

Cleaving Antimicrobial Peptides to determine the mature region

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Overview

❖ Background

- Antimicrobial peptides
- Introduction to the pipeline
- Biological significance of cleaving

❖ Methodology

- What current cleaving methods are and why sliding window is needed

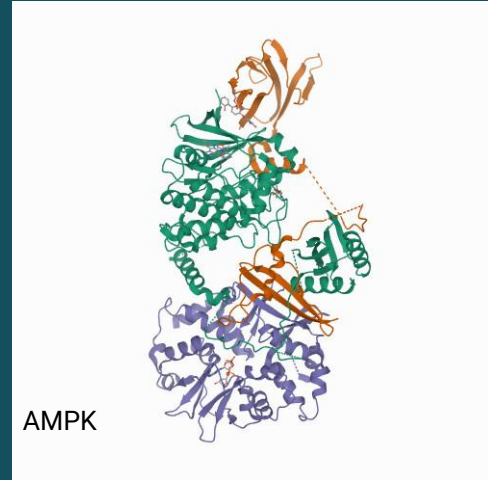
❖ Results

❖ Conclusion

Background

❖ Antimicrobial peptides

- Short, peptide sequences
- Often positively charged
- Amphipathic
- Produced by all life forms
- Part of the innate immune system



Source: Darcy Sutherland, PeptAID AIM 1 Science Meeting; image from: <https://www.rcsb.org/structure/4CFF>

