Modelling amyloidogenic scaffolds of biofilms

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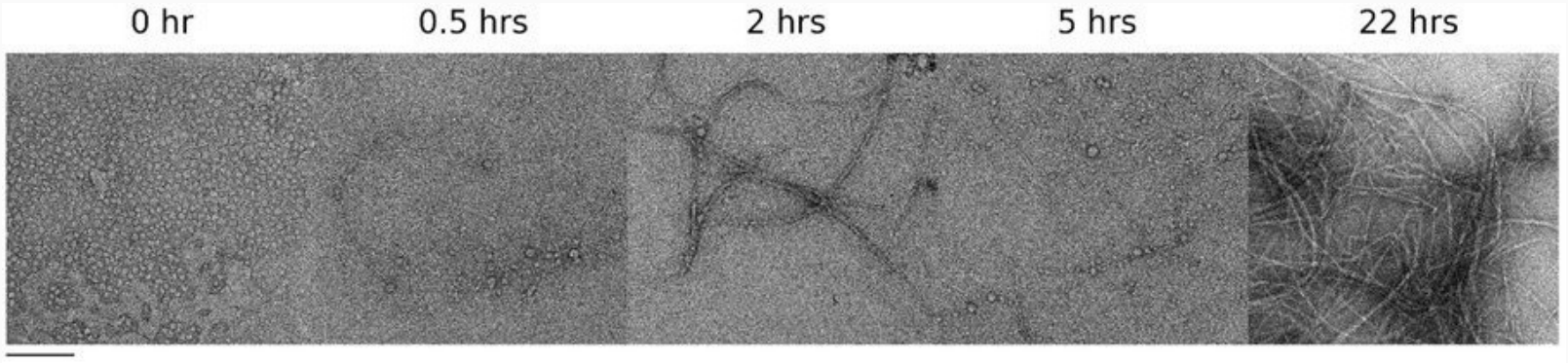
The role of amyloids in biofilm formation

Curli fibers:

- involved in adhesion to surfaces, cell aggregation, and biofilm formation.
- formed by highly aggregative structural subunits, e.g, curlin (CsgA).

CsgA fibres form through self-assembly (amyloidogenic aggregation).



Scale bar = 200nm. Taylor et al. (2016).

Three out of five very similar regions of CsgA (R1, R3 and R5) are able to aggregate on their own.

CsgA protein (E. coli K12):

