signalHsmm: prediction of malarial signal peptides

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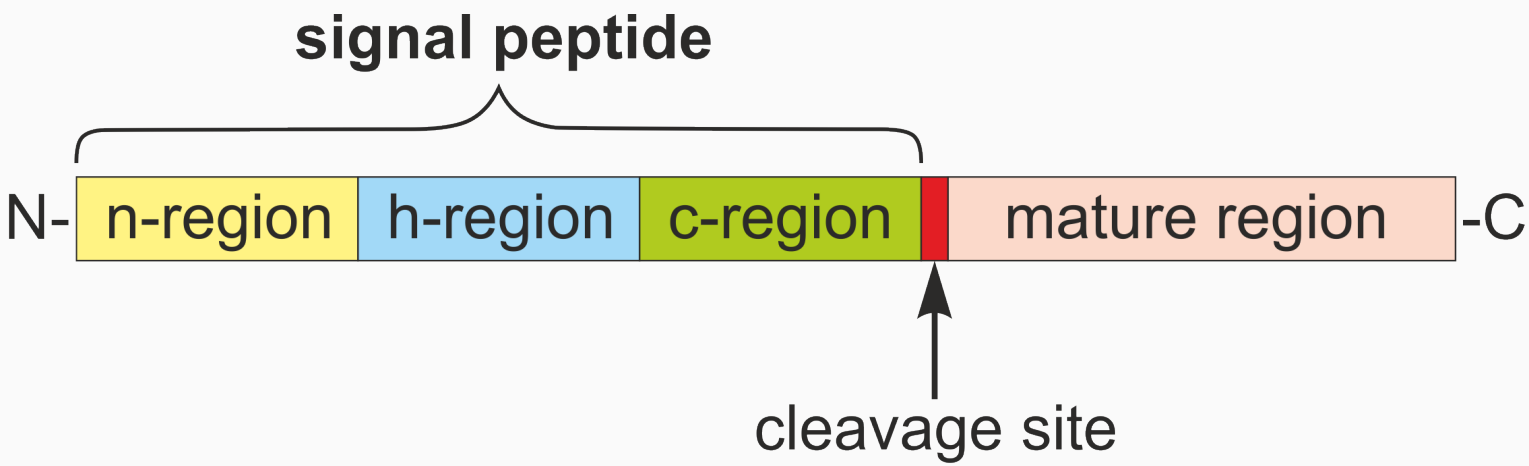
Signal peptides

Secretory signal peptides:

- are short (20-30 residues) N-terminal amino acid sequences forming α-helices,
- direct a protein to the endomembrane system and next to the extracellular localization,
- are universal enough to direct properly proteins in different secretory systems; artifically introduced bacterial signal peptides can guide proteins in mammals (Nagano and Masuda, 2014) and plants (Moeller et al., 2009),
- tag hormons, immune system proteins, structural proteins, and metabolic enzymes.

Architecture



Signal peptides possess three distinct domains with variable length and characteristic amino acid composition (Hegde and Bernstein, 2006):

- n-region: mostly basic residues (Nielsen and Krogh, 1998),
- h-region: strongly hydrophobic residues (Nielsen and Krogh, 1998),
- c-region: a few polar, uncharged residues (Jain et al., 1994).

