biogram: a toolkit for n-gram analysis

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Outline

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Aim: convert **bio**logical sequences to n-**grams**, continuous or discontinuous sub-sequences.

	P1	P2	P3	P4	P5	P6
S1	a	c	c	c	t	a
S2	c	t	c	c	c	a
S3	a	a	a	t	t	t

Sample sequences.

	a	c	g	t
S1	2	3	0	1
S2	1	4	0	1
S3	3	0	0	3

Unigrams.

	a.a	c.a	g.a	t.a
S1	0	0	0	1
S2	0	1	0	0
S3	2	0	0	0

Bigrams.

	1_a	2_a	3_a	4_a	5_a	6_a	1_c
S1	1	0	0	0	0	1	0
S2	0	0	0	0	0	1	1
S3	1	1	1	0	0	0	0

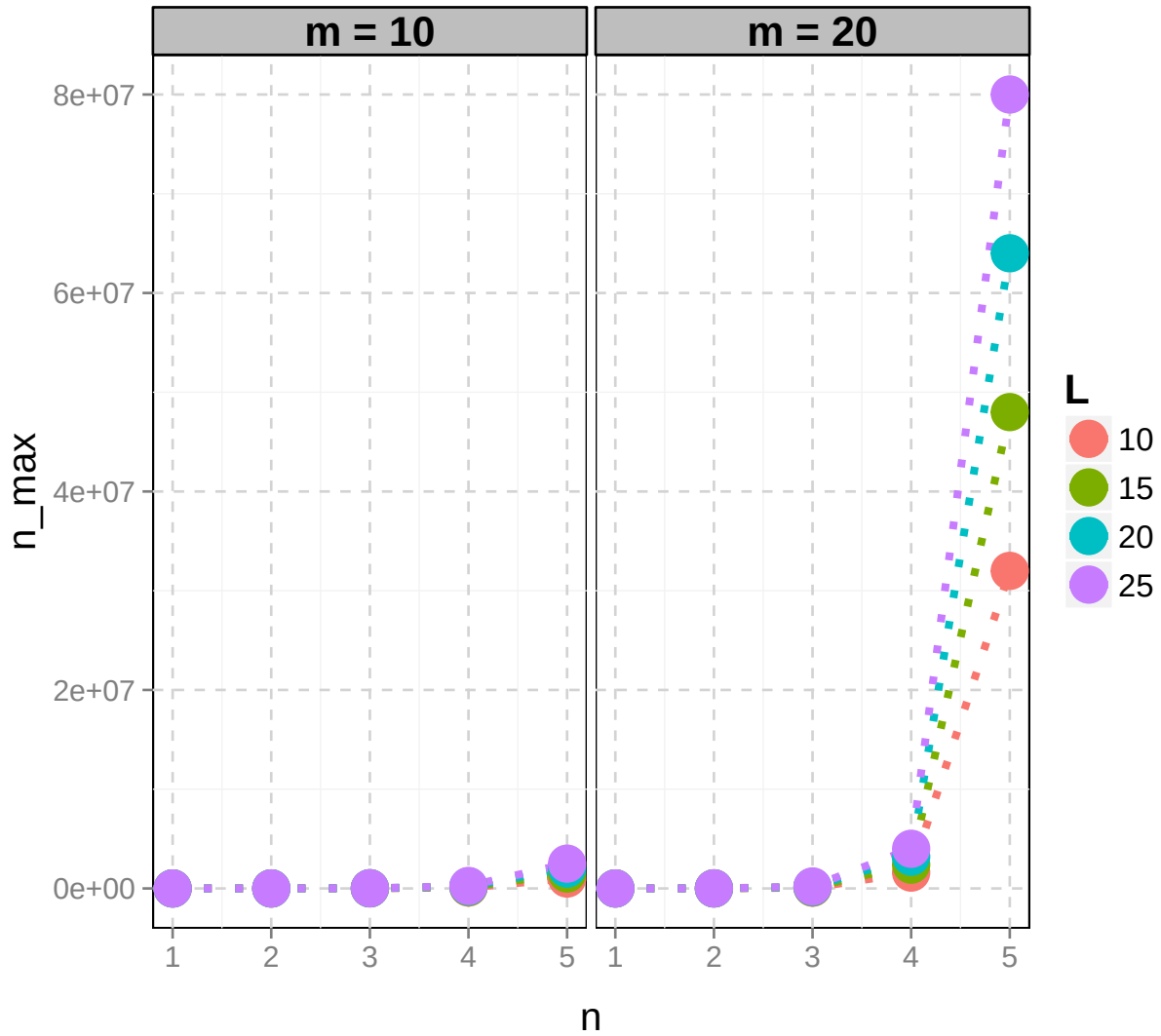
A fraction of possible unigrams with position information.

