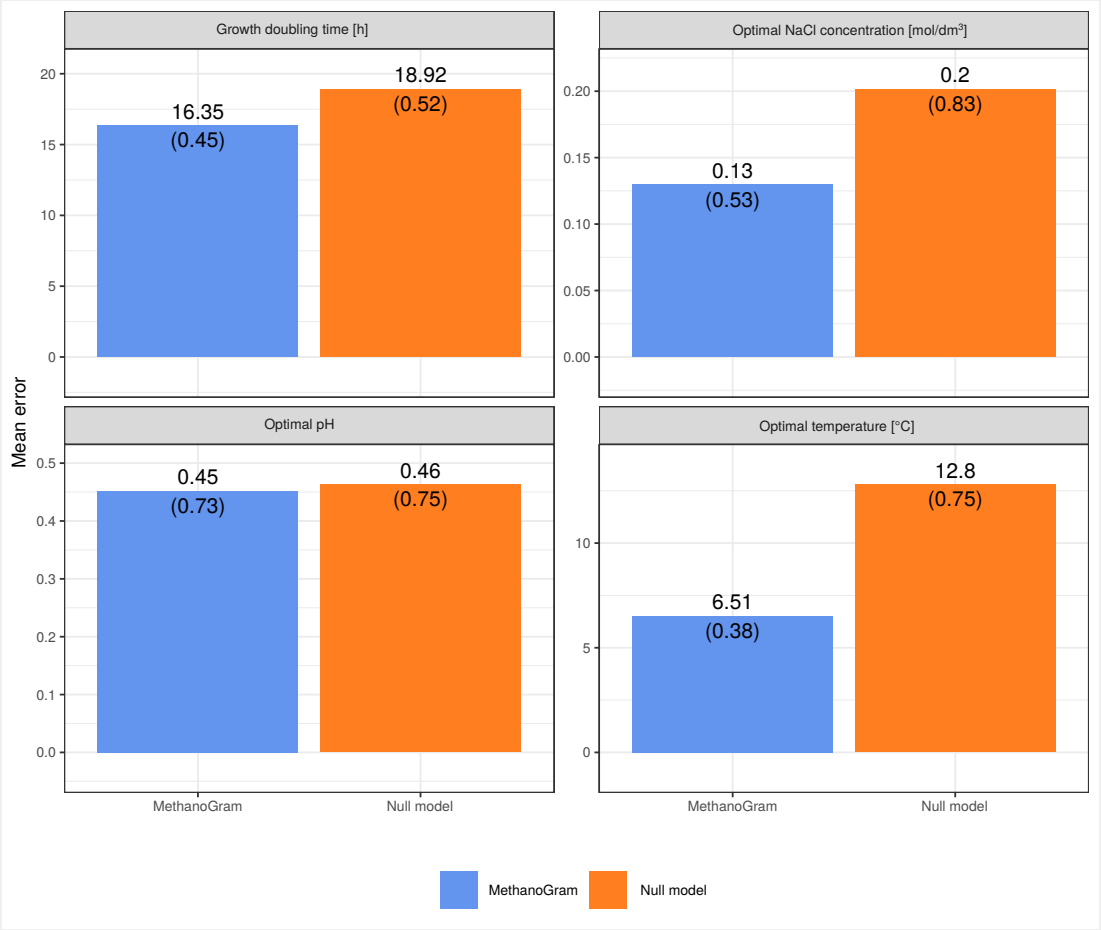
Methanogram: predicting methanogens culturing conditions

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.

Summarise()

Webservers:

- 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/.

Pakiety R:

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

Summarise()

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

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Mentors:

- Michał Burdukiewicz (Politechnika Warszawska).
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- Federal Ministry of Education and Research (InnoProfile-Transfer-Projekt 03IPT611X).

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