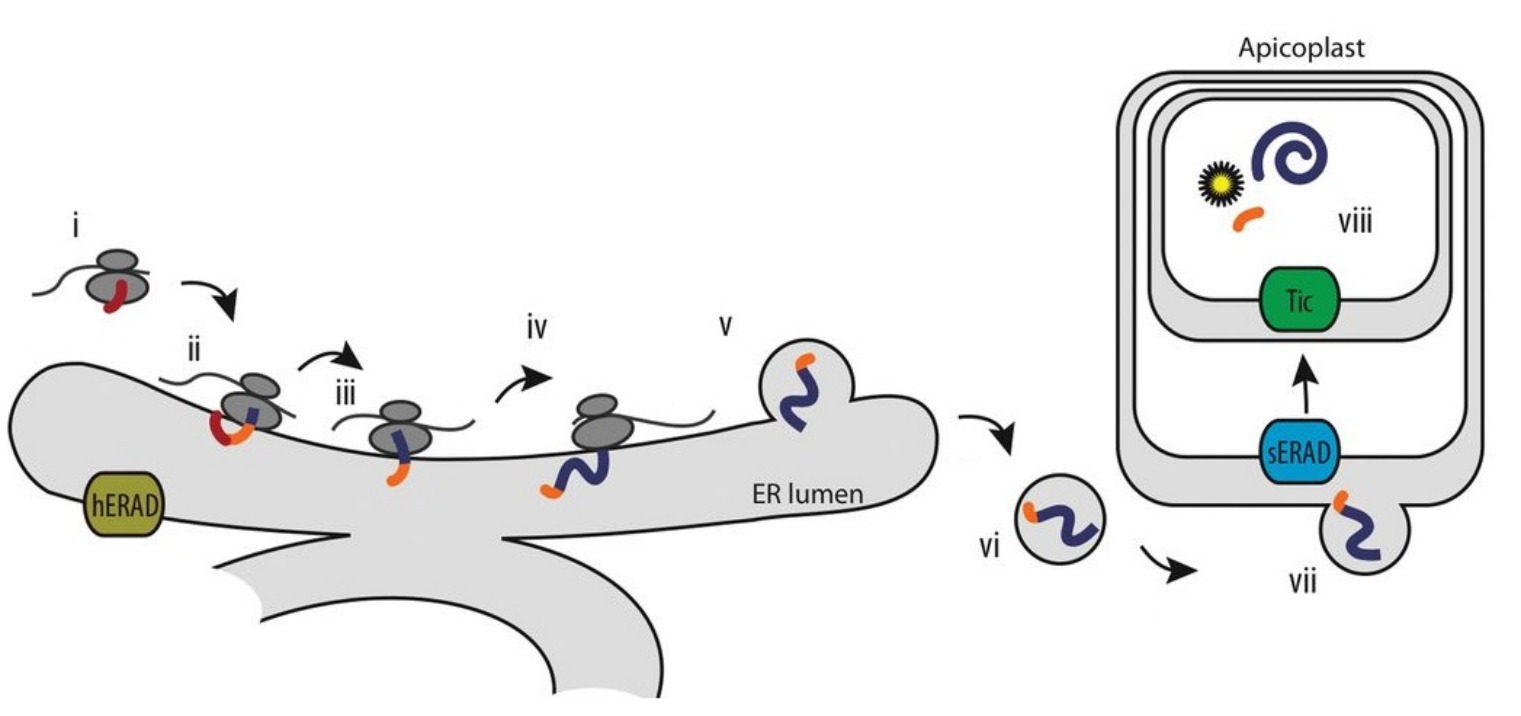
Malarial signal peptides

Apicoplast

Four membrane-bounded plastid responsible for several biochemical pathways including the biosynthesis of fatty acids, isoprenoids and haem.

Apicoplast

The signal peptide is required for targeting the apicoplast protein to the endomembrane system, whereas the transit peptide is required to traffic the protein to the apicoplast.