Positioned n-gram data is binary.

Number of possible positioned n-grams:

$$n_{max} = L \times m^n$$

- n_{max}: total number of n-grams.
- L: length of the sequence.
- m: number of unique symbols in the alphabet.
- n: length of the n-gram.


m = 10
m = 20
8e+07
6e+07
4e+07
2e+07
0e+00
n_max
1
2
3
4
5
n
L
10
15
20
25

The n-gram data is cumbersome to use without the reduction of the dimensionality. Solutions:

- Reduce alphabet (in case of amino acid sequences).
- Filter features.

Existing encodings of amino acids:

- Orthogonal (20 bits, Ala = 10000000000000000000, Cys = 01000000000000000000, ...).
- Orthogonal (5 bits, Ala = 00001, Cys = 00011, ...).
- Exchange groups.

Drawbacks:

- Does not take into account relationships between amino acids (orthogonal encodings) or employs only selected relationships (exchange group).
- Sparse encoding requires large data sets (Lin, May, & Taylor, 2002).

Clustering of amino acids using a set of physicochemical properties specific for a given problem removes redundant information and simplifies model.

1. Calculate test statistic for the given positioned n-gram and etiquettes (T_R).
2. Permute counts of n-grams and calculate permuted test statistic (T_P).
3. Repeat step 2. N times.
4. Calculate p-value using:

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

$N_{T_P > T_R}$ is number of times when T_P was bigger than T_R

Advantages:

- Model independent.

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- Computationally expensive (number of cases, number of features).
- Single feature analysis (no feature interaction).
- Unfeasible precise estimation of low p-values (the number of permutations is inversely proportional to the interval between p-values).

The binary positioned n-gram data tabulated by binary label can be easily described in 2d contingency table.

sequence ID	feature	target
1	1	0
2	1	0
3	0	0
4	1	1
5	0	1
...	...	...

Positioned n-grams with a label.

sequence ID	feature	target
1	1	0
2	1	0
3	0	0
4	1	1
5	0	1
...	...	...

Positioned n-grams with a label.

	target	feature
0	$n_{1,1}$	$n_{1,0}$
1	$n_{0,1}$	$n_{0,0}$

Contingency table.

Test statistics used by QuiPT (information gain, Kullback-Leibler divergence) measure inbalance of contingency tables.

The probability of certain contingency table is given as the conditional distribution, as impose restrictions on two parameters $n_{.,1}$ and $n_{1,.}$. The test statistic is computed for each possible value of $n_{1,1}$.

Advantages over permutation test

- Speed.

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- Speed.
- Using the exact distribution of possible values of the criterion QuiPT yields precise small p-values without increasing the computation time.

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Amyloids:

- Short proteins associated with the number of clinical disorders, for example Alzheimer's or Creutzfeldt-Jakob's diseases.

