

AmyloGram: Analysis of proteins in R

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PRESENTATION PLAN

- 1 Aminoacids and proteins
- 2 n-grams and simplified alphabets
- 3 Amyloid prediction
- 4 Shiny application

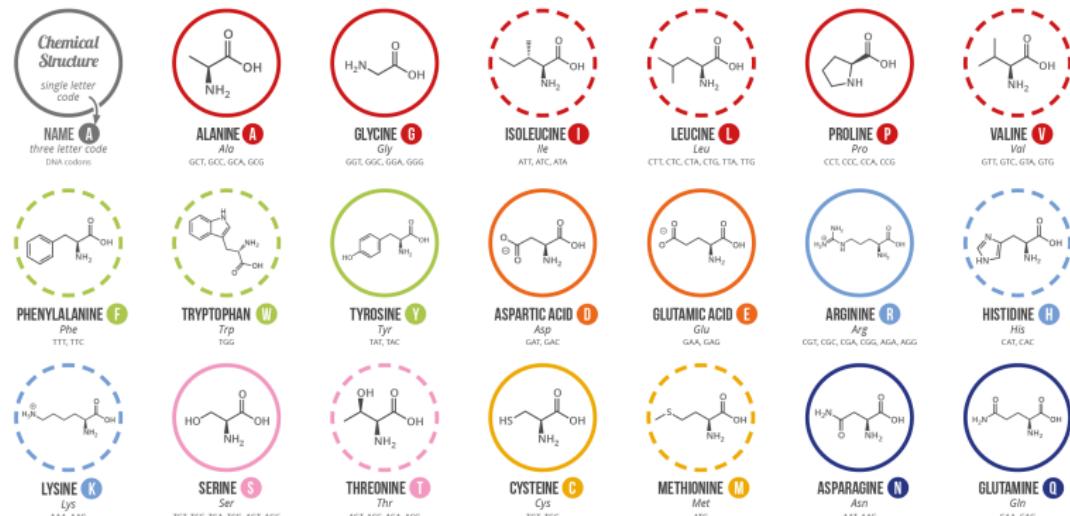
Aminoacids and proteins

AMINOACIDS

A GUIDE TO THE TWENTY COMMON AMINO ACIDS

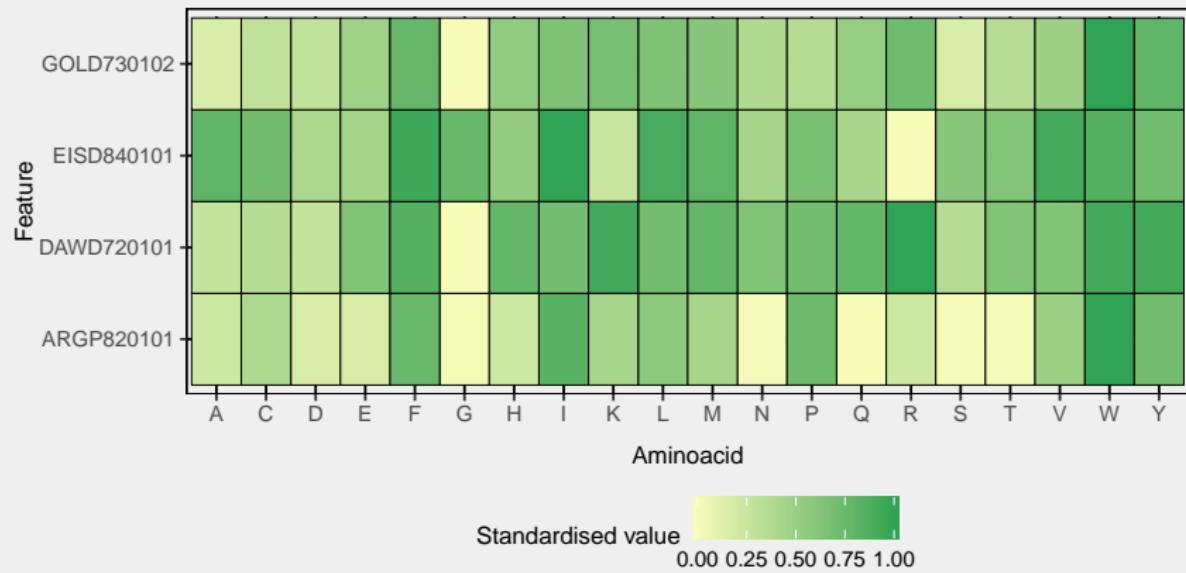
AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

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

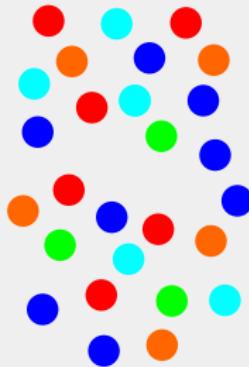


Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In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

AMINOACIDS



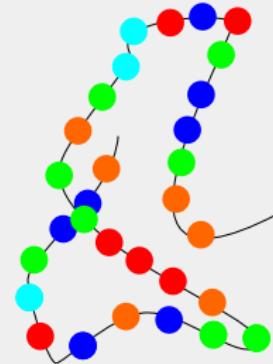
PROTEINS



Aminoacids

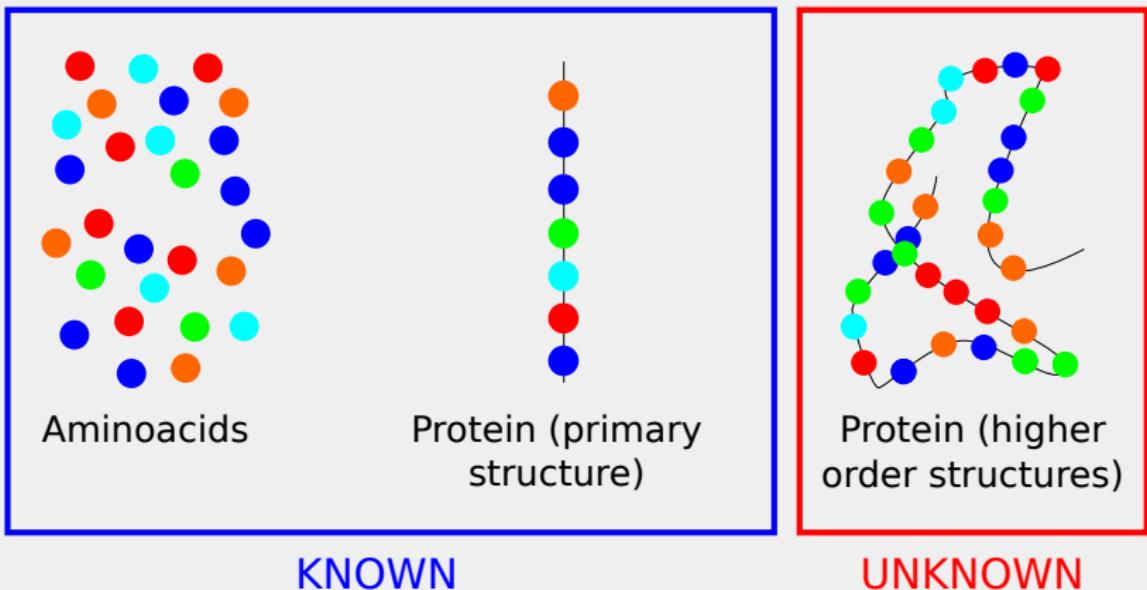


Protein (primary structure)