AMP discovery Pipeline

INPUT

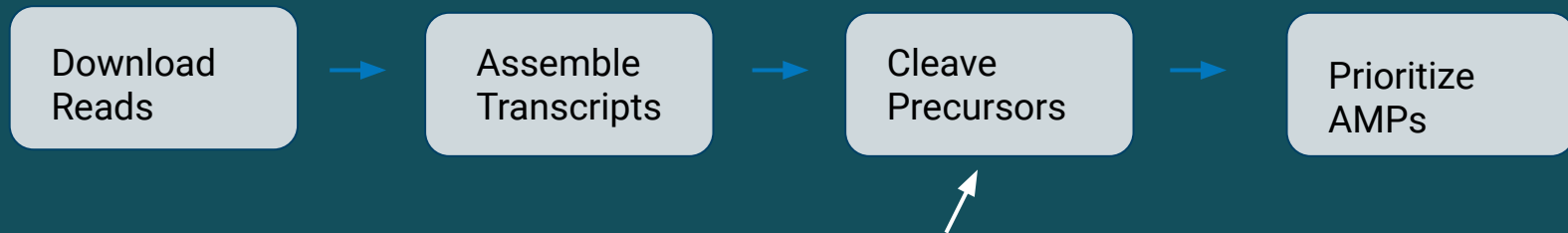
Publicly available
RNA-seq reads



OUTPUT

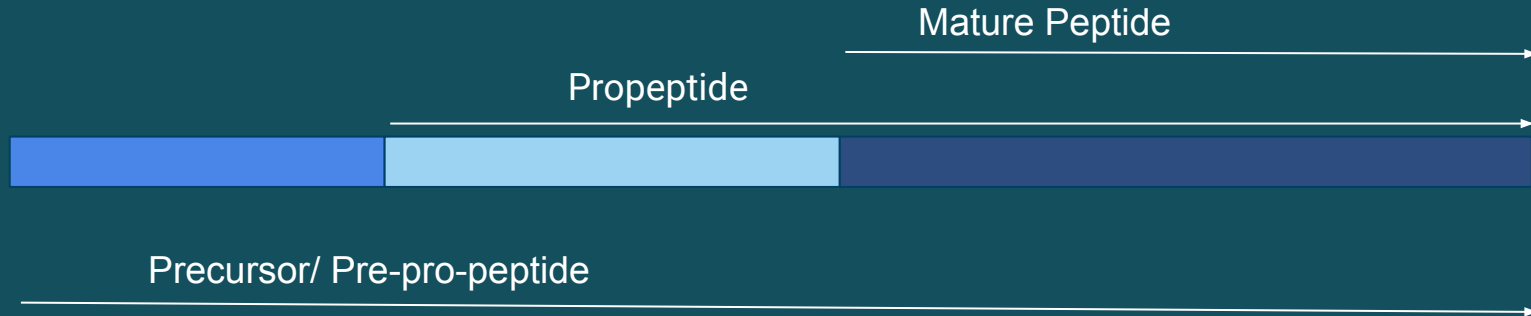
Potential AMP
Protein sequences

AMP discovery Pipeline



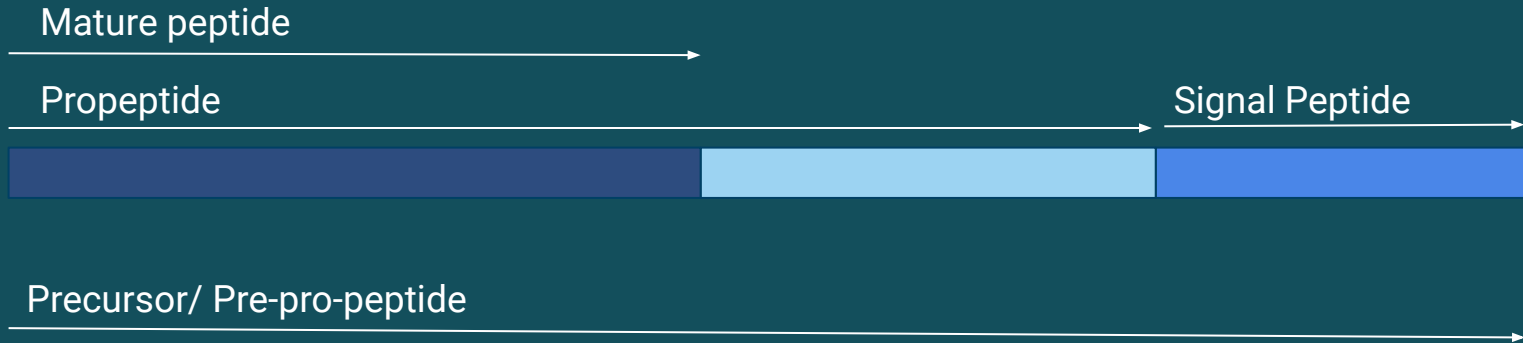
Antimicrobial Peptide (AMPs)

Structure:

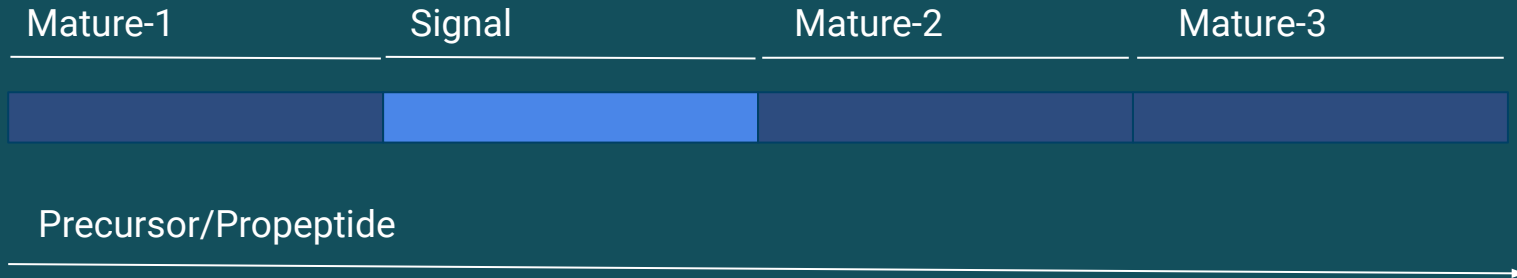


More on AMP structures...

Structures:



AMP structures...



Current Challenge and Motivation

Current Cleaving Technologies

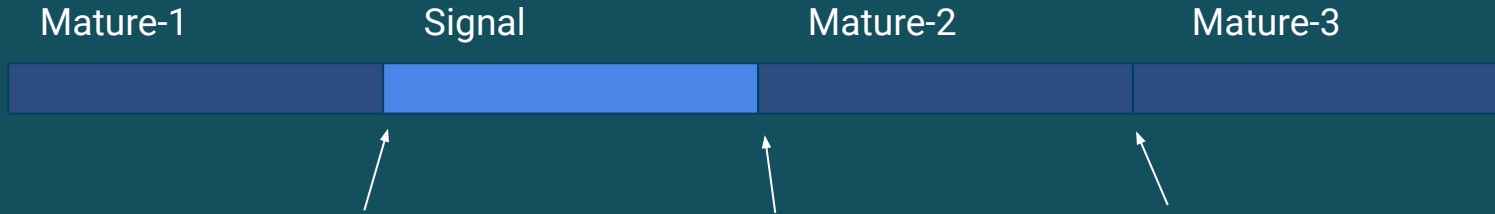
- ❖ Tool currently in use
 - ProP
- ❖ Other Bioinformatics tool available
 - MatureP