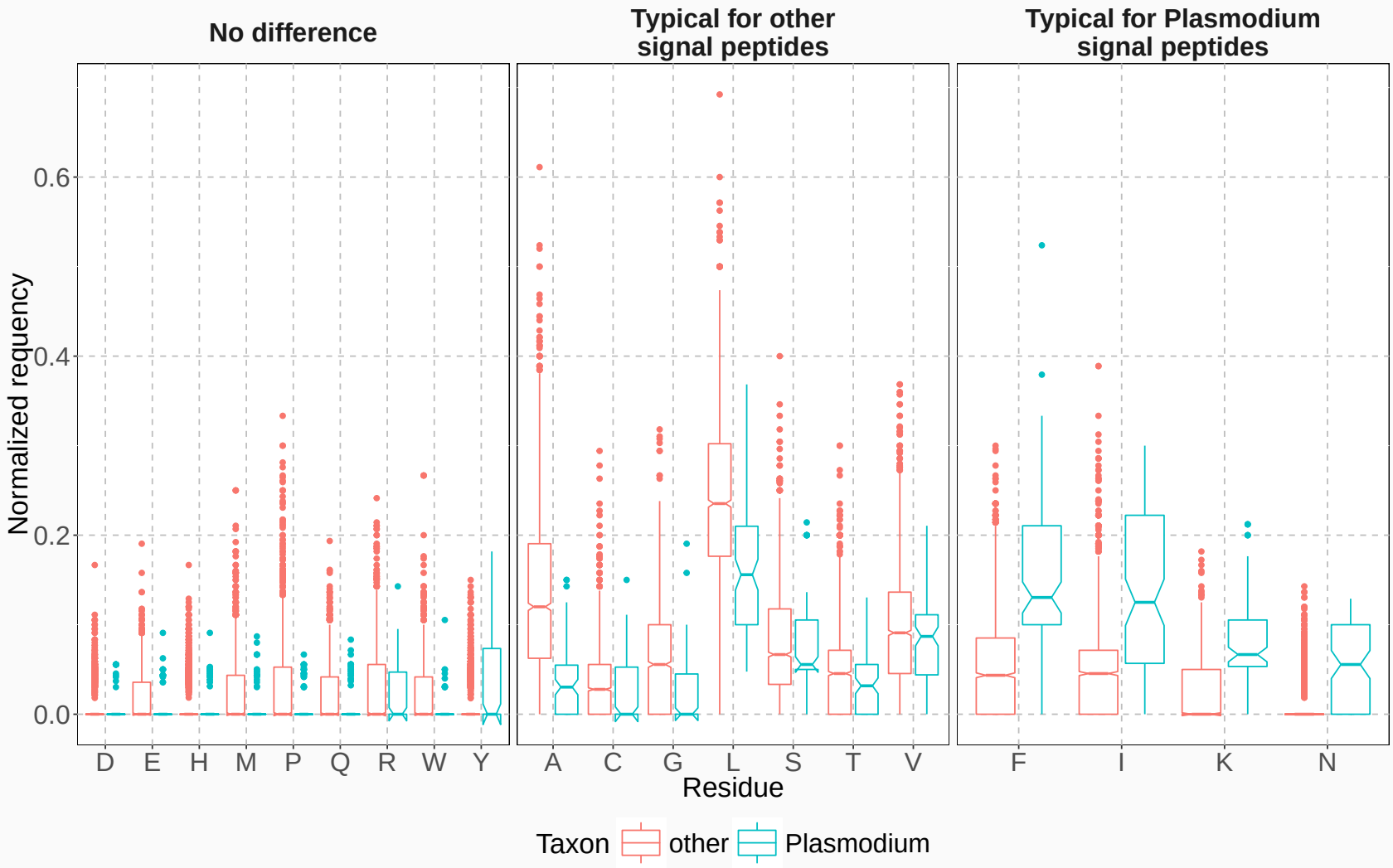


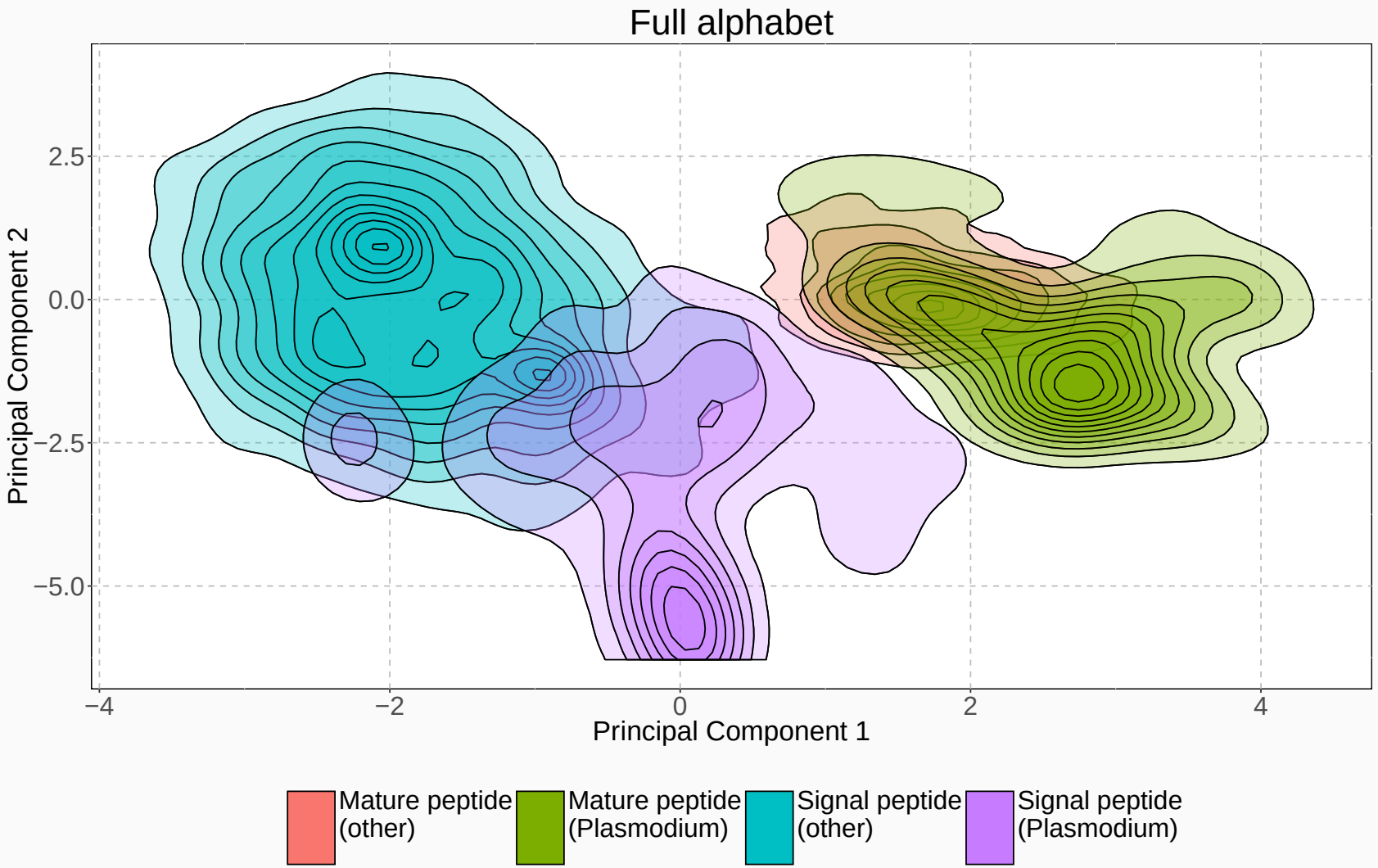
Kalanon and McFadden (2010)