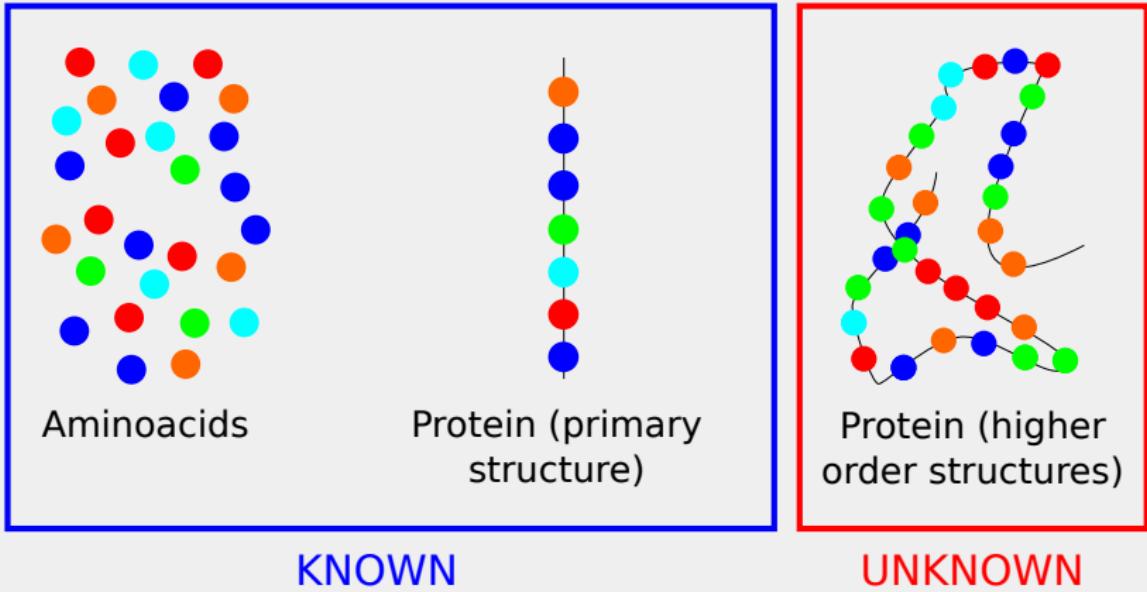


Protein (higher order structures)

PROTEINS

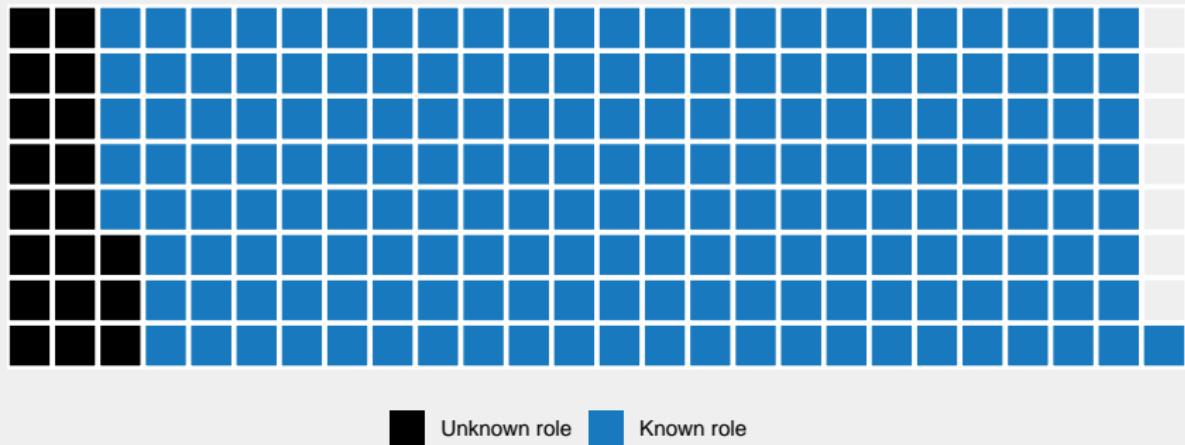


PROTEINS



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 simplified alphabets

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

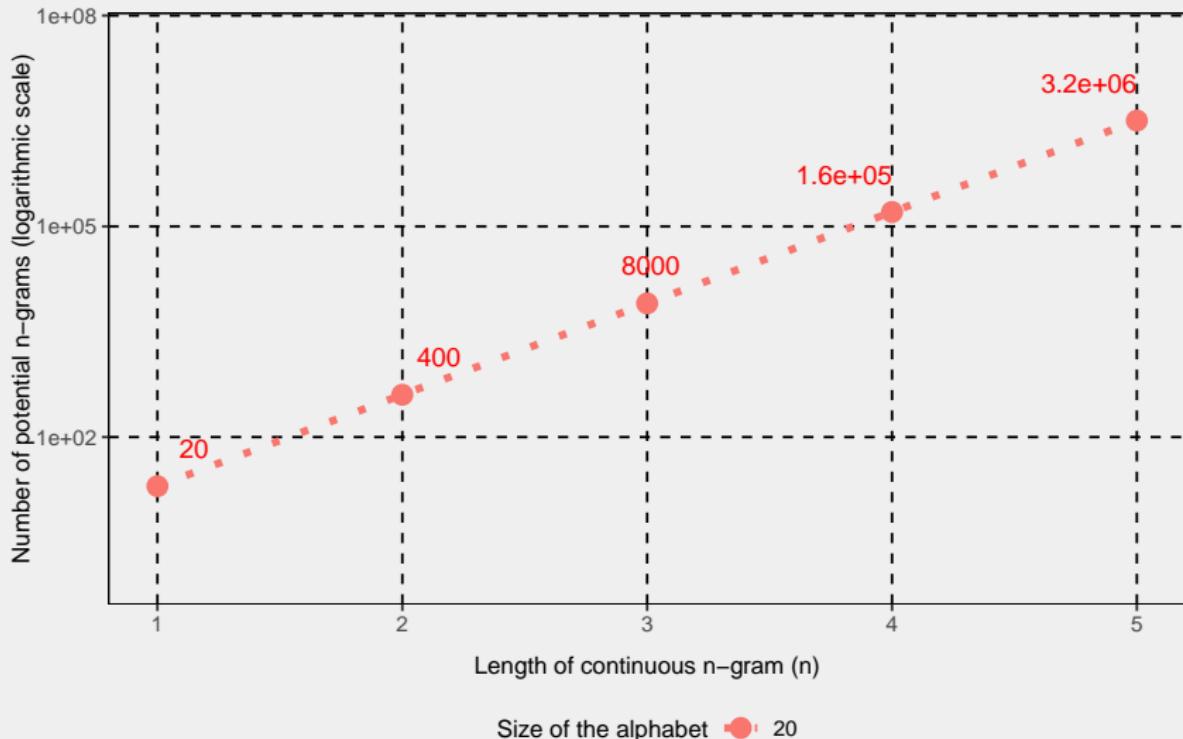
- subsequences (continuous or discontinuous) n aminoacid or nucleotide residues,
- more informative than the individual residues.

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.**

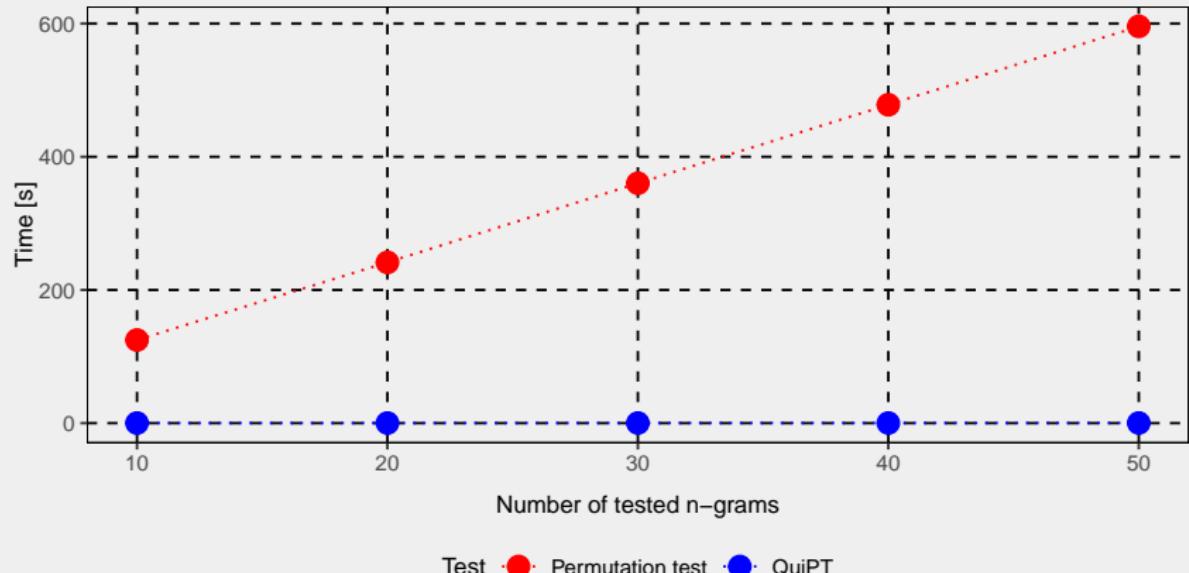


Longer n-grams are more informative, but create larger attribute spaces that are more difficult to analyze.