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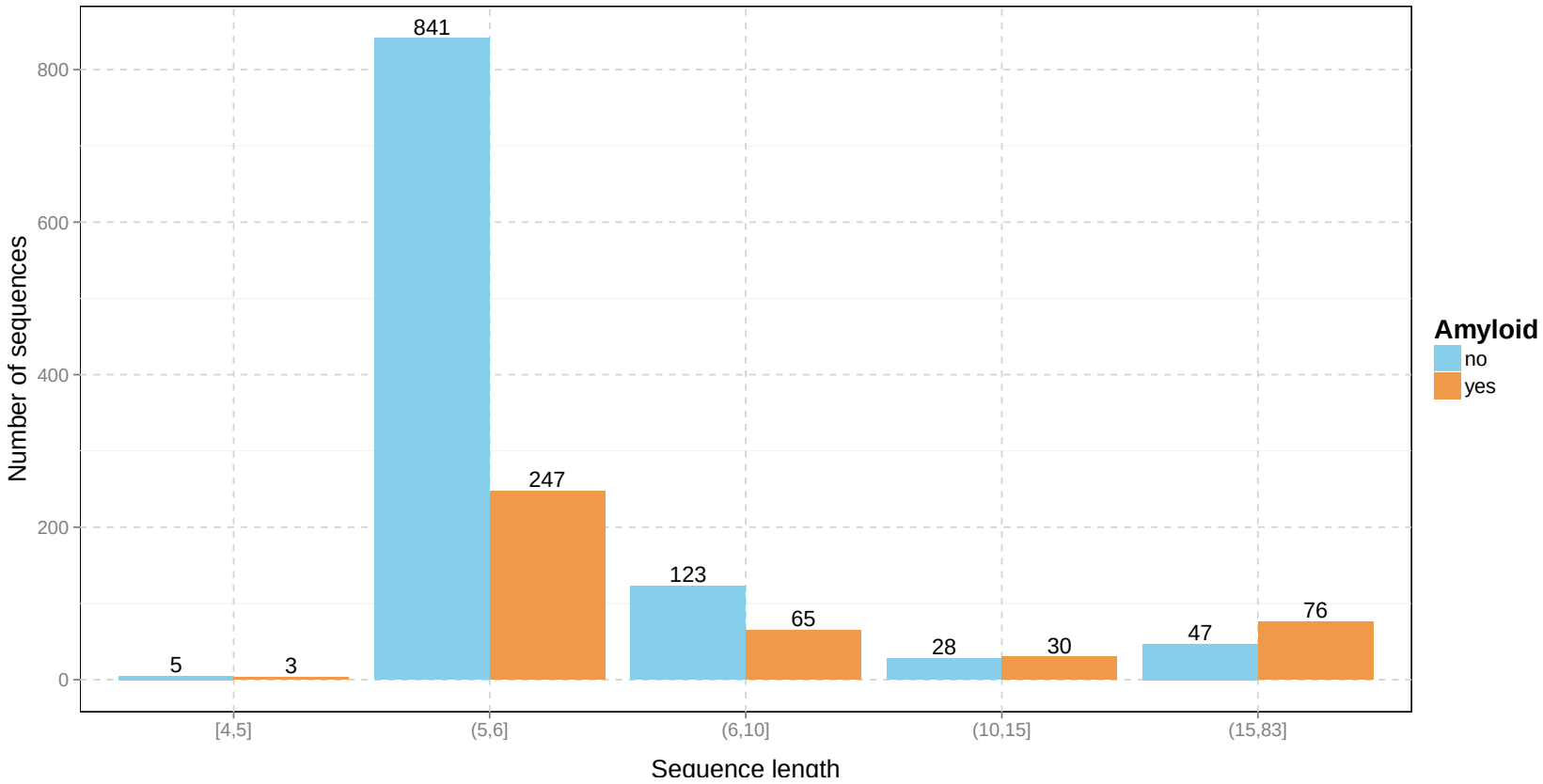
- Short proteins associated with the number of clinical disorders, for example Alzheimer's or Creutzfeldt-Jakob's diseases.
- Create harmful zipper-like -structures through characteristic short subsequences of amino acids (hot-spots).



Source of physicochemical properties: Kawashima et al. (2008). Source of amyloid and non-amyloid sequences: Wozniak and Kotulska (2015).

Training length	Number of groups	AUC	Specificity	Sensitivity
6	4	0.8183	0.9014	0.5038
<16	6	0.8320	0.5186	0.9195

Encodings with the best sensitivity and specificity.