Apicoplast

The absence of a metabolic counterpart in human host make the apicoplast proteins promising targets for anti-malarial drug development.

Heavy adenine-thymine bias of parasitic genomes alters amino acid composition of malarial signal peptides making them hard to predict using software trained on other Eukaryotes.


No difference
Typical for other signal peptides
Typical for Plasmodium signal peptides
Normalized requency
0.0
0.2
0.4
0.6
D E H M P Q R W Y
A C G L S T V
F I K N
Residue
Taxon
other
Plasmodium


Full alphabet
Principal Component 2
2.5
0.0
−2.5
−5.0
−4
−2
0
2
4
Principal Component 1
Mature peptide (other)
Mature peptide (Plasmodium)
Signal peptide (other)
Signal peptide (Plasmodium)

Methods

Do signal peptides require the exact sequence of amino acids?

Even nonbiological sequences can be effective signal peptides provided they fulfill general requirements (Tonkin et al., 2008).

NH_2-SKINNYSLINKYKINKYTHING-COOH - targets apicoplast.

NH_2-ITWILLNEVERTARGETPLASTID-COOH - does not target apicoplast.

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.

Novel reduced amino acid alphabets

13 physicochemical properties handpicked from AAIndex database relevant to

Novel reduced amino acid alphabets

Property name	Amino acid scale
Size	Size
Size	Molecular weight
Size	Residue volume
Size	Bulkiness
Hydrophobicity	Normalized hydrophobicity scales for α-proteins
Hydrophobicity	Consensus normalized hydrophobicity scale
Hydrophobicity	Hydropathy index
Hydrophobicity	Surrounding hydrophobicity in α-helix
Polarity	Polarity
Polarity	Mean polarity
Occurrence in α-helices	Signal sequence helical potential
Occurrence in α-helices	Normalized frequency of N-terminal helix
Occurrence in α-helices	Relative frequency in α-helix

Cross-validation

We built 96 reduced amino acid alphabets (each based on one scale per a given property category) and evaluated them in cross-validation experiment using eukaryotic sequences.