SLAM: SPARSE LIGHTWEIGHT ARRAYS AND MATRICES

Counting n-grams creates sparse matrices, that are causing dimensional problems.

	m	storage	value
1	1.00	base	0.000214 Mb
2	1.00	slam	0.001122 Mb
3	10.00	base	0.000969 Mb
4	10.00	slam	0.001312 Mb
5	100.00	base	0.0765 Mb
6	100.00	slam	0.002625 Mb
7	1000.00	base	7.629601 Mb
8	1000.00	slam	0.016357 Mb
9	10000.00	base	762.939659 Mb
10	10000.00	slam	0.153687 Mb



QuiPT (available 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

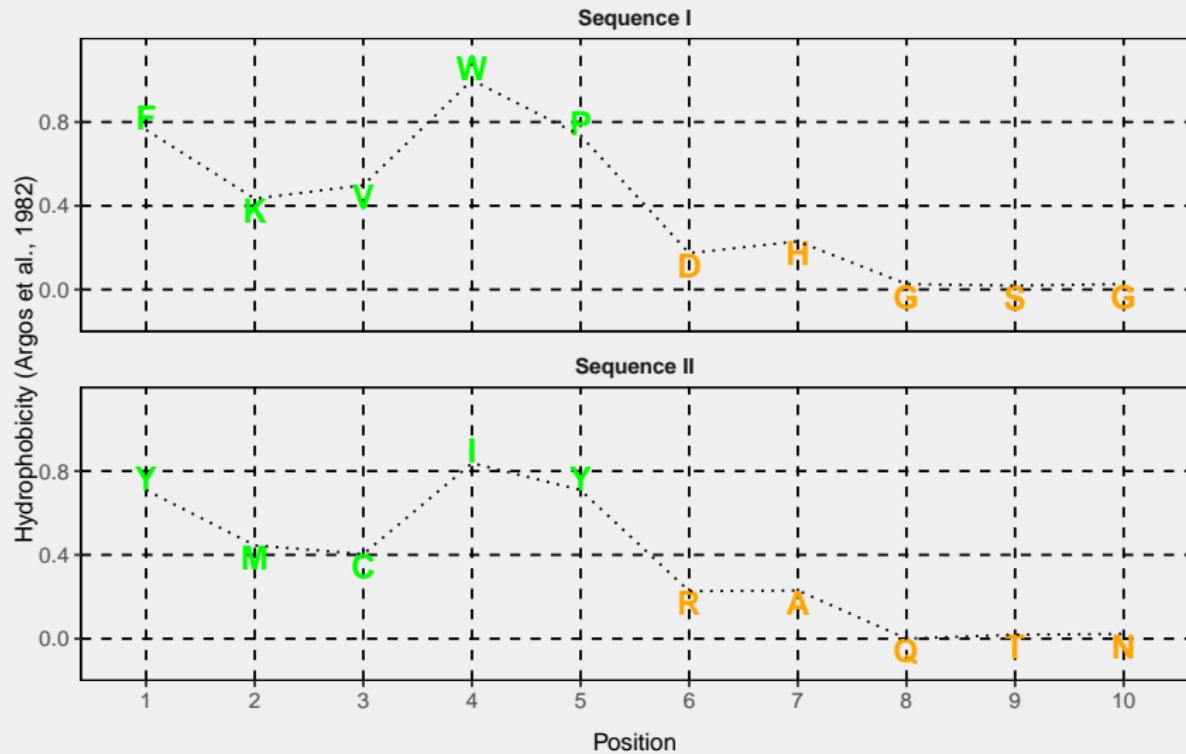
Following peptides appear to be completely different in terms of amino acid composition.

Peptide I:

FKVWPDHGSG

Peptide II:

YMCIYRAQTN



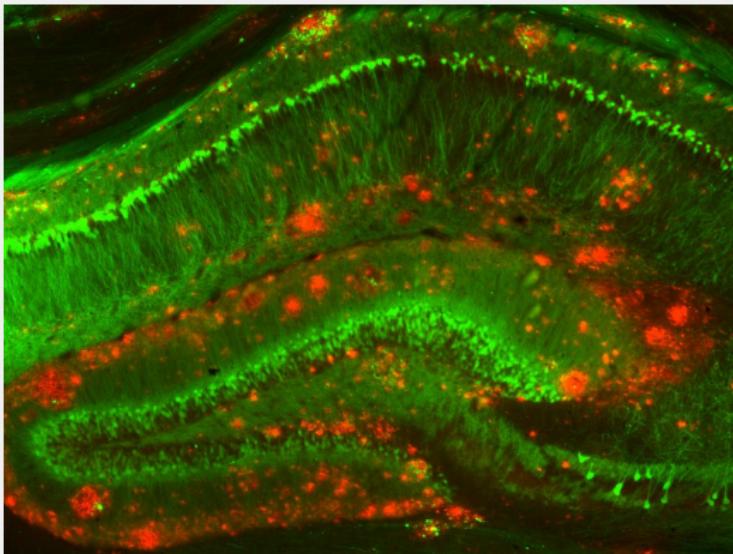
Group	Aminoacids
1	C, I, L, K, M, F, P, W, Y, V
2	A, D, E, G, H, N, Q, R, S, T

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

Amyloid prediction

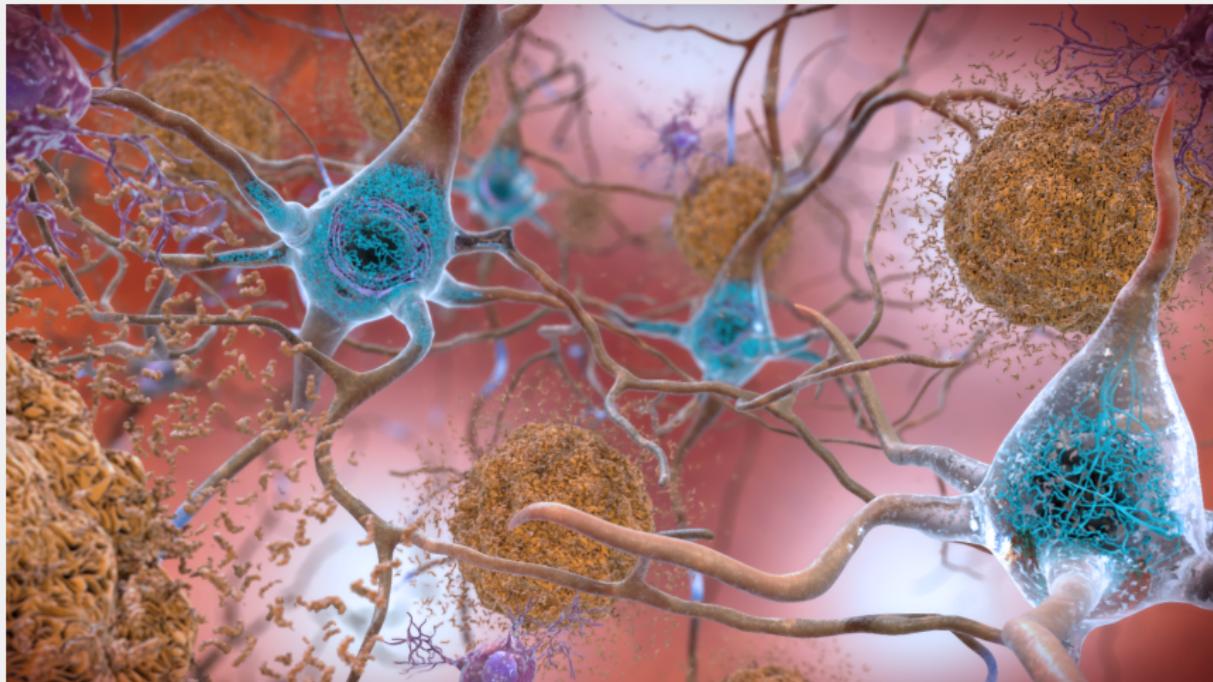
AMYLOIDS

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.