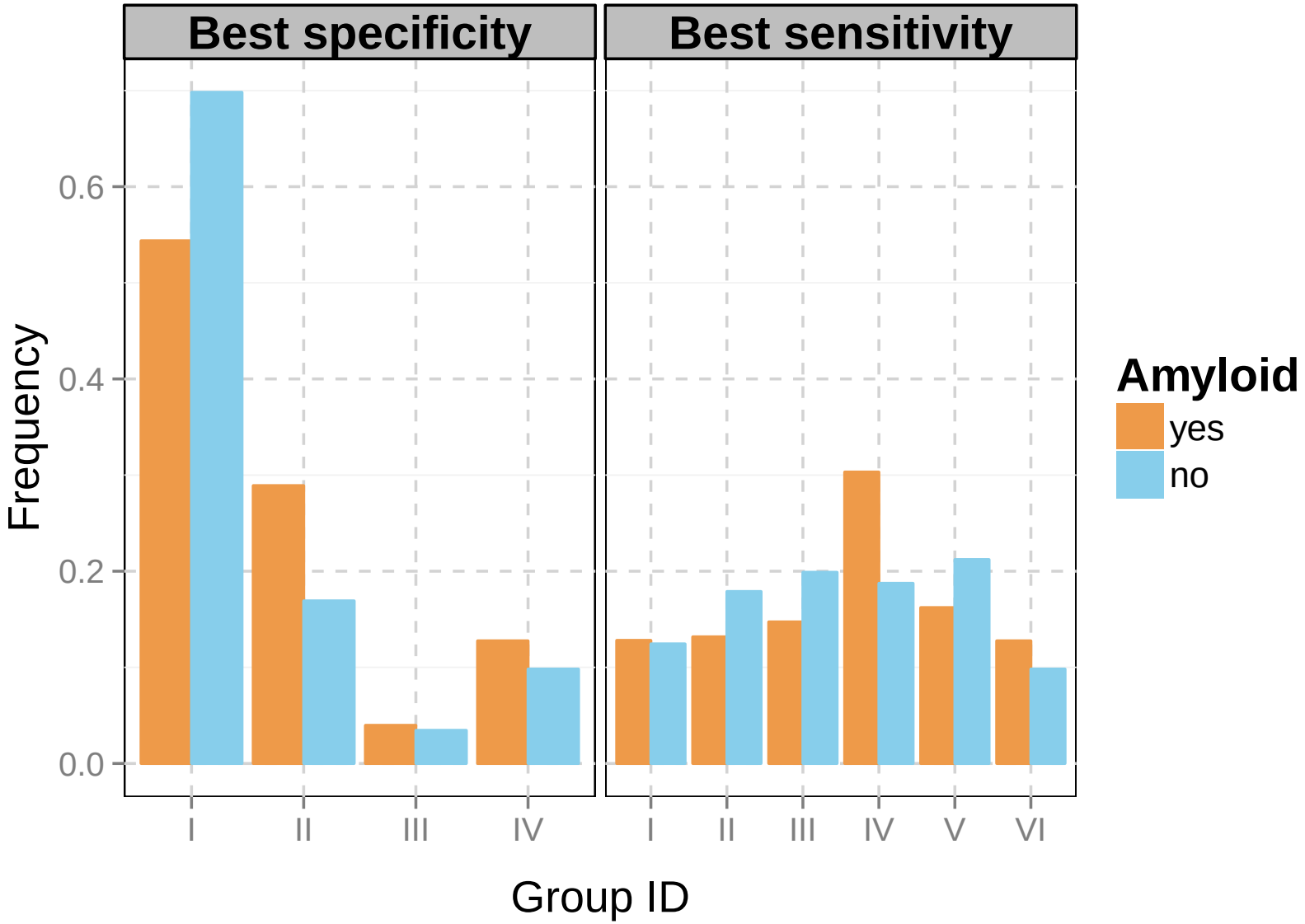
The committee of the best specificity and best sensitivity classifiers has overall 0.8911 AUC, 0.7473 sensitivity and 0.8684 specificity.

The best specificity encoding.

ID	Amino acids
I	H, M
II	F, W, Y
III	C, I, L, V
IV	A, D, E, G, K, N, P, Q, R, S, T

The best sensitivity encoding.

ID	Amino acids
I	A, T
II	D, E, N
III	G, P, S
IV	F, W, Y
V	H, K, Q, R
VI	C, I, L, M, V


Best specificity
Best sensitivity
Frequency
0.0
0.2
0.4
0.6
I
II
III
IV
I
II
III
IV
V
VI
Group ID
Amyloid
yes
no

The committee was compared to the two best-performing predictors of amyloids

Benchmark of three best-performing classifiers on Pep424 data set (Walsh et al., 2014).