43-SELNIYQYGGGNSALALQTDARN-

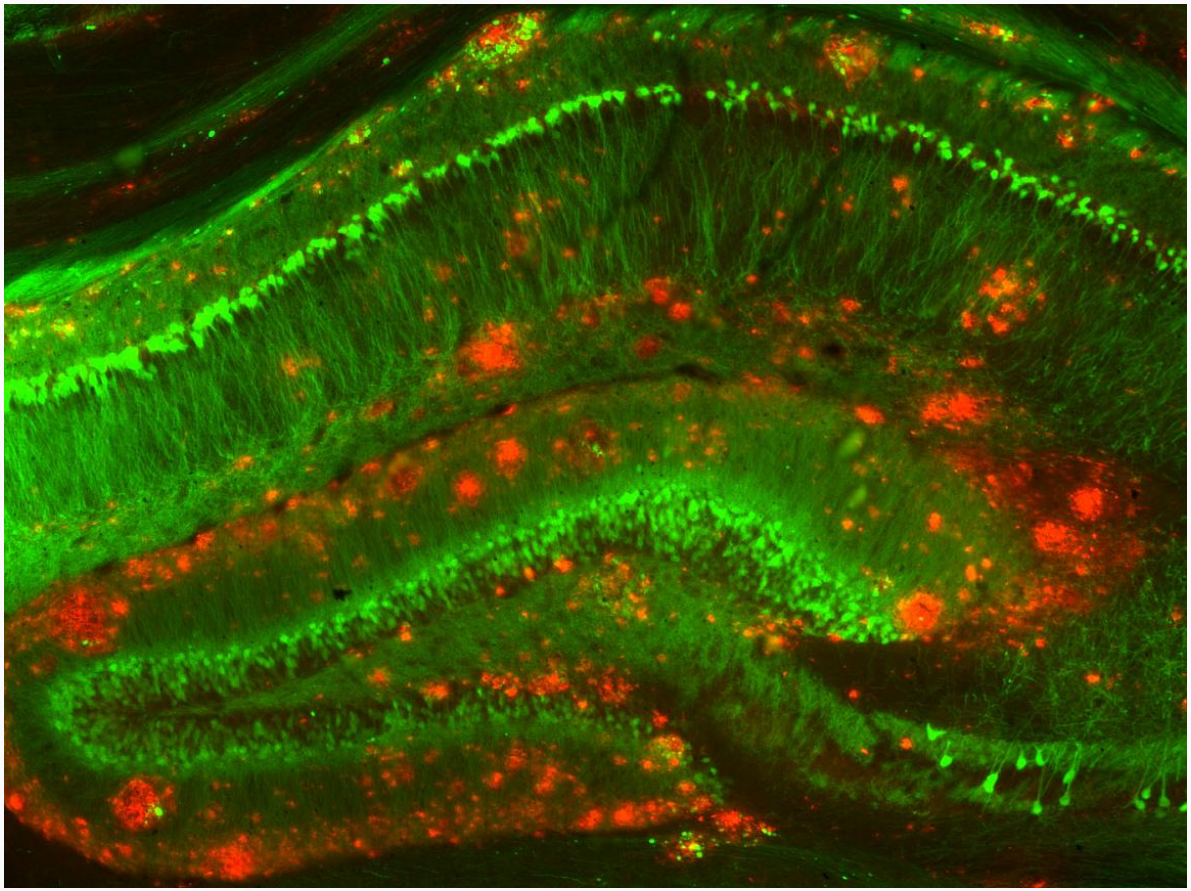
-SDLTITQHGGGNGADVGQGSDD-

-SSIDLTQRGFGNSATLDQWNGKN-

-SEMTVKQFGGGNGAAVDQTASN-

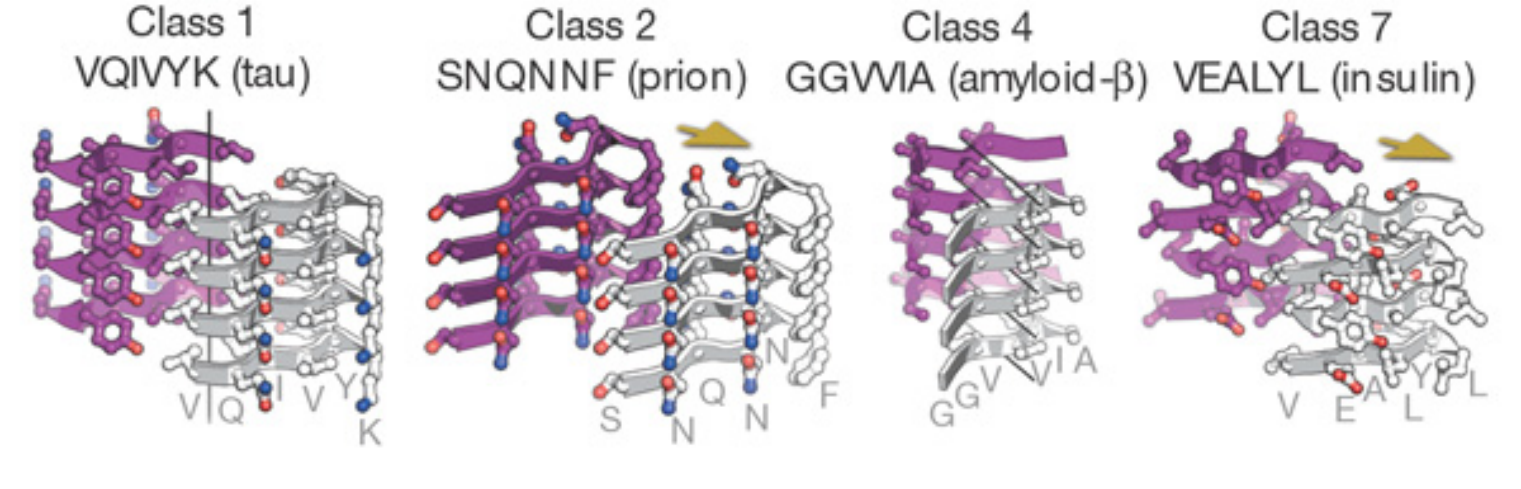
-SSVNVTQVGFGNNATAHQY

A similar aggregation mechanism is demonstrated by proteins associated with various neurodegenerative disorders (e.g., Alzheimer's, Parkinson's, Creutzfeldta-Jakob's diseases).



β-amyloid aggregates (red) around neurons (green). Strittmatter Laboratory, Yale University.

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

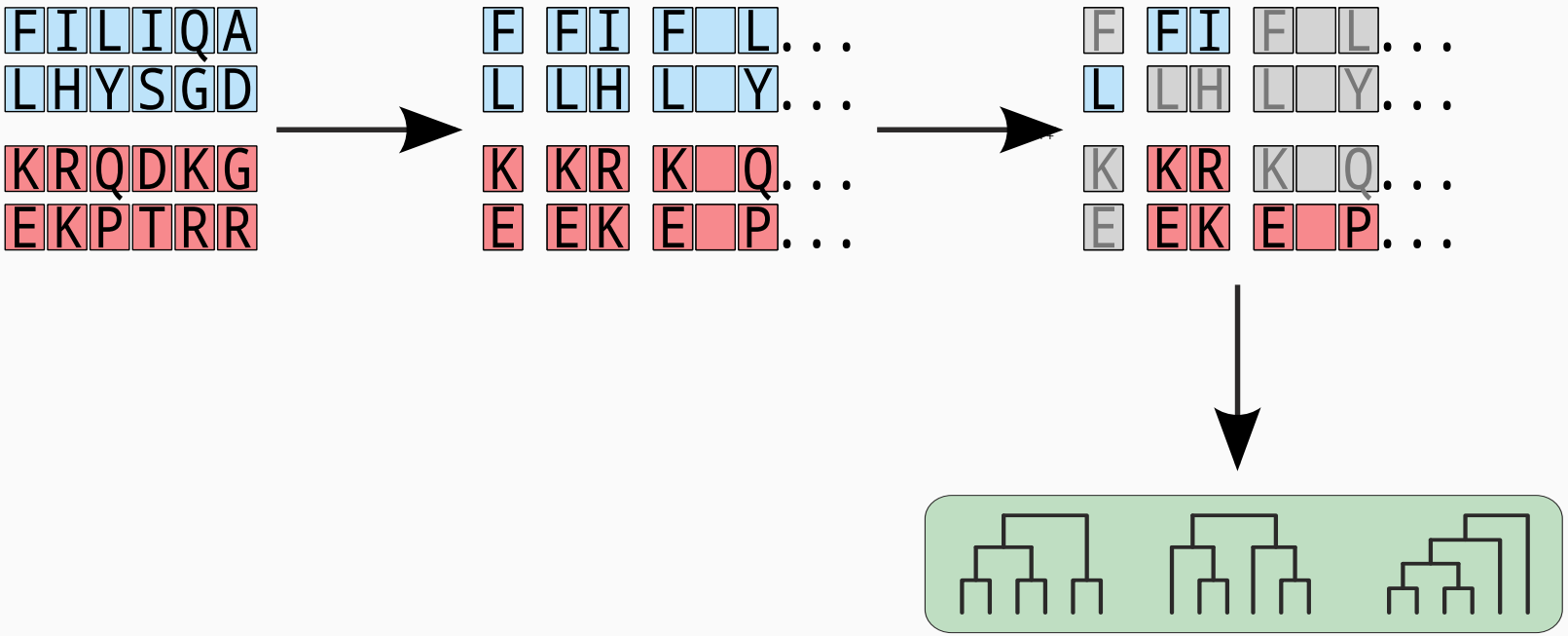


Sawaya et al. (2007)

Amyloidogenic motifs

Which motifs (n-grams, countinous or gapped subsequences of amino acids) are associated with amyloidogenicity?

Can we build a predictor of amyloidogenicity using these motifs?