Problem

Recombining cleaved pieces and determining the mature sequence more accurately

Source: Duckert, P., Brunak, S. & Blom, N. Prediction of proprotein convertase cleavage sites. Protein Eng. Des. Sel. 17, 107–112 (2004).

Current Challenge and Motivation



Objective

To more accurately determine the mature region of precursor peptides using a sliding window

Methods Overview

- ❖ Sliding window of various “kmer” sizes is used to cleave the entire precursor sequence
- ❖ The cleaved pieces are then run on AMPlify and their score patterns are observed

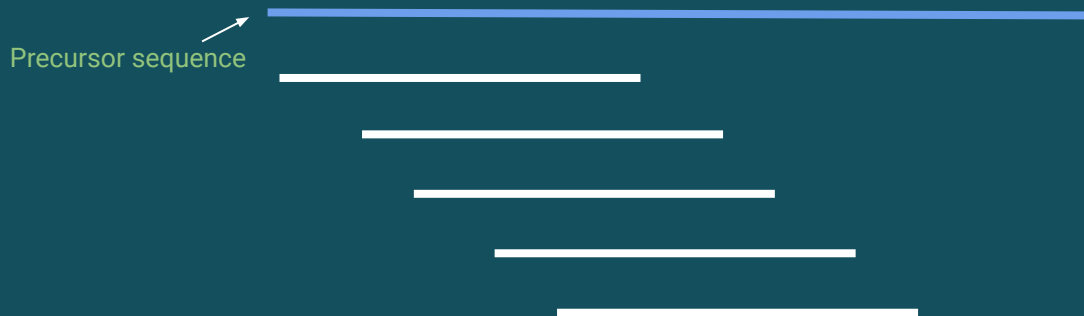
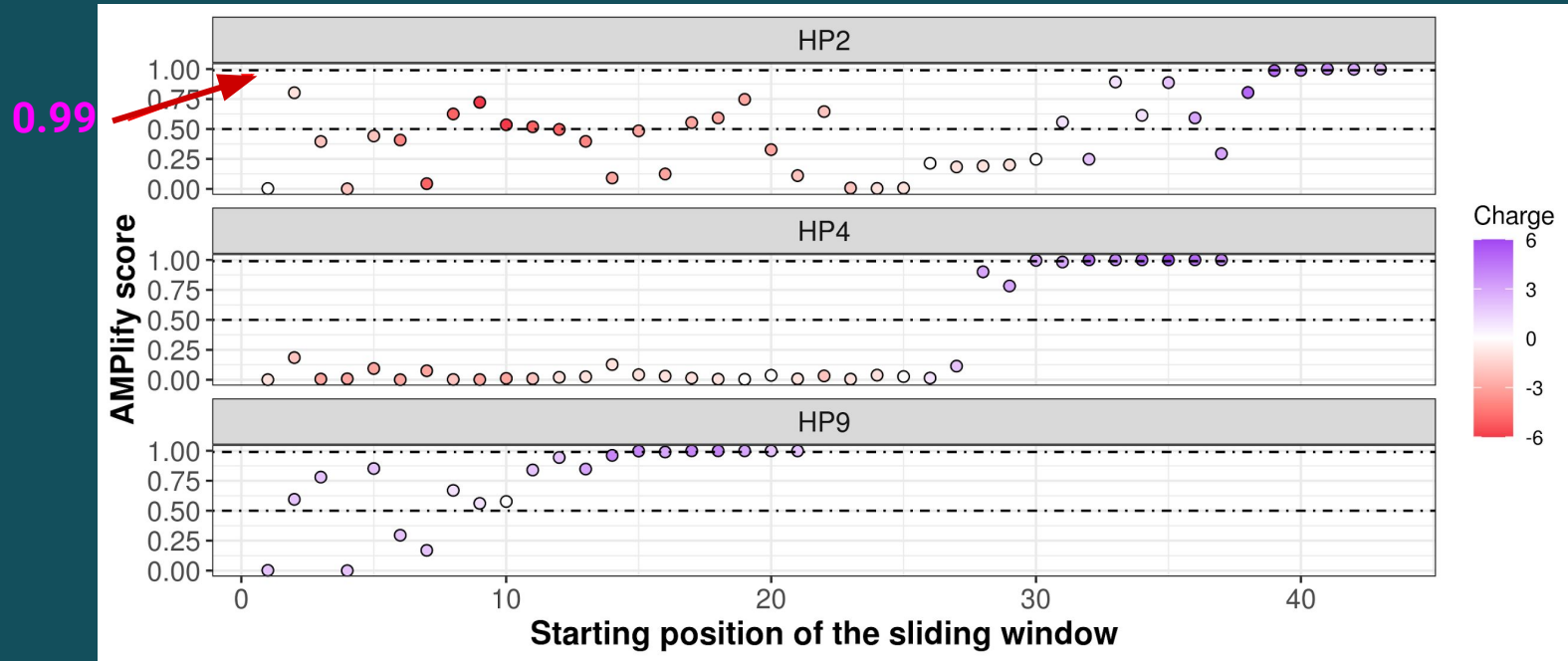