Name	AUC
PASTA 2.0 (Walsh et al., 2014)	0.8573
Committee	0.8410
FoldAmyloid (Garbuzynskiy et al., 2010)	0.8331

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Secretory signal peptides:

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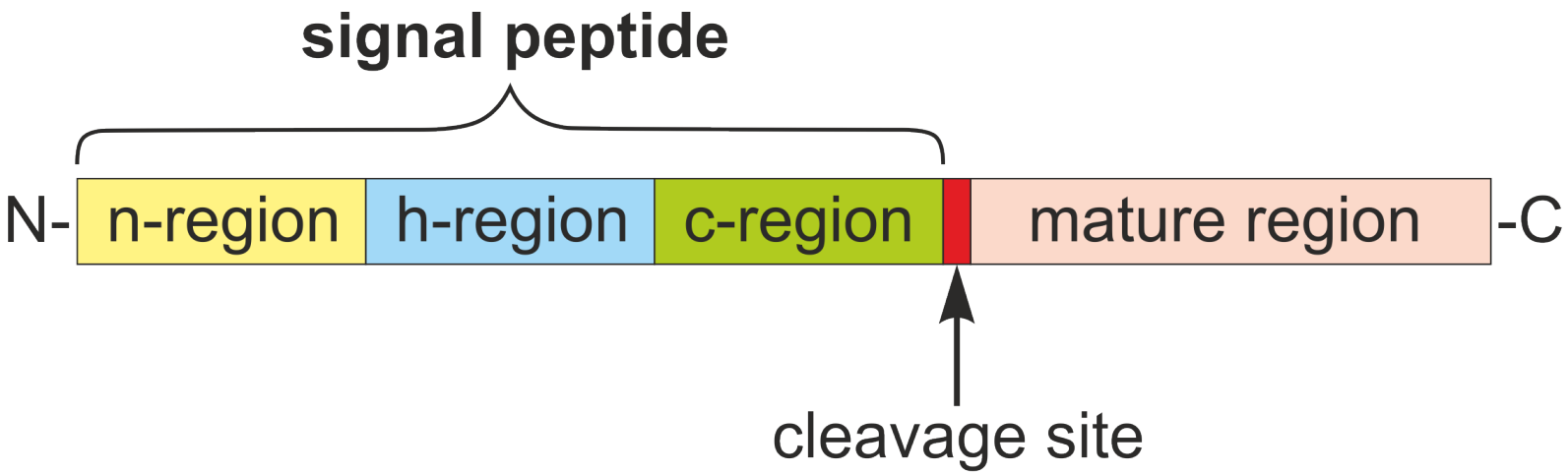
- Short (20-30 residues) N-terminal amino acid sequences.
- Direct a protein to the endomembrane system and next to the extracellular localization.
- Possess three distinct domains with variable length and specific amino acid composition (Hegde & Bernstein, 2006).



Organization of signal peptide

Hidden semi-Markov models assumptions (Rabiner, 1989; Koski, 2001):

- The current region (state) of the sequence (process) depends on the previous region.

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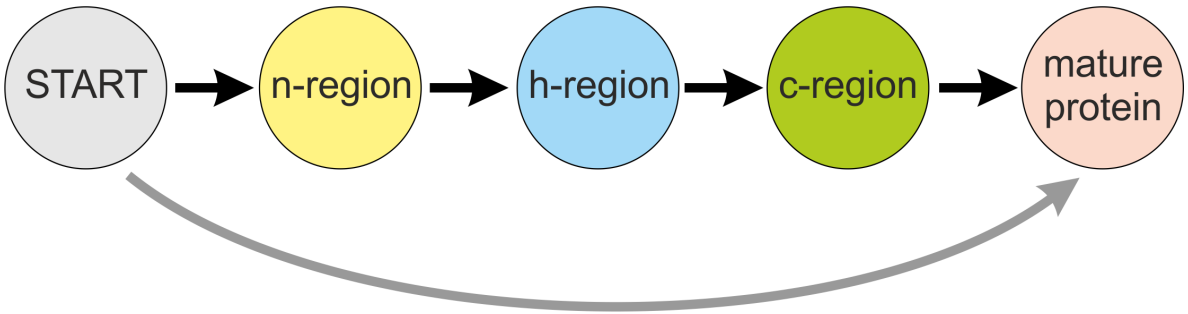
- The current region (state) of the sequence (process) depends on the previous region.
- Regions may be only indirectly determined using amino acid residues (observations).
- Probability of staying in a region is modeled by a probability distribution.