AMYLOIDS

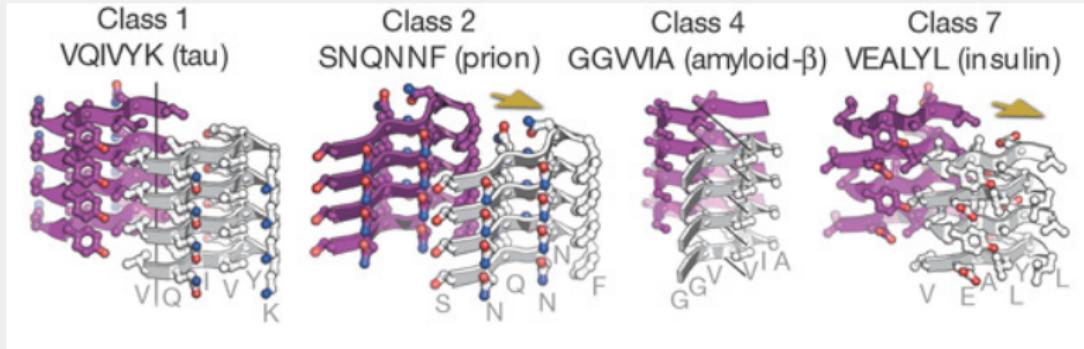


Source: National Institute on Aging (NIA) | National Institutes of Health (NIH)

AMYLOID PROTEINS

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

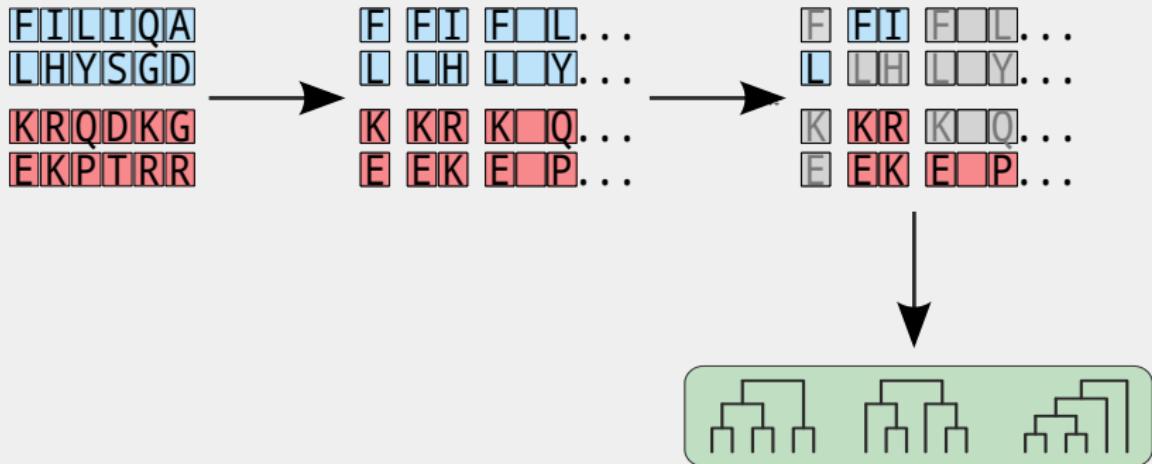
- short (6-15 aminoacids),
- very variable, usually hydrophobic, aminoacid composition,
- create unique β -structures.



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

RANGER: A FAST IMPLEMENTATION OF RANDOM FORESTS

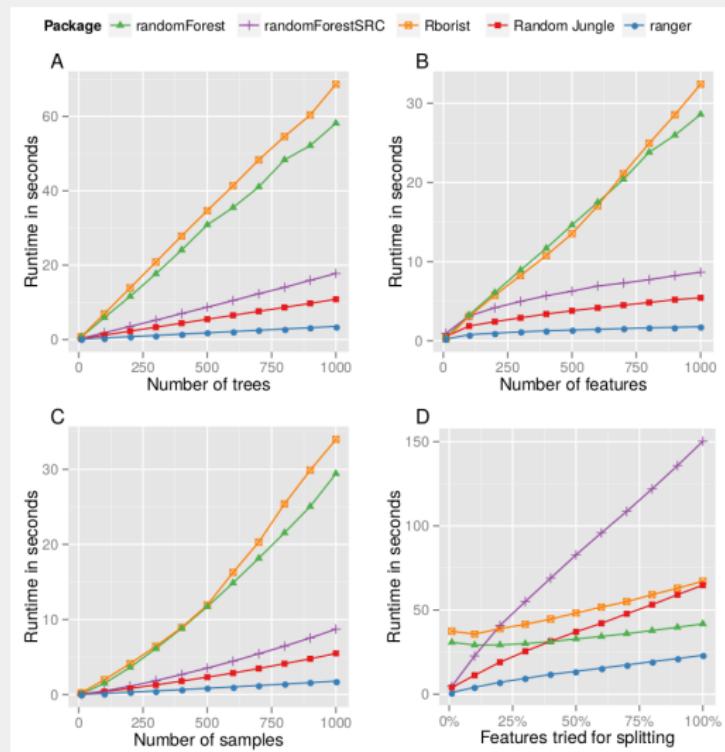
Package	Runtime [h]			Memory usage [GB]
	mtry=	5000	15,000	
randomForest	101.24	116.15	248.60	39.05
randomForest (MC)	32.10	53.84	110.85	105.77
bigrf	NA	NA	NA	NA
randomForestSRC	1.27	3.16	14.55	46.82
Random Jungle	1.51	3.60	12.83	0.40
Rborist	NA	NA	NA	>128
ranger	0.56	1.05	4.58	11.26
ranger (save.memory)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

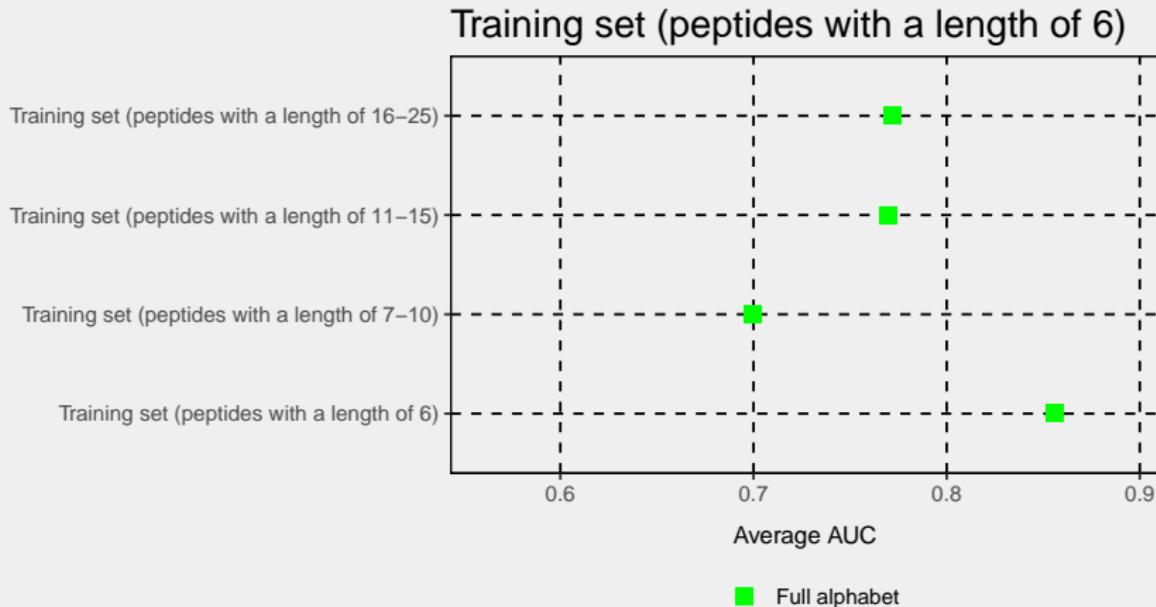
without disk caching failed because of memory shortage for all mtry values and number of CPU cores.

With disk caching, we stopped bigrf after 16 days of computation.