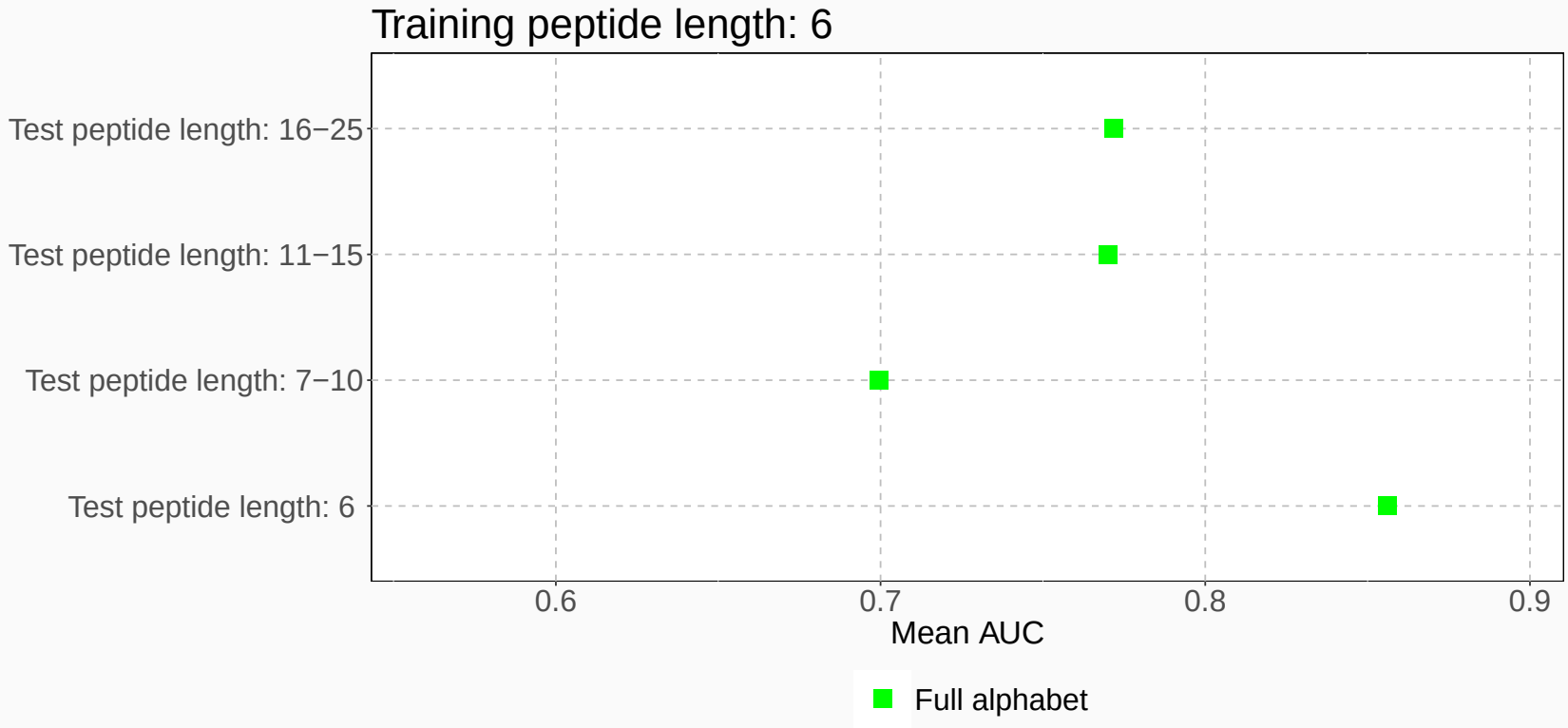

FILIQA
LHYSGD
KRQDKG
EKPTRR
F FI F L...
L LH L Y...
K KR K Q...
E EK E P...
F FI F L...
L LH L Y...
K KR K Q...
E EK E P...

QuiPT

Quick **P**ermutation **T**est is a fast alternative to permutation tests for motifs. It also allows more precise estimation of p-value.

QuiPT is avaible as a part of the **biogram** R package.

Cross-validation



Full alphabet: 20 standard amino acids (A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y).

Area Under the Curve (AUC): a performance measure between 0 (completely wrong predictions) and 1 (completely right predictions).

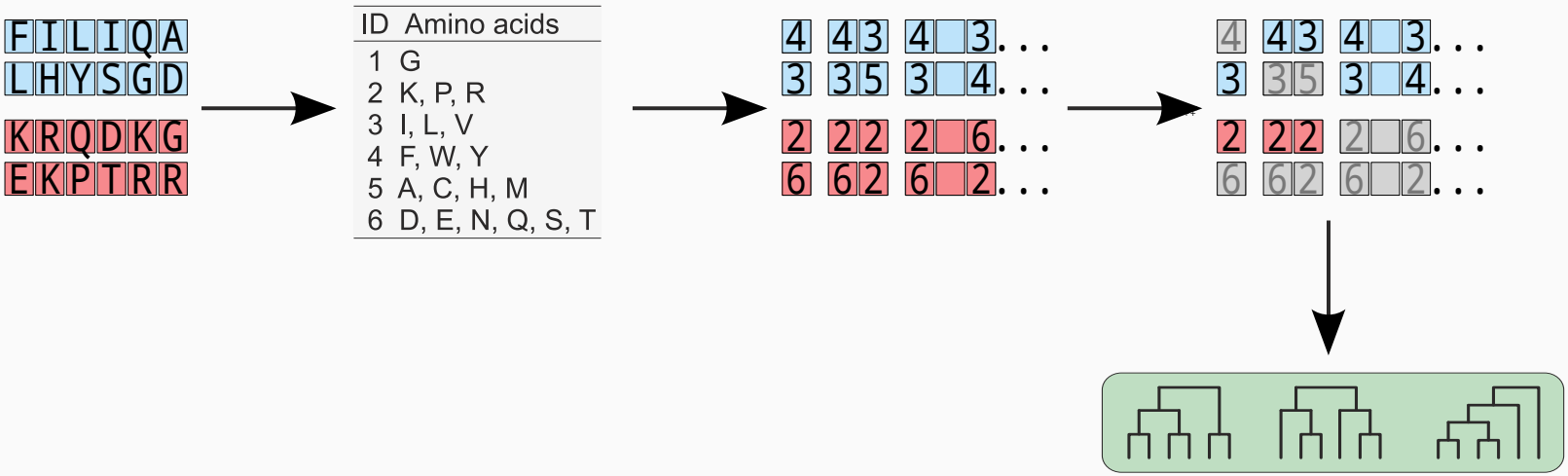
Reduced amino acid alphabets

Does amyloidogenicity depend on the exact sequence of amino acids?