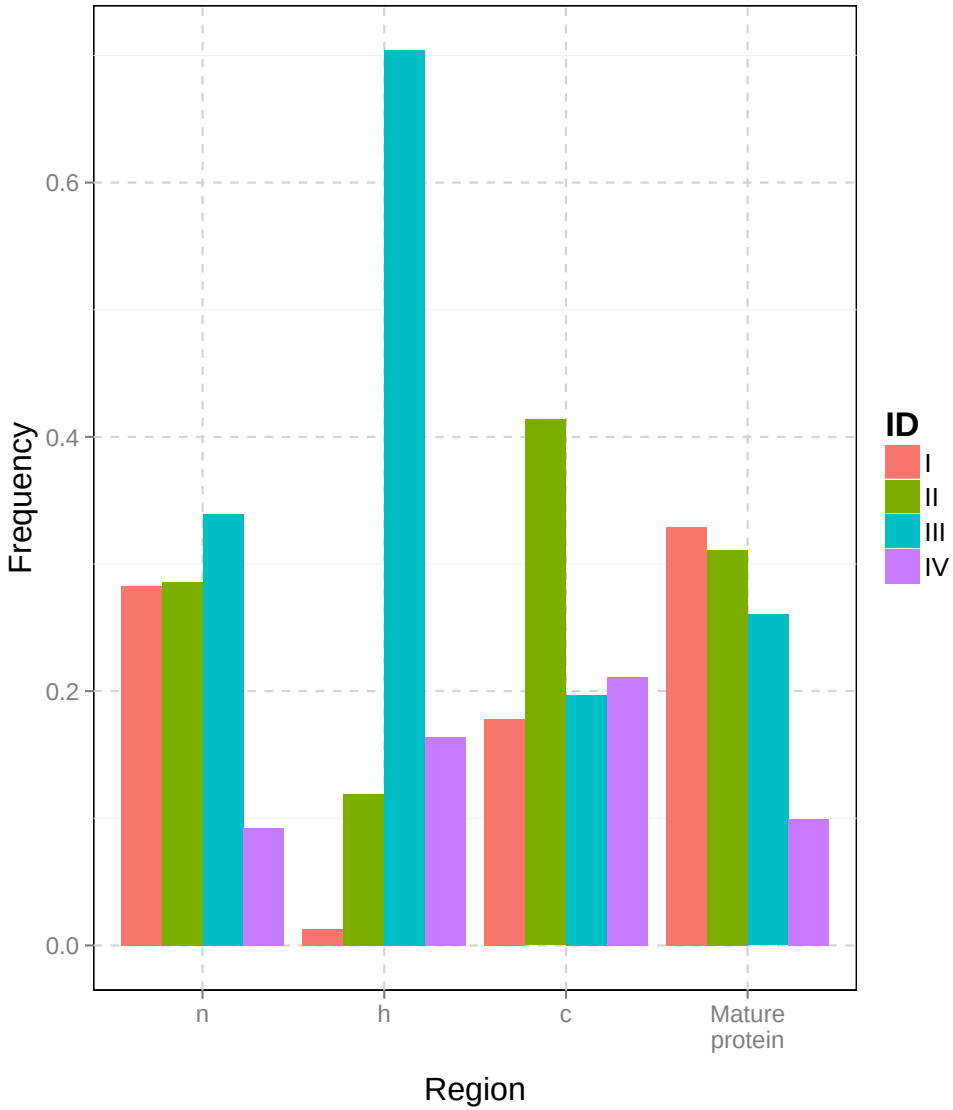
signalHsmm predictive model



During the test phase, each protein was fitted to two models. The outcome consists of probabilities that a particular residue belongs to a given model and predicted cleavage site.