RANGER: A FAST IMPLEMENTATION OF RANDOM FORESTS



CROSS-VALIDATION

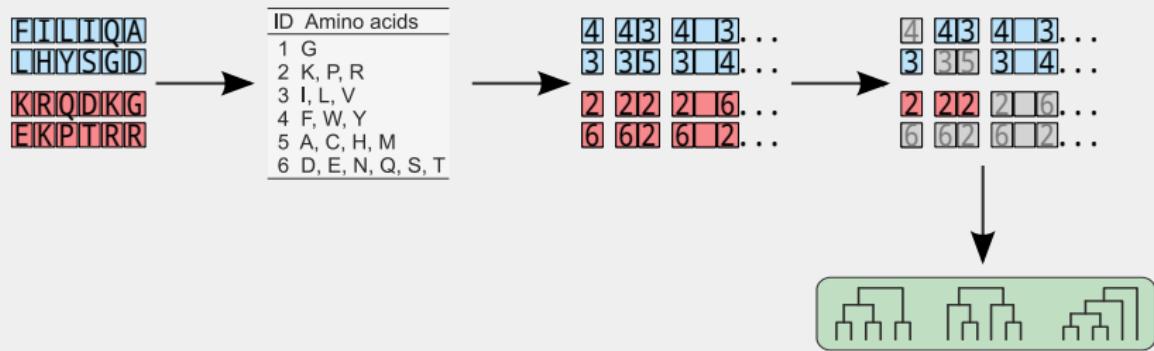


Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. *Scientific Reports* 7, 12961

STANDARD SIMPLIFIED ALPHABETS

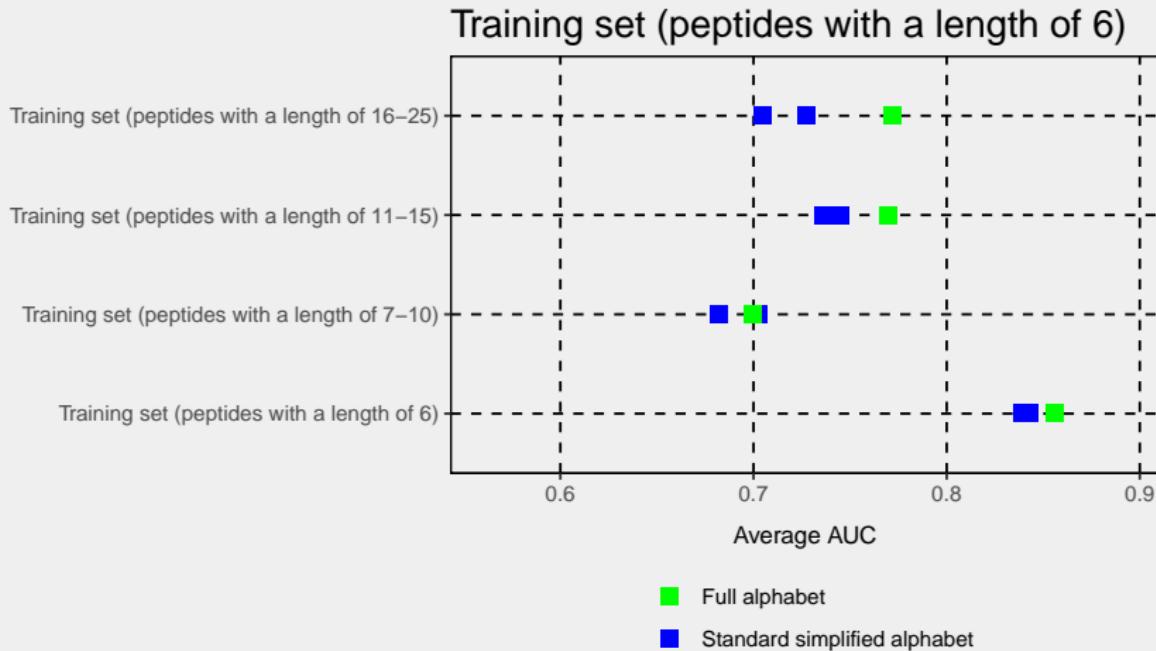
Do standard simplified alphabets developed for different biological issues help to improve amyloid prediction?

STANDARD SIMPLIFIED 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 SIMPLIFIED ALPHABET



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

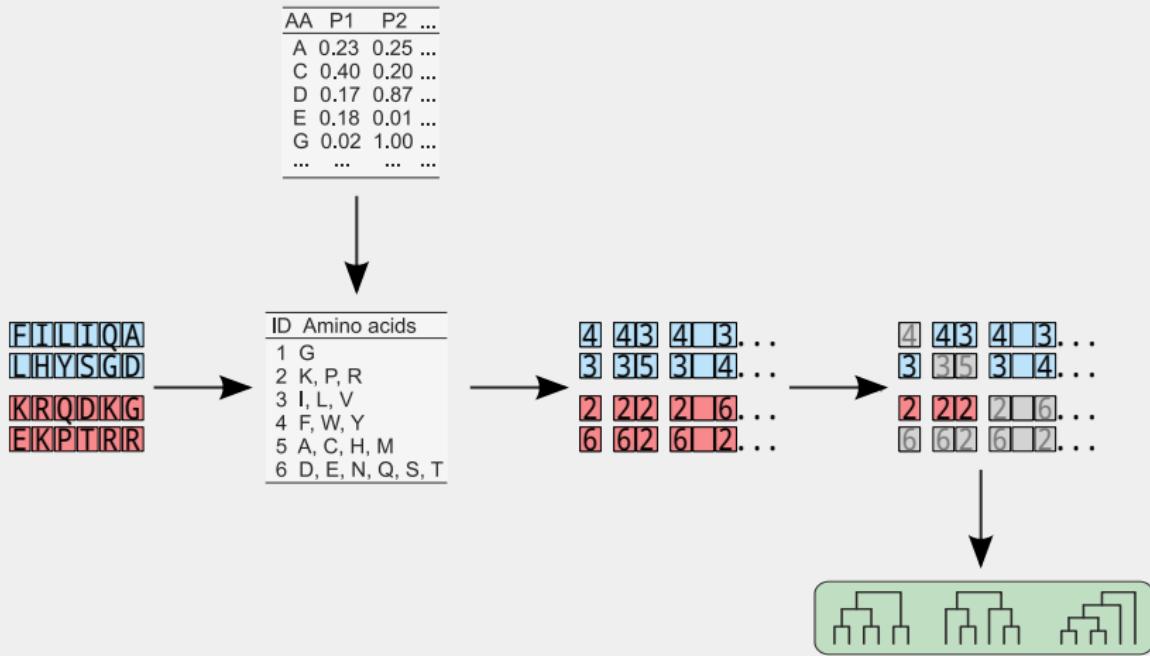
Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. *Scientific Reports* 7, 12961

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)

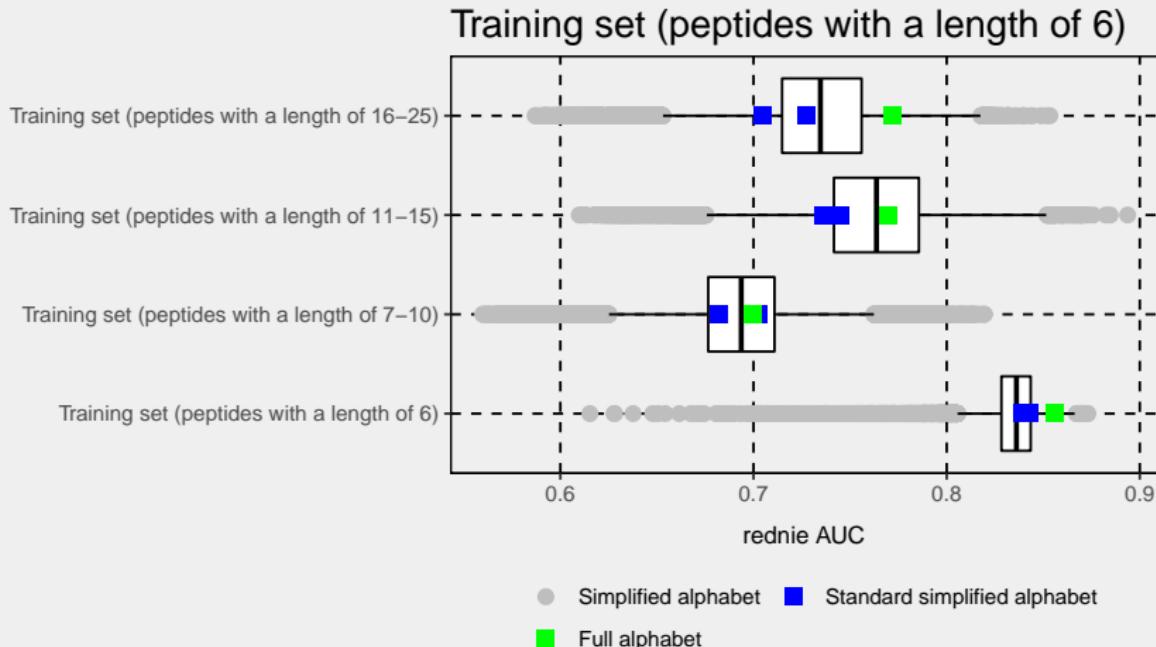
Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. *Scientific Reports* 7, 12961