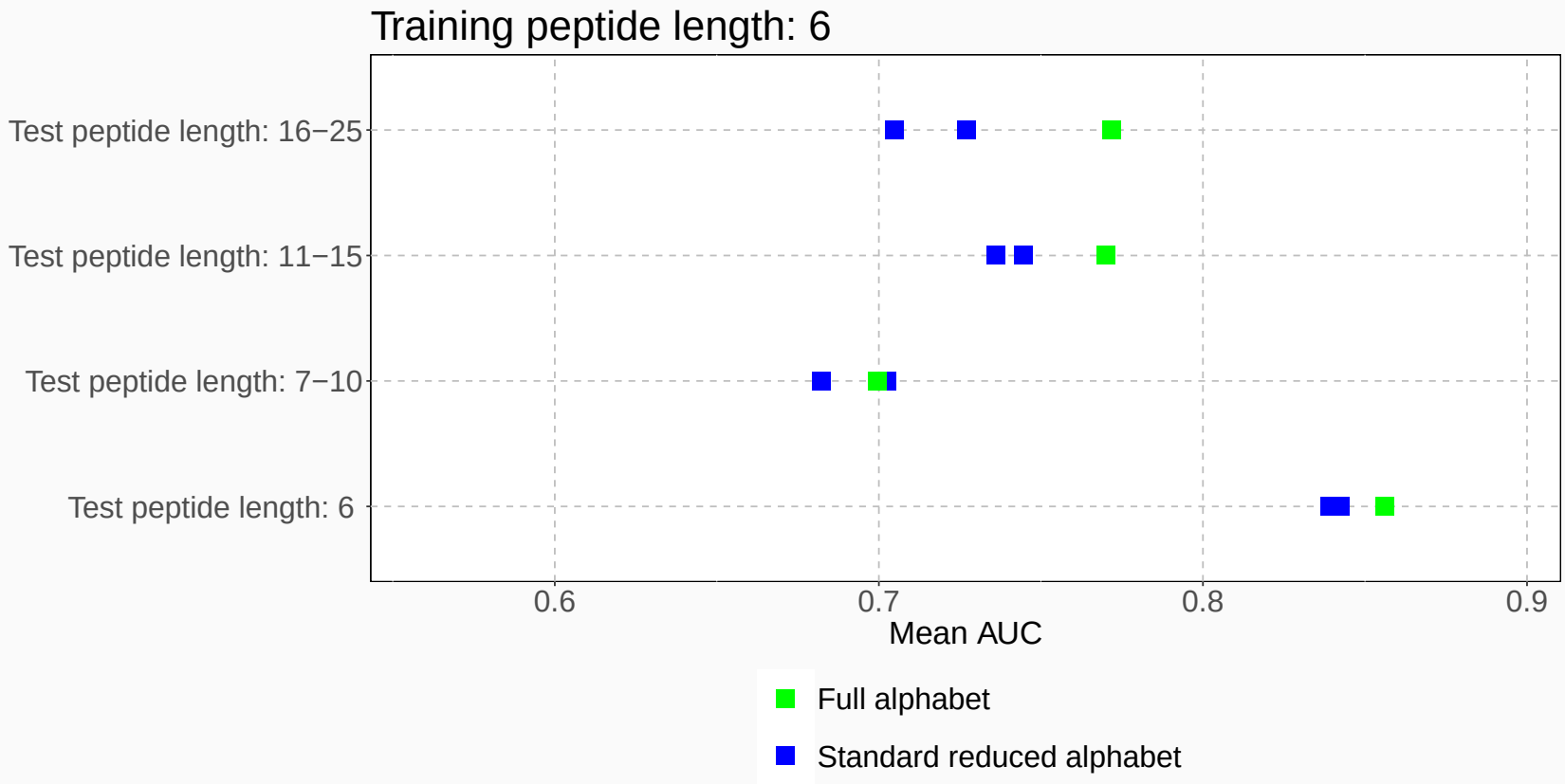
Standard reduced amino acid alphabets

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

Standard reduced amino acid alphabets



Cross-validation

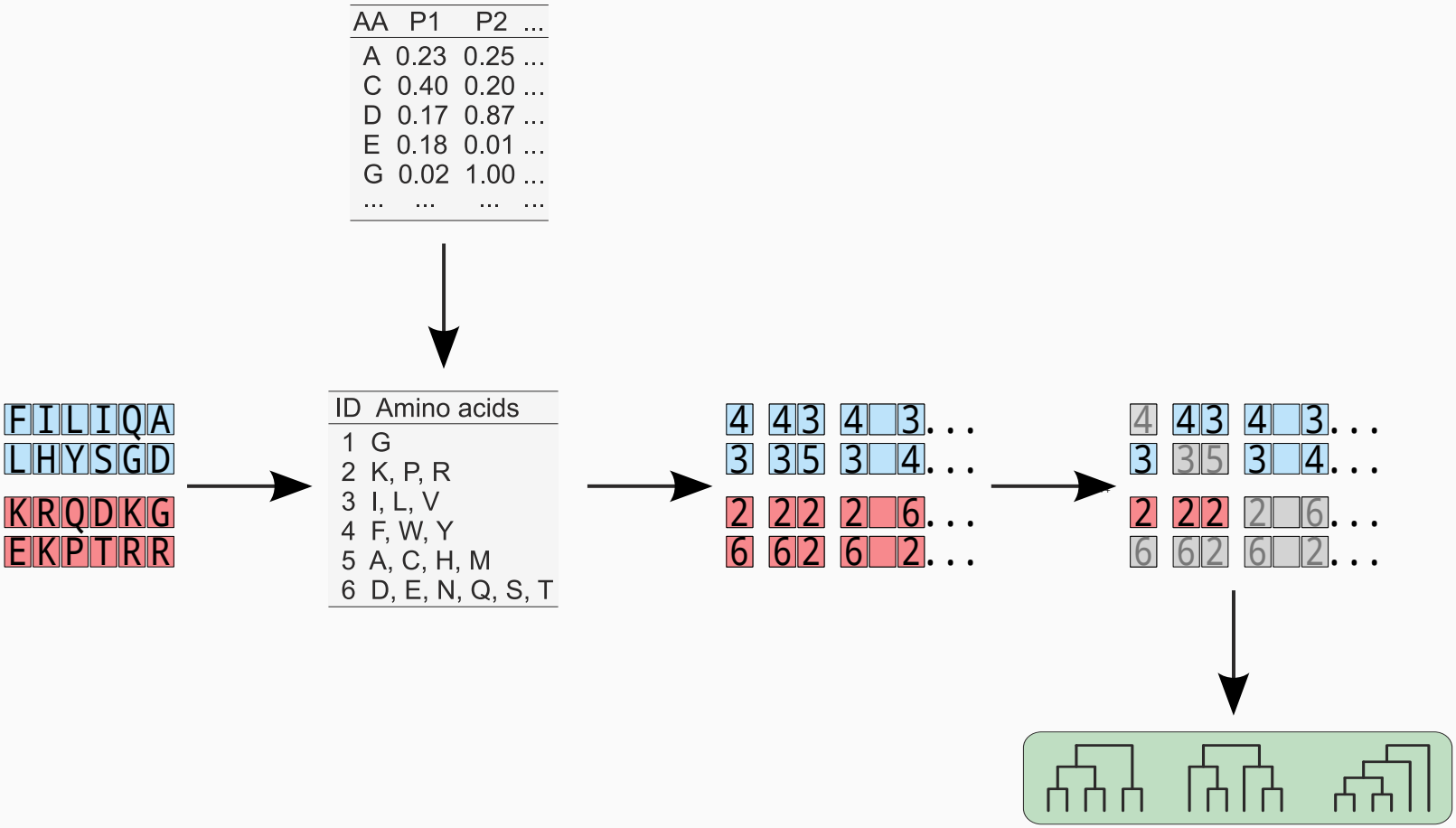


Standard reduced amino acid alphabets do not enhance discrimination between amyloidogenic and non-amyloidogenic proteins.