The best AUC encoding.

ID	Amino acids
I	D, E, H, K, N, Q, R
II	G, P, S, T, Y
III	F, I, L, M, V, W
IV	A, C



Comparison of Area Under the Curve, H-measure and Matthews Correlation Coefficient for different classifiers considering only proteins belonging to Plasmodiidae.

Software name	AUC	Sensitivity	Specificity
signalP 4.1 (no tm) (Petersen et al., 2011)	0.8356	0.7745	0.8966
signalP 4.1 (tm) (Petersen et al., 2011)	0.7928	0.6471	0.9385
PrediSi (Hiller et al., 2004)	0.6597	0.3725	0.9469
Phobius (Käll et al., 2004)	0.7963	0.6765	0.9162
Philius (Reynolds et al., 2008)	0.7753	0.6176	0.9330
signalHsmm-2010	**0.9340**	**1.0000**	0.8436
signalHsmm-1989	0.9326	0.9510	**0.8631**

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biogram is a flexible toolkit for analysis of biological sequences. It reduces efficiently feature space by extracting important features and removing redundant information.

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biogram R package:
`http://cran.r-project.org/web/packages/biogram/`

biogram source: `https://github.com/michbur/biogram`

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Wozniak, P. P., & Kotulska, M. (2014). Characteristics of protein residue-residue contacts and their application in contact prediction. *Journal of Molecular Modeling*, *20*(11). Retrieved 2015-07-27, from `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4221654/` doi: 10.1007/s00894-014-2497-9

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Comparison of Area Under the Curve, Sensitivity, Specificity and Matthews Correlation Coefficient for different classifiers.

Software name	AUC	Sensitivity	Specificity
signalP 4.1 (no tm) (Petersen et al., 2011)	0.9416	**0.9720**	0.9112
signalP 4.1 (tm) (Petersen et al., 2011)	**0.9673**	0.9579	**0.9766**
PrediSi (Hiller et al., 2004)	0.8949	0.9065	0.8832
Phobius (Käll et al., 2004)	0.9509	0.9673	0.9346
Philius (Reynolds et al., 2008)	0.9369	0.9533	0.9206
signalHsmm-2010	0.9526	0.9533	0.8832
signalHsmm-1989	0.9562	0.9626	0.8972

If probability that target equals 1 is p and probability that feature equals 1 is q and feature and target are independent then each of them has the following probabilities