NEW SIMPLIFIED ALPHABETS



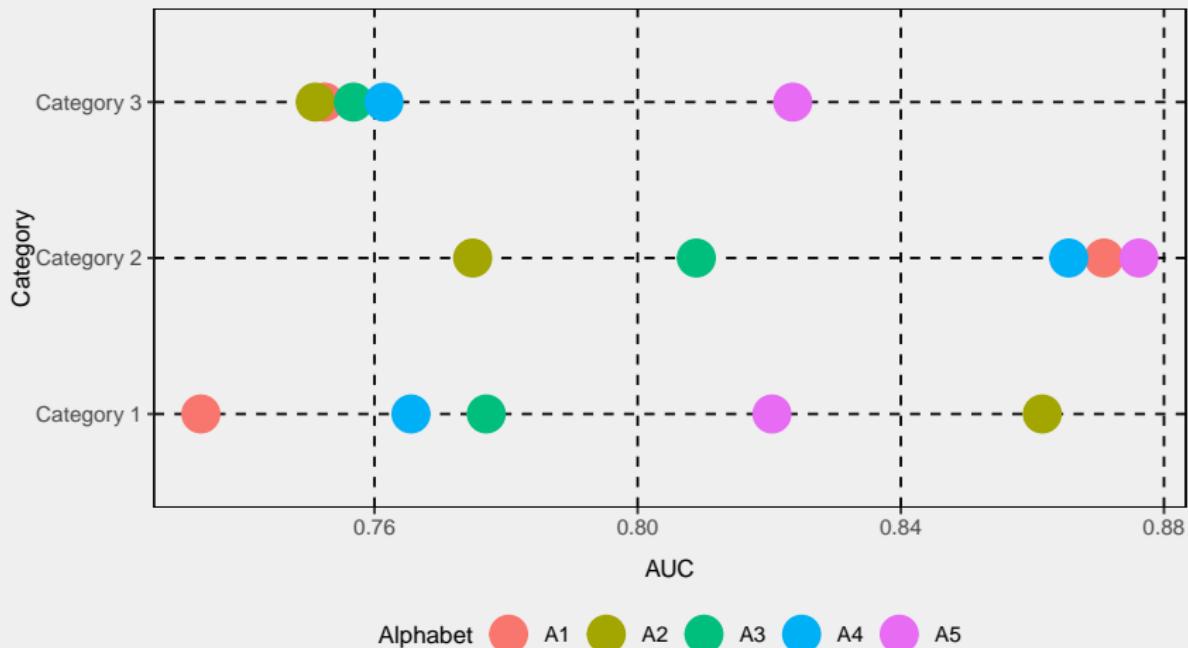
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STANDARD SIMPLIFIED ALPHABET

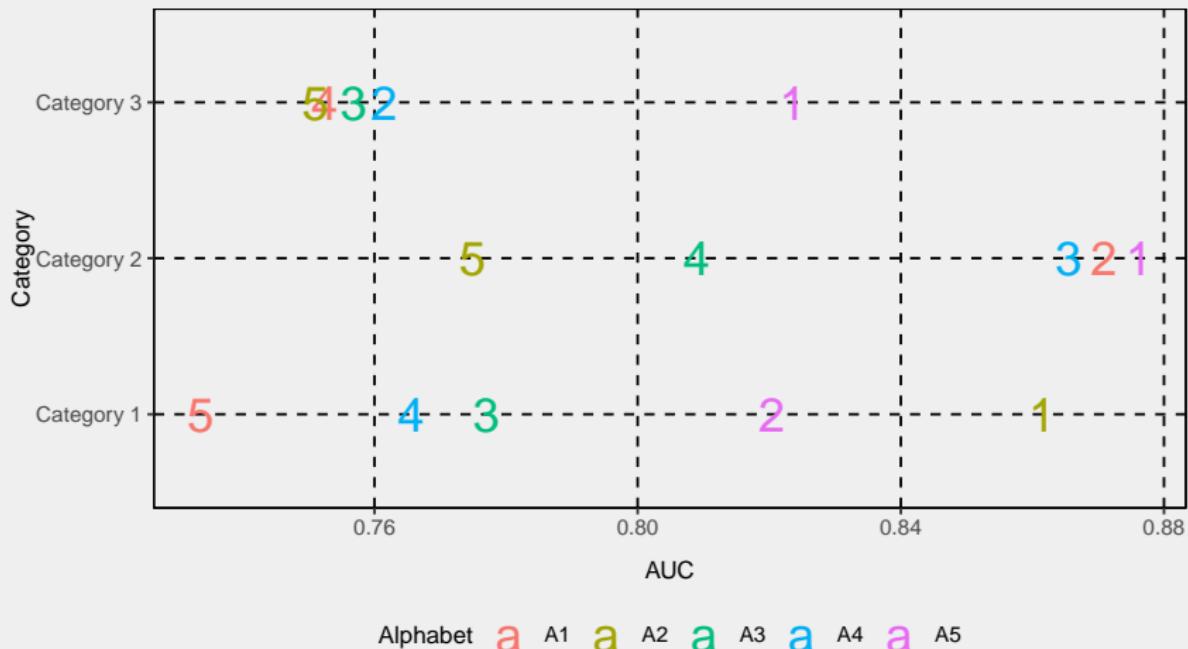


Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. *Scientific Reports* 7, 12961