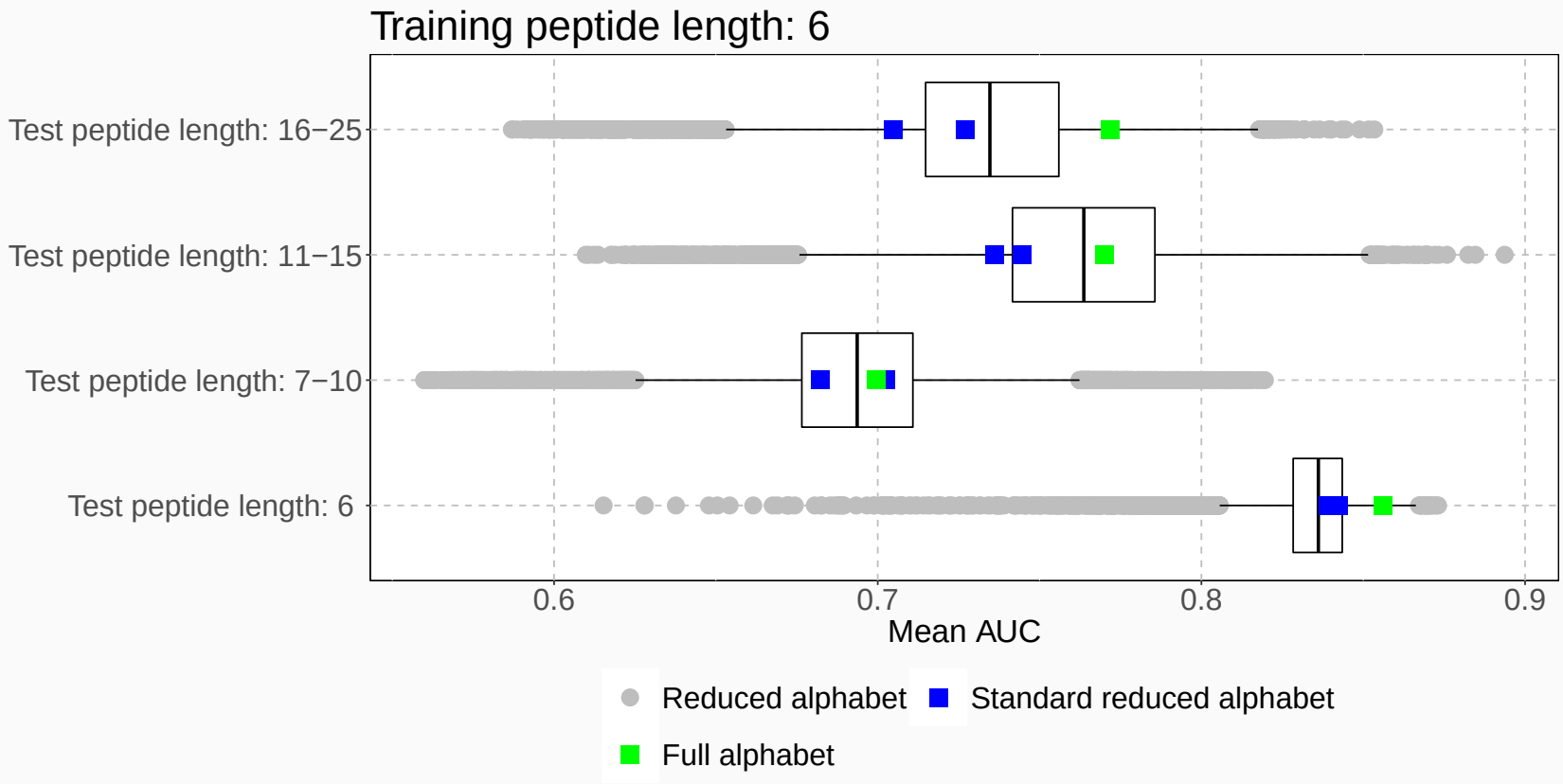
Novel reduced amino acid alphabets

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

Novel reduced amino acid alphabets

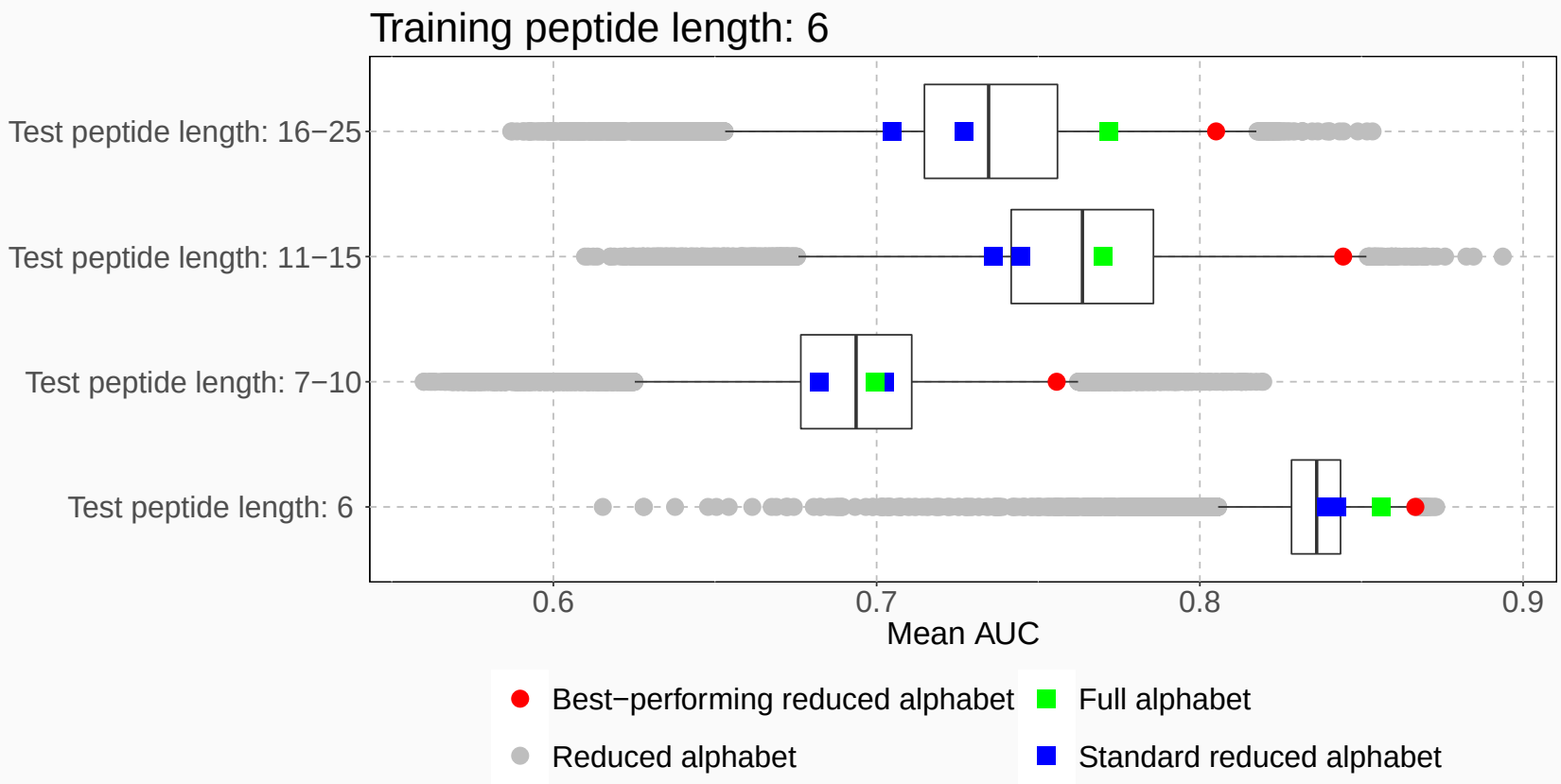


Cross-validation



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 reduced alphabets with the AUC outside the 0.95 confidence interval.

The best-perfoming reduced alphabet



Knowledge-discovery

Are groups of amino acids in the best performing reduced alphabet related to amyloidogenicity?