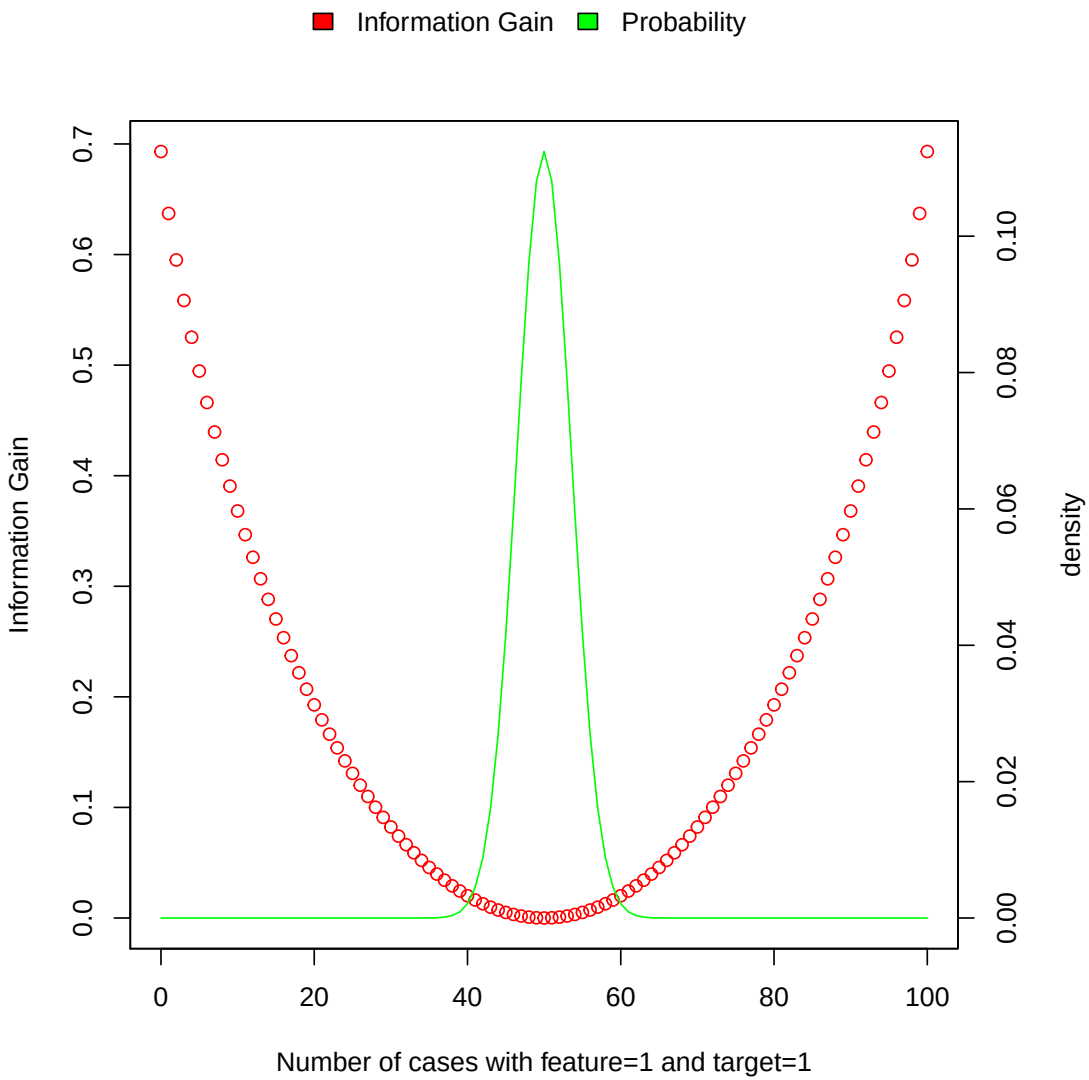
$$P(Target, Feature) = (1, 1)) = p \cdot q$$

$$P(Target, Feature) = (1, 0)) = p \cdot (1 - q)$$

$$P(Target, Feature) = (0, 1)) = (1 - p) \cdot q$$

$$P(Target, Feature) = (0, 0)) = (1 - p) \cdot (1 - q)$$

- $n_{1,1}$ is from range $[0, min(n_{.,1}, n_{1,.})]$.
- The probability of certain contingency table is given as the conditional distribution, as impose restrictions on two parameters $n_{.,1}$ and $n_{1,.}$.
- The test statistic is computed for each possible value of $n_{1,1}$.
- The distribution of test statistics under hypothesis that target and feature are independant is computed using values from 3.



	Target	Feature	Freq
1	0	0	50
2	1	0	50
3	0	1	50
4	1	1	50

1. Nine scales representing properties important in the amylogenicity: hydrophobicity, size polarity and solvent accessibility from AAIndex databasewere chosen. Additionally, two frequencies of forming contact sites (Wozniak & Kotulska, 2014) were added. All scales were normalized.
2. All combinations of characteristics (each time selecting only one scale per the property) were clustered using Euclidean distance and Ward's method.
3. Each clustering was divided into 3 to 6 groups creating 144 encodings of amino acids.
4. Redundant 51 encodings (identical to other encodings) were removed.

1. Sequences shorter than 6 amino acids were discarded.
2. From each sequence overlapping 6-grams were extracted. All n-grams were labelled as their sequence of the origin (e.g. all 6-grams extracted from amyloid sequence were labelled as positive).
3. For each encoding features were filtered by the QuiPT and used to train the Random Forests (Liaw & Wiener, 2002). This procedure was performed independently on three training sets: a) 6 amino acids, b) 10 amino acids or shorter, c) 15 amino acids or shorter creating three classifiers.
4. All classifiers were evaluated in the 5-fold cross-validation eight times. The sequence was labelled as positive (amylogenic), if at least one 6-gram was assessed as amylogenic.