SELECTION OF BEST-PERFORMING SIMPLIFIED ALPHABET

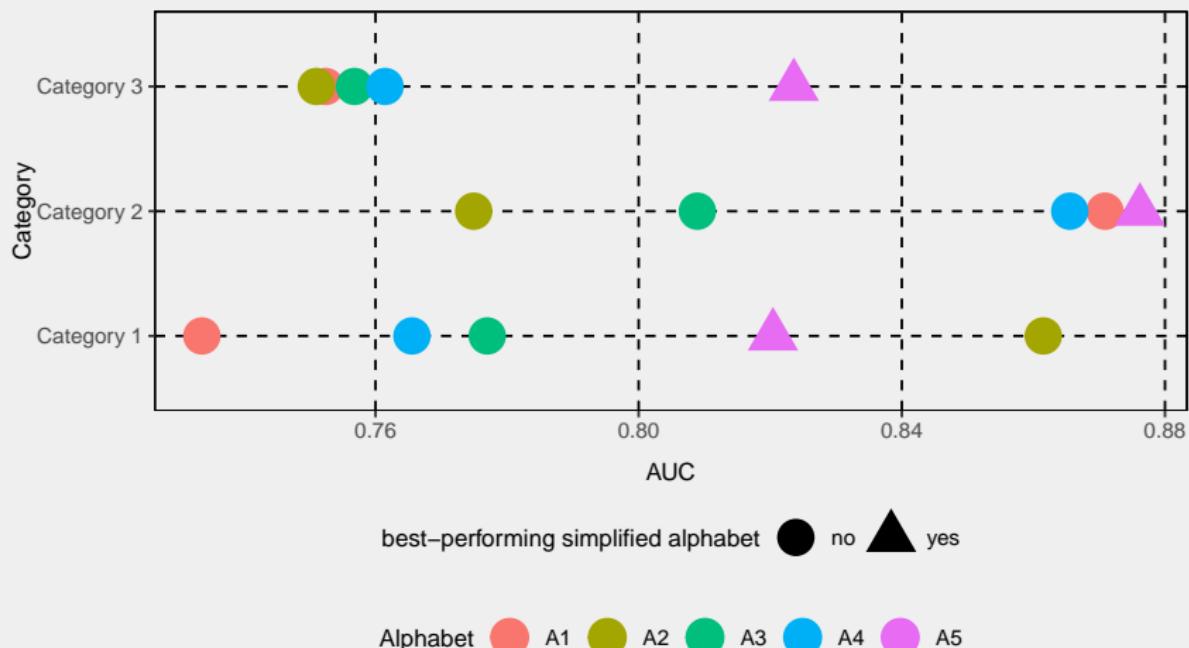


SELECTION OF BEST-PERFORMING SIMPLIFIED ALPHABET



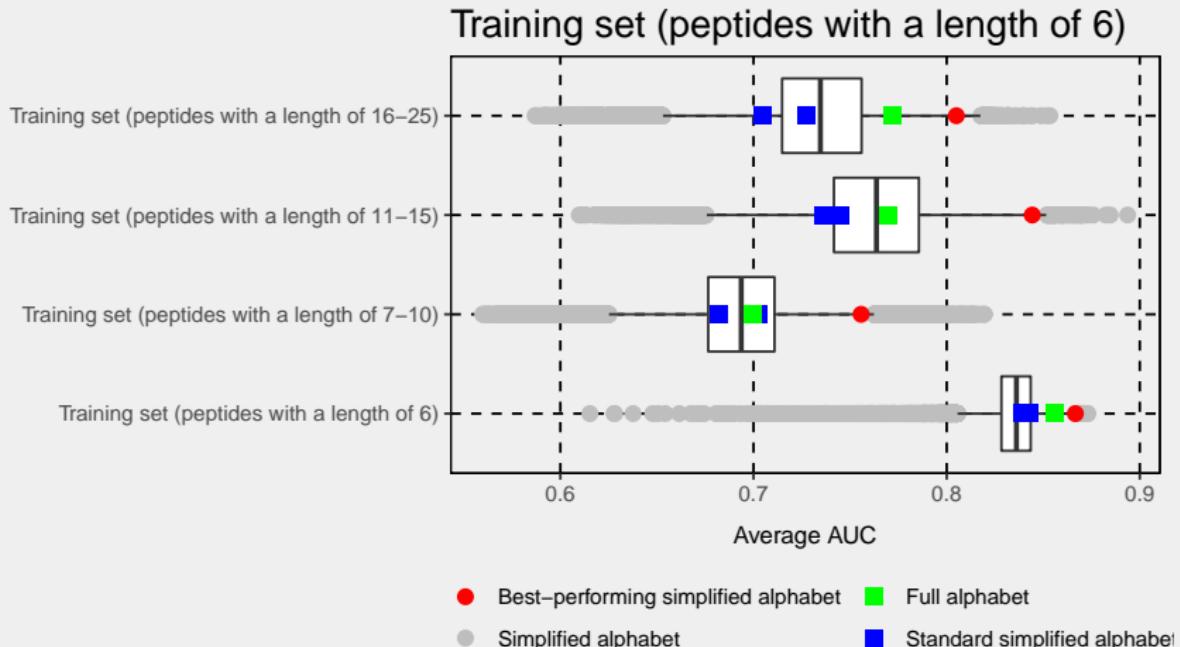
For each category the alphabets have been ranked (rank 1 for the best AUC, etc.)

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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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

Grupy 3 i 4 - hydrophobic aminoacids.

BEST-PERFORMING SIMPLIFIED ALPHABET

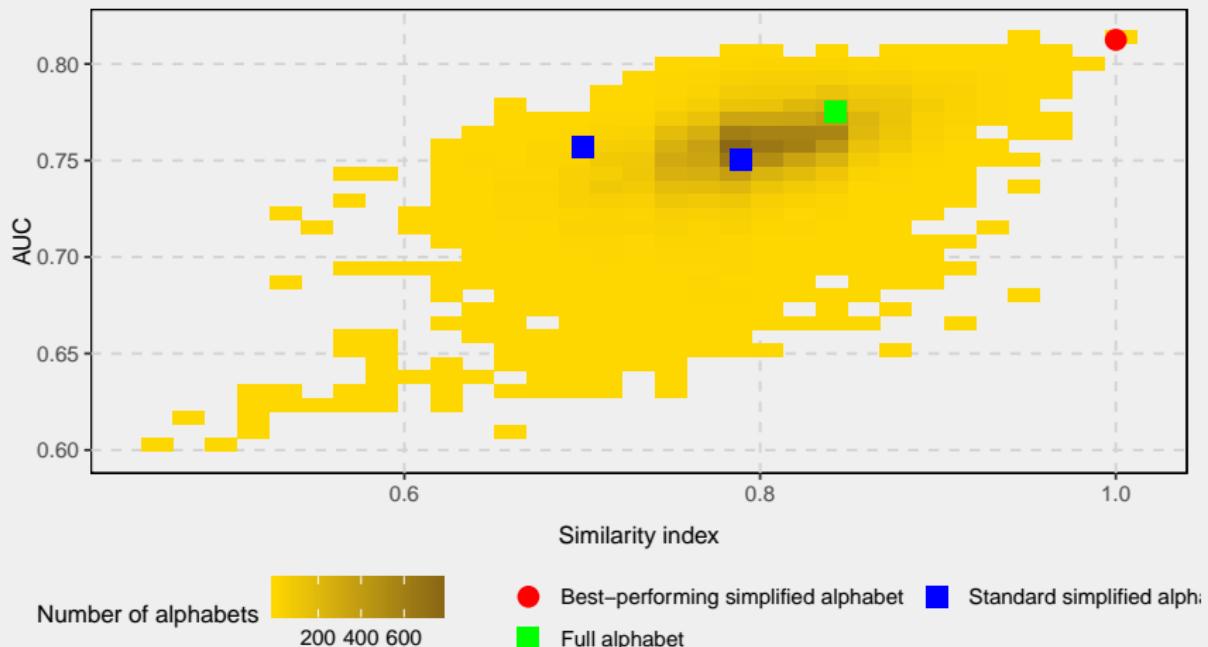
Group	Aminoacids
1	G
2	K, P, R
3	I, L, V
4	F, W, Y
5	A, C, H, M
6	D, E, N, Q, S, T

Group 2 - aminoacids disrupting the β -structure.

ALPHABET SIMILARITY AND QUALITY OF PREDICTION

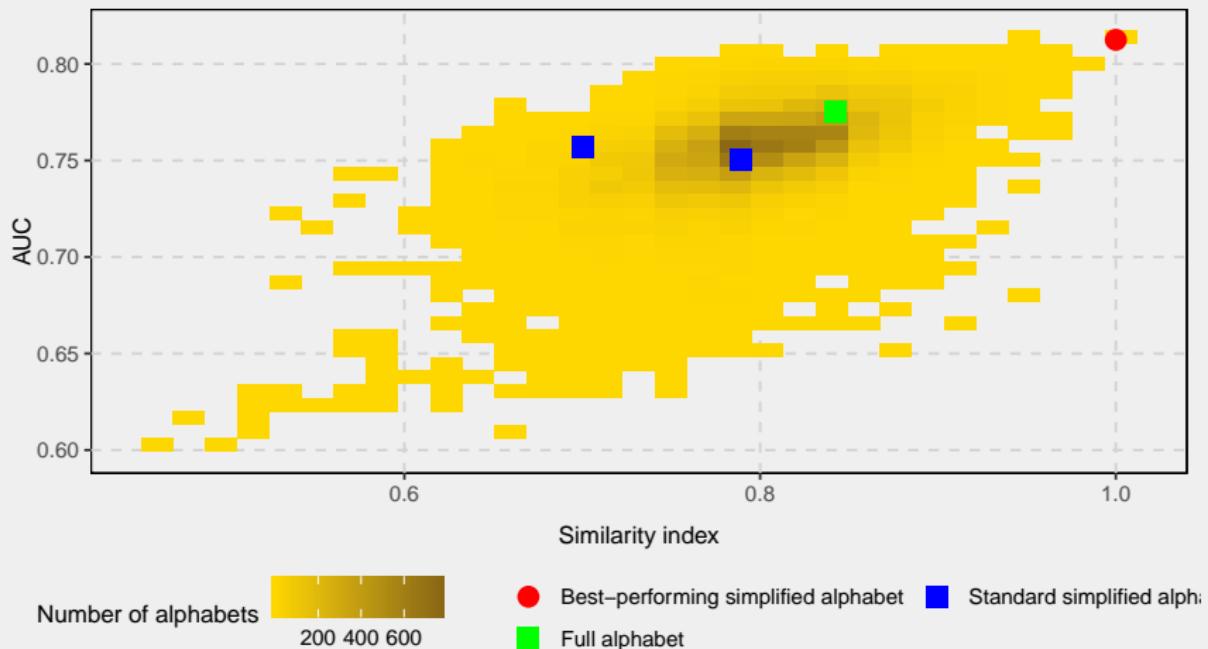
Do alphabets similar to the best simplified alphabet also support amyloid predictions?