The best-perfoming reduced alphabet

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

The best-perfoming reduced alphabet

Subgroup ID	Amino acids
1	G
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5	A, C, H, M
6	D, E, N, Q, S, T

Group 3 and 4 - hydrophobic amino acids.

The best-perfoming reduced alphabet

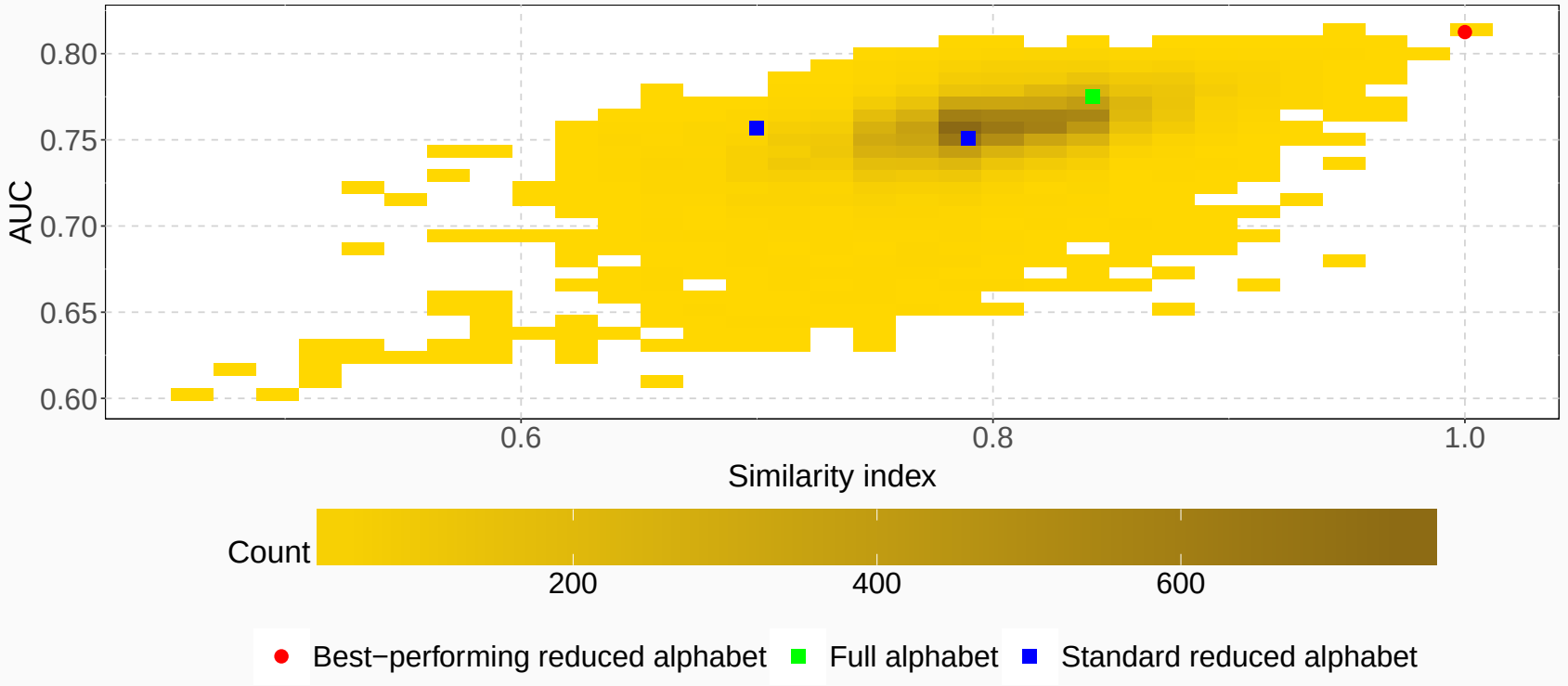
Subgroup ID	Amino acids
1	G
2	K, P, R
3	I, L, V
4	F, W, Y
5	A, C, H, M
6	D, E, N, Q, S, T

Group 2 - charged breakers of β-structures.