Results

Group	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

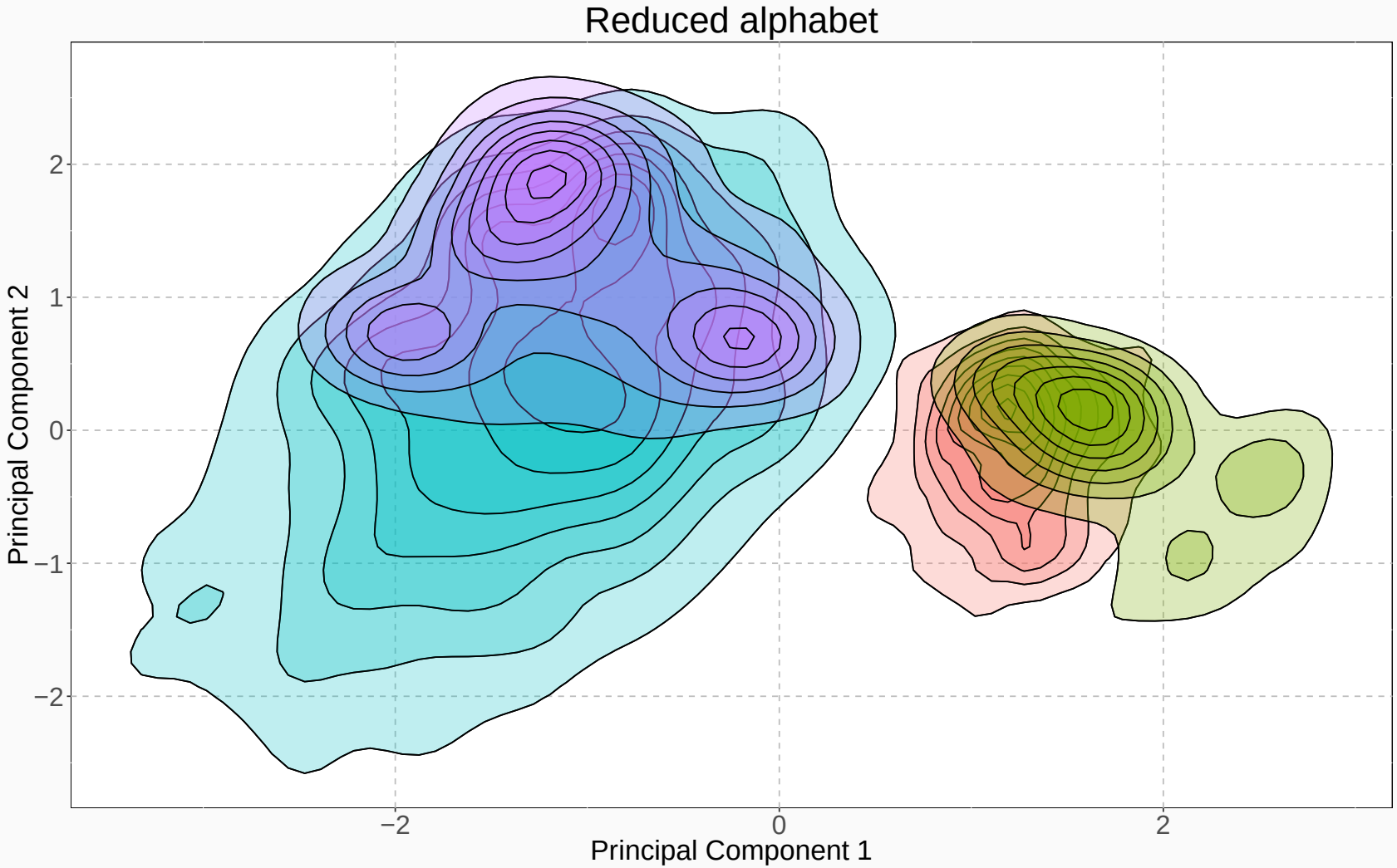
I. Amino acids absent in h-region.