SIMILARITY INDEX



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

SIMILARITY INDEX

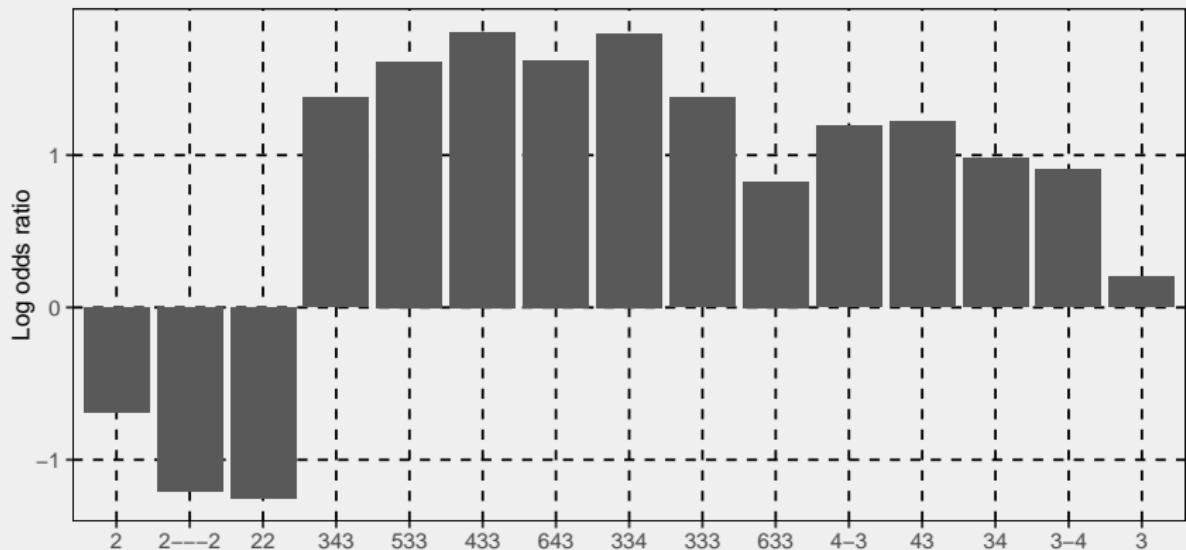


The correlation between the similarity index and the average AUC is important ($p\text{-value} \leq 2.2^{-16}$; $\rho = 0.51$).

Burdzkiekiewicz, 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).

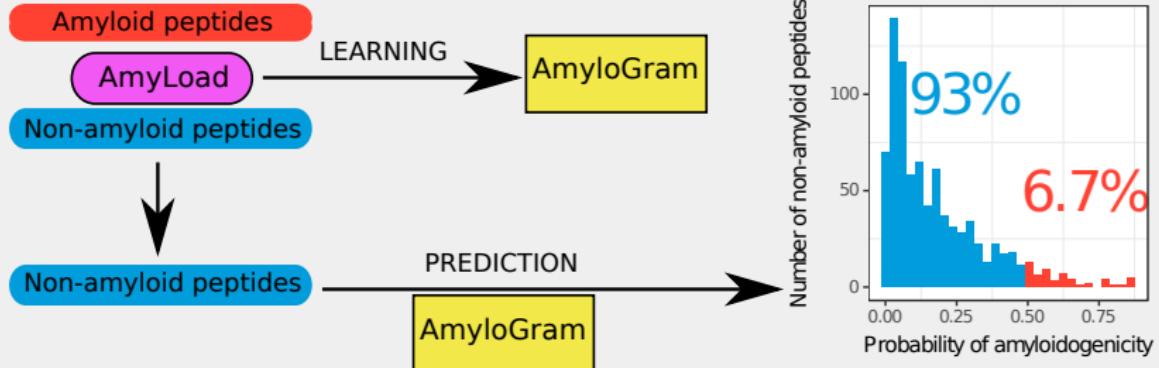
Burdukiewicz, M., Sobczyk, P., Rödiger, S., Duda-Madej, A., Mackiewicz, P., and Kotulska, M. (2017). Amyloidogenic motifs revealed by n-gram analysis. *Scientific Reports* 7, 12961

BENCHMARK WITH OTHER SOFTWARE

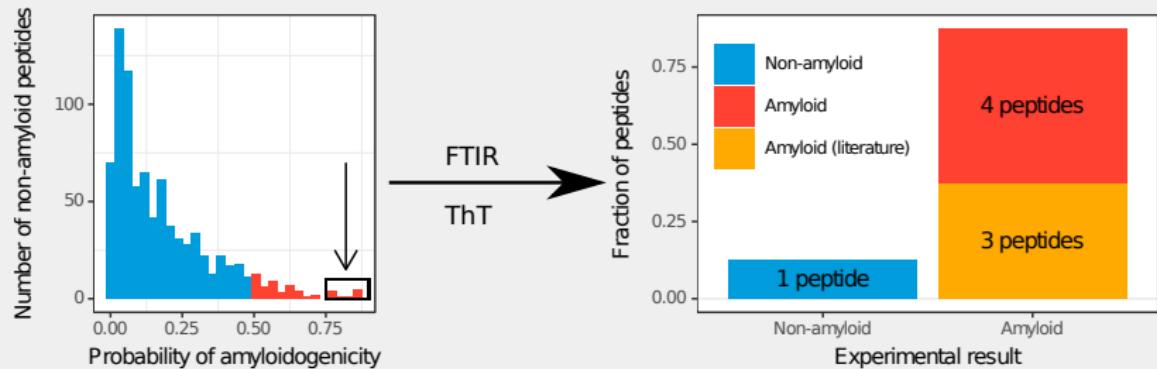
Program	AUC	MCC
AmyloGram	0.8972	0.6307
PASTA 2.0 (Walsh et al., 2014)	0.8550	0.4291
FoldAmyloid (Garbuzyntsiy et al., 2010)	0.7351	0.4526
APPNN (Família et al., 2015)	0.8343	0.5823

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

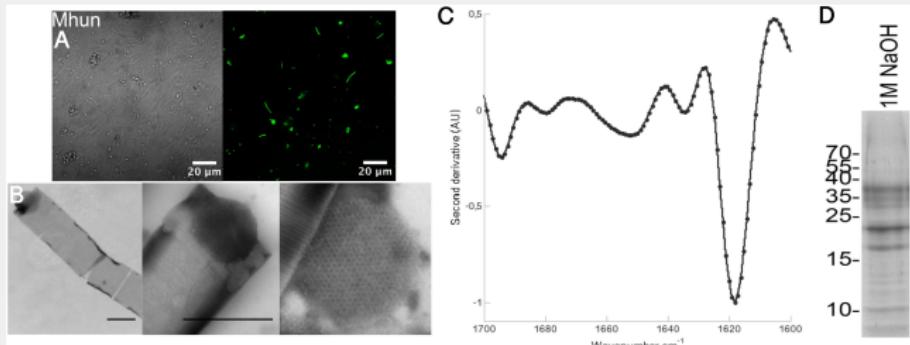
EXPERIMENTAL VALIDATION



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 application

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: Michał Burdakiewicz, Piotr Sobczyk.

Citation: Burdakiewicz, 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  
>Ado-2h  
VPSNEEQIKNLLQLEAQEHLQY  
>AMY138|Alpha 6(Glutathione S Transeferase P domain  
QISFADVNLLDLLRIHQVLN  
>AMY143|M8|Spectrin SH3  
DILTLNLNSTNKDWKKVEVND  
>CsgA|region 1  
SELNIYQYGGGN SALALQTDARN
```

Paste sequences (FASTA format required) here...

Submit data from field above

Submit .fasta or .txt file:

Browse... No file selected

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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	Sensitivity: 0.8658
	Specificity: 0.7852
	MCC: 0.6268

[Start a new query](#)

Results (tabular) [Detailed results](#)

Copy CSV Excel Print

Input name	Amyloid probability	Is amyloid?
All	All	All
AMY133 Alpha	0.6725	yes
Ada-2h	0.4702	no
AMY138 Alpha	0.7515	yes
AMY143 M8 Spectrin	0.6488	yes
CsgA region	0.8216	yes

Showing 1 to 5 of 5 entries

Previous 1 Next

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Authors: Michał Burakiewicz, Piotr Solcik.

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

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Cutoff
 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

All All

Protein	Fraction of amyloid residues
AMY1331Alpha	0.6364
Ada-2h	0.0000
AMY138Alpha	0.7500
AMY141MB/Spectrin	0.3500
CspARegion	0.6087

Showing 1 to 5 of 5 entries

Previous 1 Next

Amyloid regions

Probability of amyloidicity

Position

SUMMARISE()

Web servers:

- AmyloGram: <http://www.smorfland.uni.wroc.pl/shiny/AmyloGram/>.

R packages:

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

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.

ACKNOWLEDGEMENTS

- Michał Burdukiewicz (Politechnika Warszawska).
- Stefan Rödiger (Brandenburg University of Technology Cottbus-Senftenberg).
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- Piotr Sobczyk (Politechnika Wrocławska).

ACKNOWLEDGEMENTS

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- KNOW Wrocław Center for Biotechnology.
- German Federal Ministry of Education and Research (InnoProfile-Transfer-Projekt 03IPT611X).

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