Alphabet similarity and performance

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

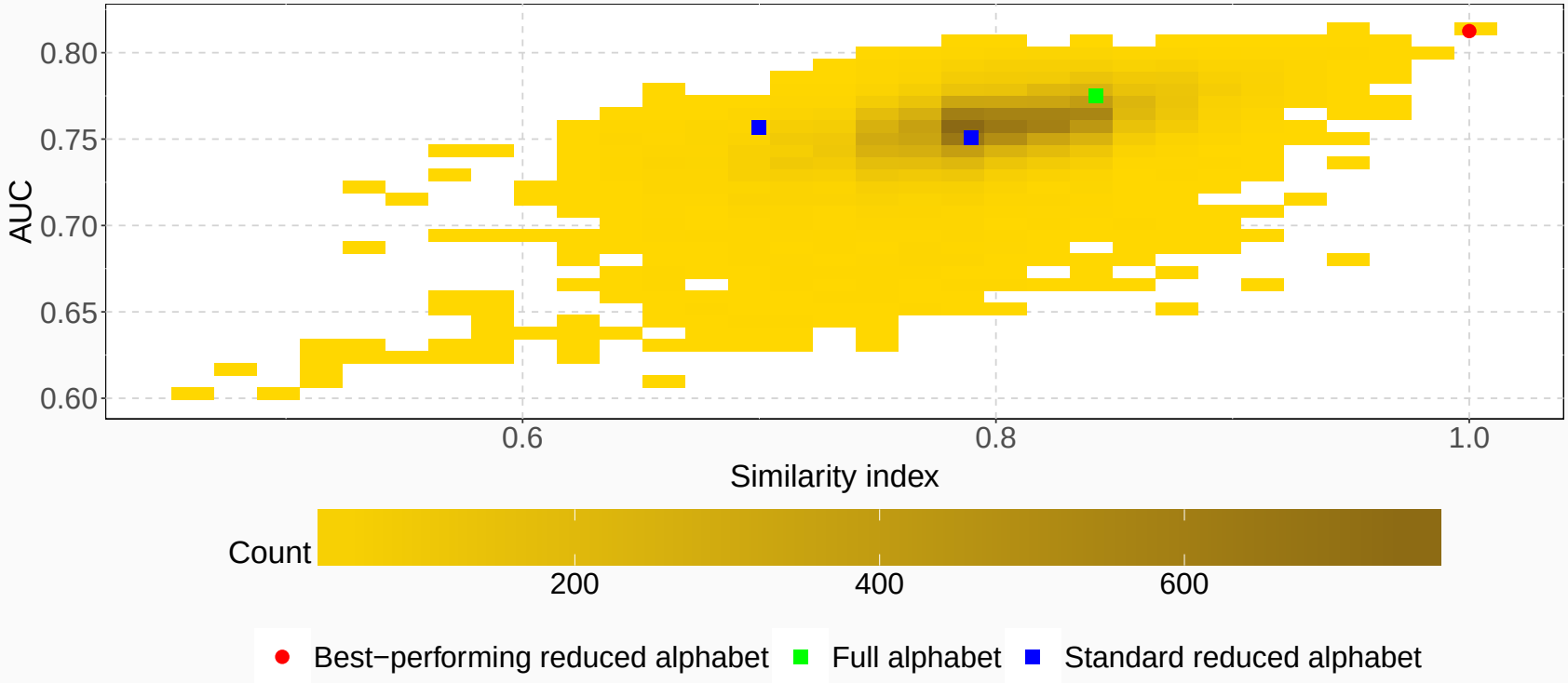
Similarity index



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

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

Similarity index



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

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

Out of 65 the most informative n-grams, 15 (23%) were also found in the motifs validated experimentally (López De La Paz et al., 2002).

Non-amyloidogenic motifs:

{K, P, R}

{K, P, R}---{K, P, R}

{K, P, R}{K, P, R}

Amyloidogenic motifs:

{I, L, V}{F, W, Y}{I, L, V}

{A, C, H, M}{I, L, V}{I, L, V}

{F, W, Y}{I, L, V}{I, L, V}

{D, E, N, Q, S, T}{F, W, Y}{I, L, V}

{I, L, V}{I, L, V}{F, W, Y}

{I, L, V}{I, L, V}{I, L, V}

{D, E, N, Q, S, T}{I, L, V}{I, L, V}

{F, W, Y}-{I, L, V}

{F, W, Y}{I, L, V}

{I, L, V}{F, W, Y}

{I, L, V}-{F, W, Y}

{I, L, V}

Benchmark and summary

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

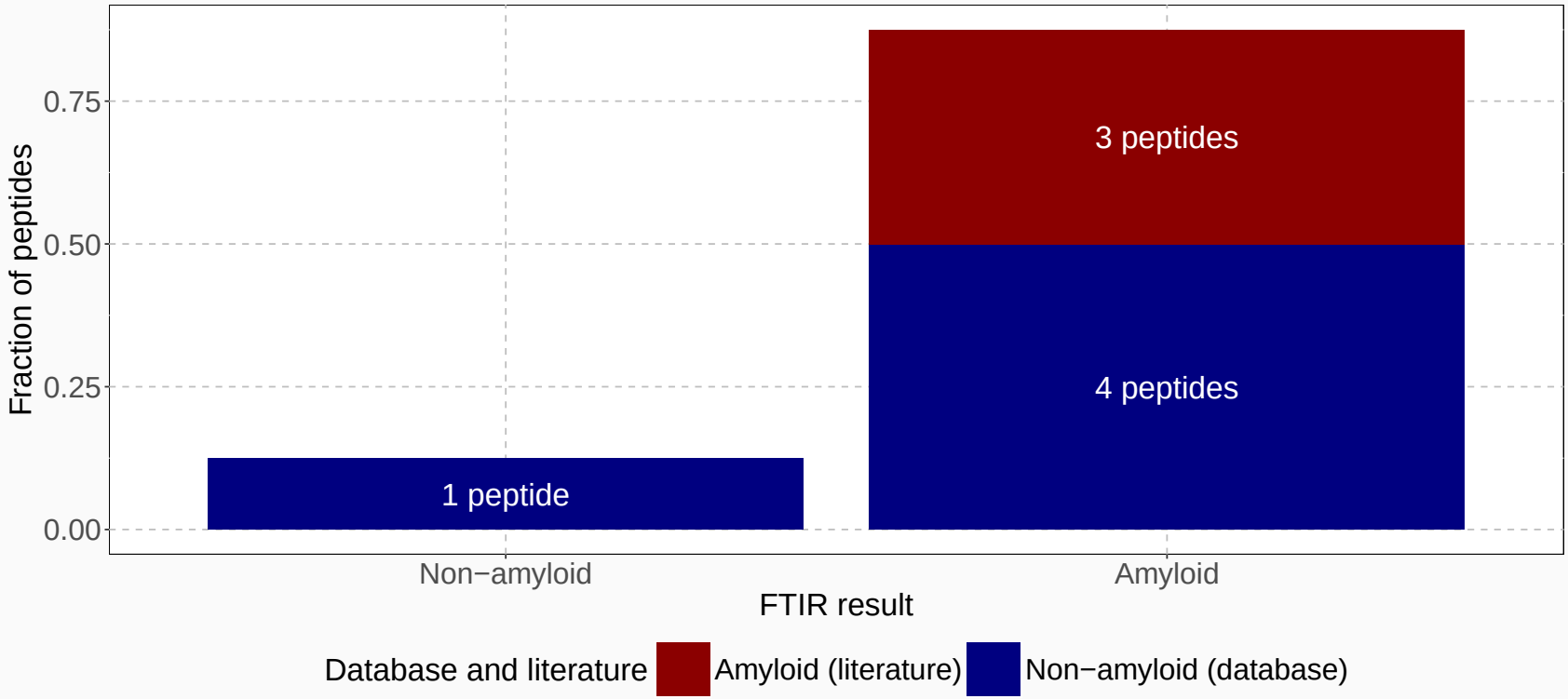
Benchmark results

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

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

Experimental verification

We verified experimentally with Fourier transform infrared spectroscopy (FTIR) **8** non-amyloidogenic peptides from the AmyLoad database that AmyloGram assessed with a high probability of amyloidogenicity.



CsgA in AmyloGram

CsgA protein (E. coli K12):

Name	Probability	Amyloidogenic (experimental)
R1	0.8216	Yes
R2	0.2739	No
R3	0.2739	Yes
R4	0.2683	No
R5	0.3362	Yes

Summary

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

Our algorithm was also capable of extracting motifs (n-grams) associated with amyloidogenicity, partially confirming experimental results.

Our software is available as a web-server: `smorfland.uni.wroc.pl/amylogram`.

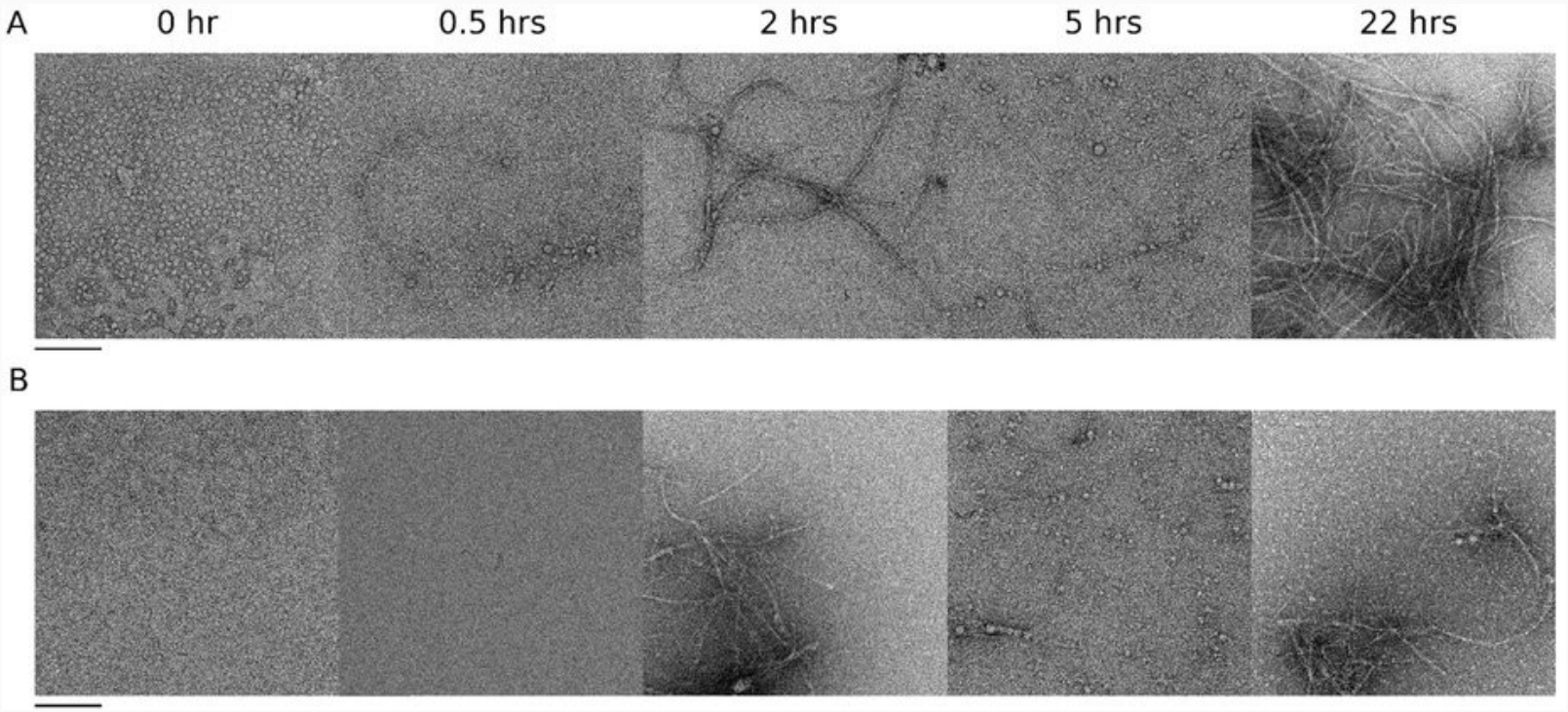
n-gram analysis workflow is implemented in the R package **biogram**: `https://cran.r-project.org/package=biogram`.

Perspectives

CsgC was reported to effectively (molar ratios as low as 1:500) inhibit CsgA aggregation. *In vivo*, it prevents CsgA from aggregating within the periplasm (Evans et al., 2015).

CsgC blocks with specificity hot-spots similar to amyloidogenic regions of CsgA (e.g., in α-synnuclein, a protein associated with Parkinson disease).

Perspectives



A. CsgA fibre formation. B. CsgA+CsgC 200:1 (molar ratio) fibre formation.

Scale bar = 200nm. Taylor et al. (2016).

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  - Chris Lauber,
- **AmyLoad** database (`comprec-lin.iiar.pwr.edu.pl/amyload`):
  - Paweł Woźniak.

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