Group	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

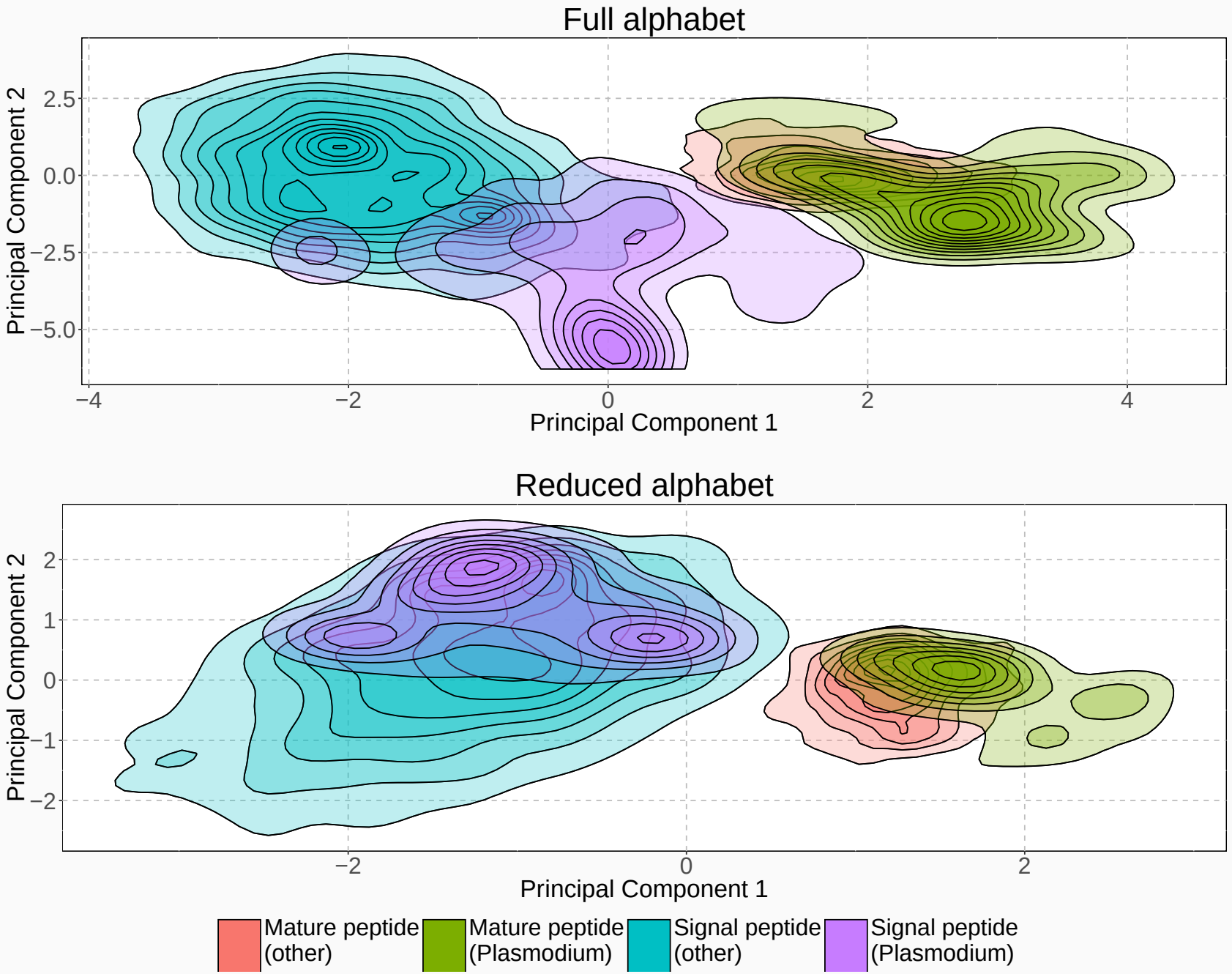
II. Amino acids common in c-region.

Group	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

III. Amino acids common in h-region.



Signal peptides, after the reduction of the amino acid alphabet, group together despite their different origins.


Full alphabet
Principal Component 2
2.5
0.0
−2.5
−5.0
−4
−2
0
2
4
Principal Component 1
Reduced alphabet
Principal Component 2
2
1
0
−1
−2
−2
0
2
Principal Component 1
Mature peptide (other)
Mature peptide (Plasmodium)
Signal peptide (other)
Signal peptide (Plasmodium)

Benchmark

Benchmark data set: 51 proteins with signal peptide and 211 proteins without signal peptide from members of *Plasmodiidae*.

Benchmark

	MCC	AUC
signalP 4.1 (no tm) (Petersen et al., 2011)	0.6872	0.8667
signalP 4.1 (tm) (Petersen et al., 2011)	0.6196	0.7951
signalP 3.0 (NN) (Bendtsen et al., 2004)	0.7220	0.8938
signalP 3.0 (HMM) (Bendtsen et al., 2004)	0.5553	0.7734
Phobius (Käll et al., 2004)	0.5895	0.7880
signalHsmm-2010	0.7409	0.9262
signalHsmm-2010 (hom. 50%)	**0.7621**	**0.9384**
signalHsmm-2010 (raw aa)	0.6853	0.8718
signalHsmm-1987	0.7271	0.9063
signalHsmm-1987 (hom. 50%)	0.7194	0.9090
signalHsmm-1987 (raw aa)	0.6350	0.8350

signalHsmm1987: trained on data set of 496 eukaryotic proteins with signal peptides added before year 1987.

Conclusions

Eukaryotic signal peptides have very similar amino acid composition in their regions considering only the physicochemical properties of residues.

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- **biogram** package (`https://cran.r-project.org/package=biogram`):
  - Piotr Sobczyk,
  - Chris Lauber.

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