Fig: How the cleaved pieces look like across the entire precursor peptide

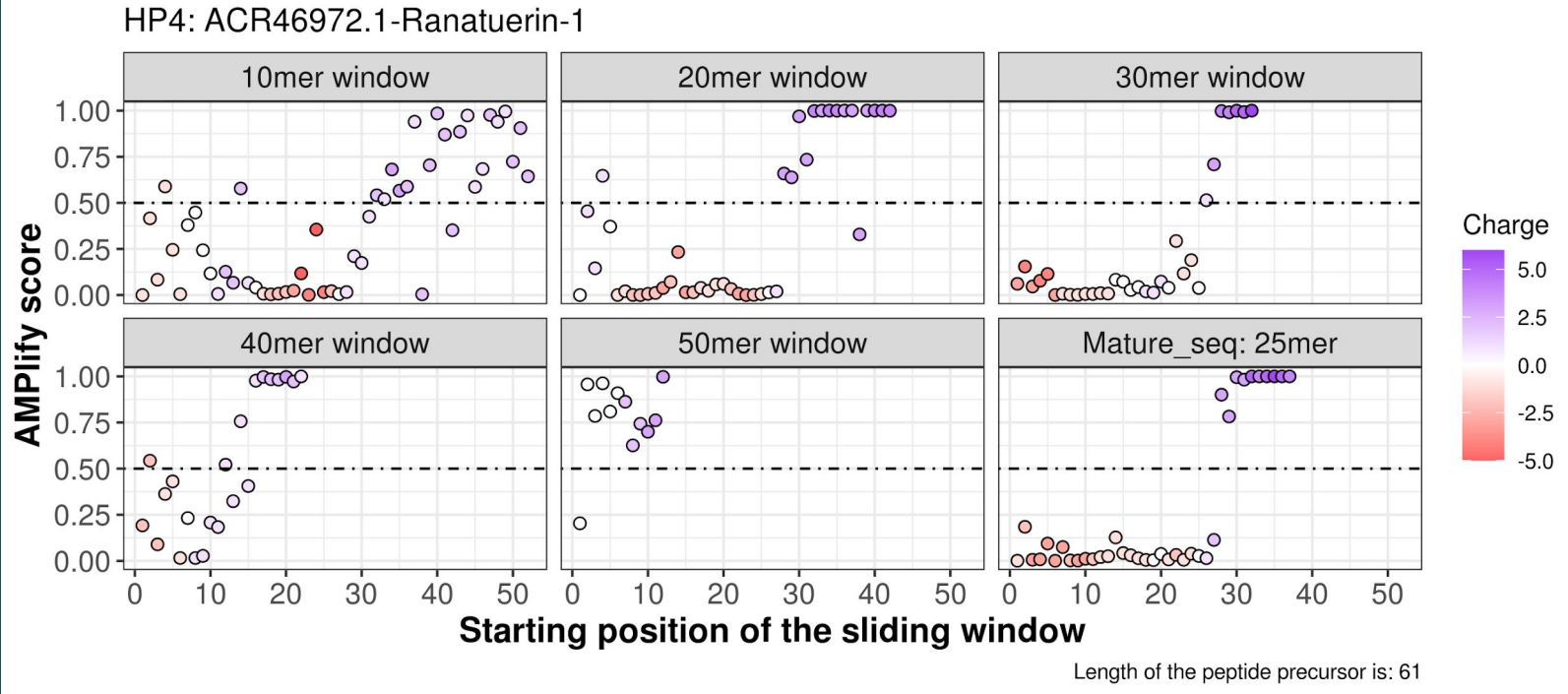
Known AMPs:

- ❖ 3 of the 7 sequences of *Rana [Lithobates] catesbeiana* were analysed from here <https://www.nature.com/articles/s41598-018-38442-1/tables/1>
- ❖ These sequences were then cleaved using the sliding window script according to the size of the mature sequence:
 - HP2 (*Ranatuerin-2PRc*) is cleaved with 29
 - HP4 (*Ranatuerin-1*) is cleaved with 25
 - HP9 (*Palustrin-Ca*) is cleaved with 31
- ❖ The sequences are then cleaved with window sizes 10,20,30,40,50

Known AMPs:



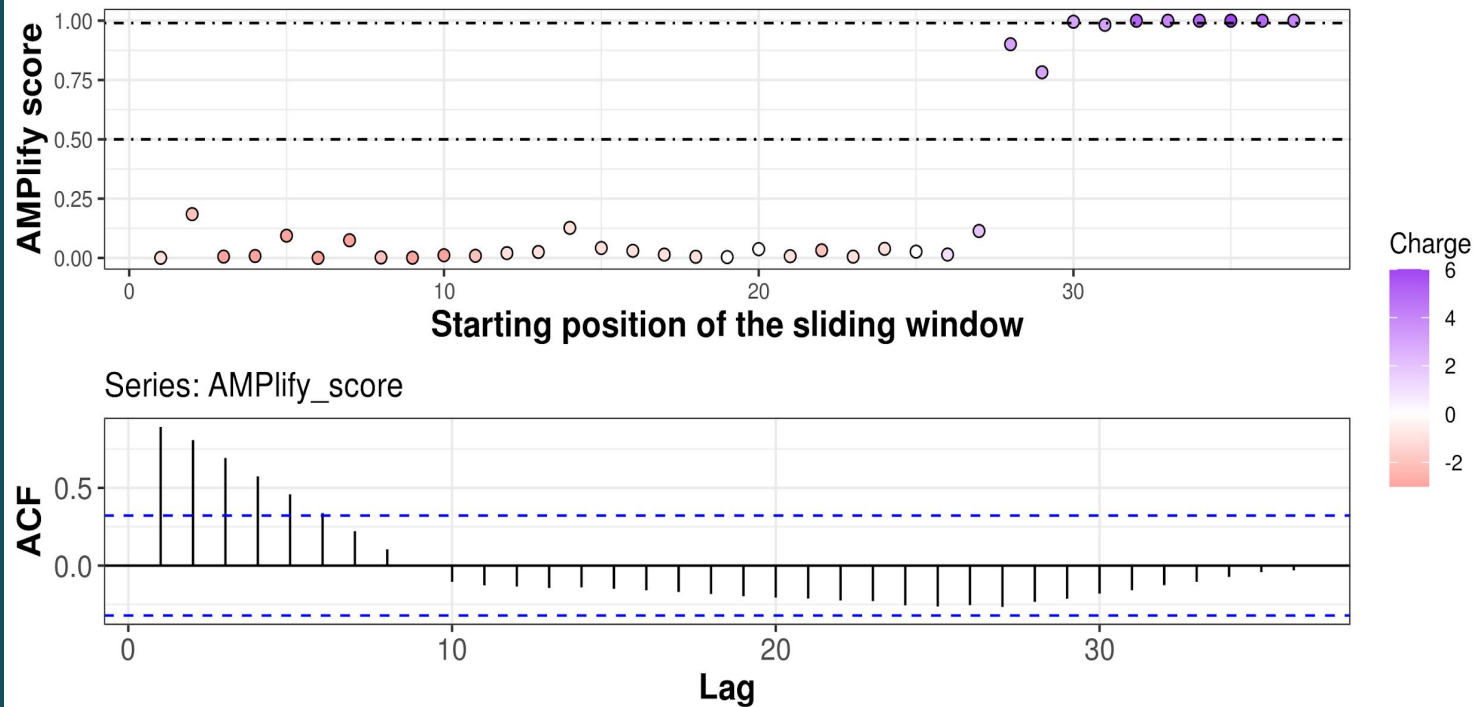
HP4: ACR46972.1-Ranatuerin-1



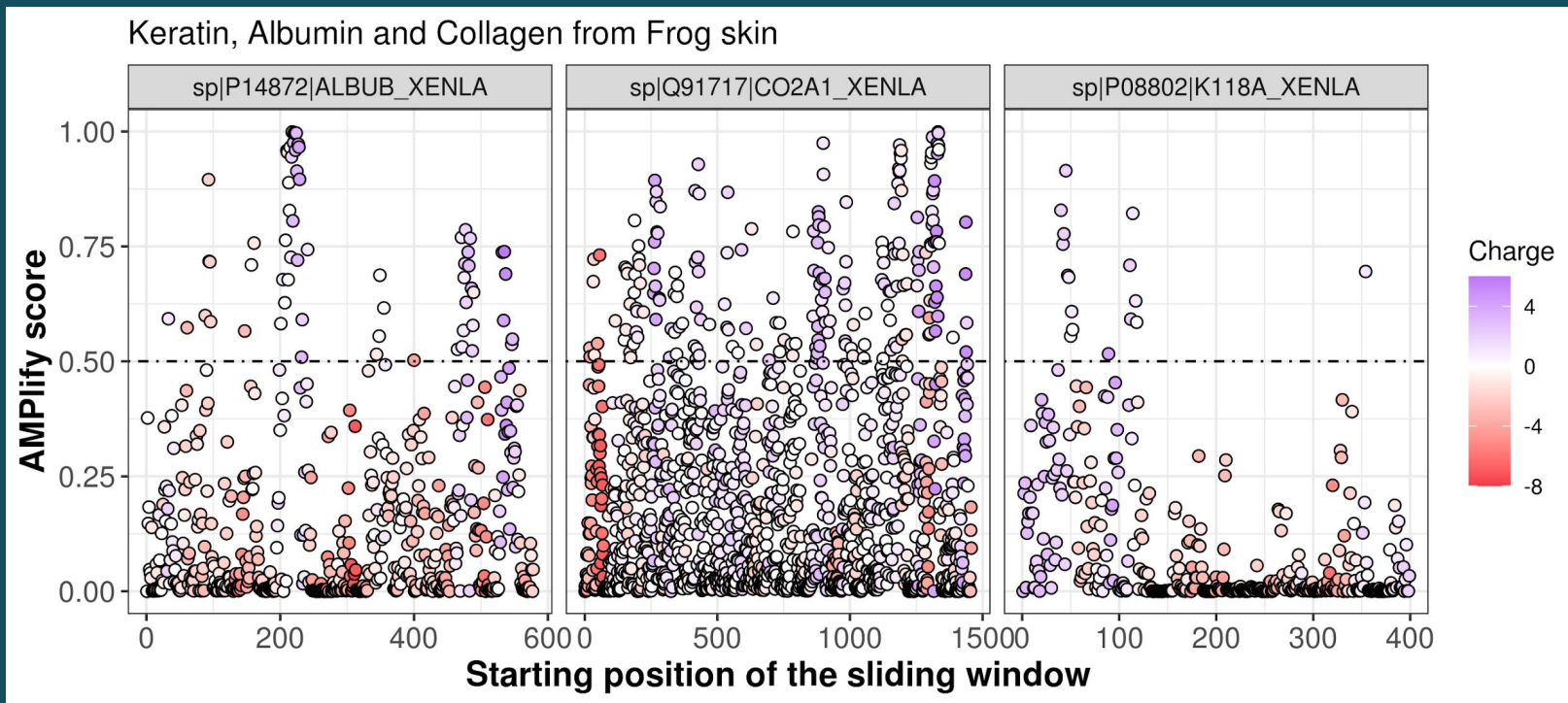
Autocorrelation:

- ❖ Whether there is a relationship between consecutive points
- ❖ What each part of the graph indicates
 - Lag
 - Height of the lines

Plot: HP4



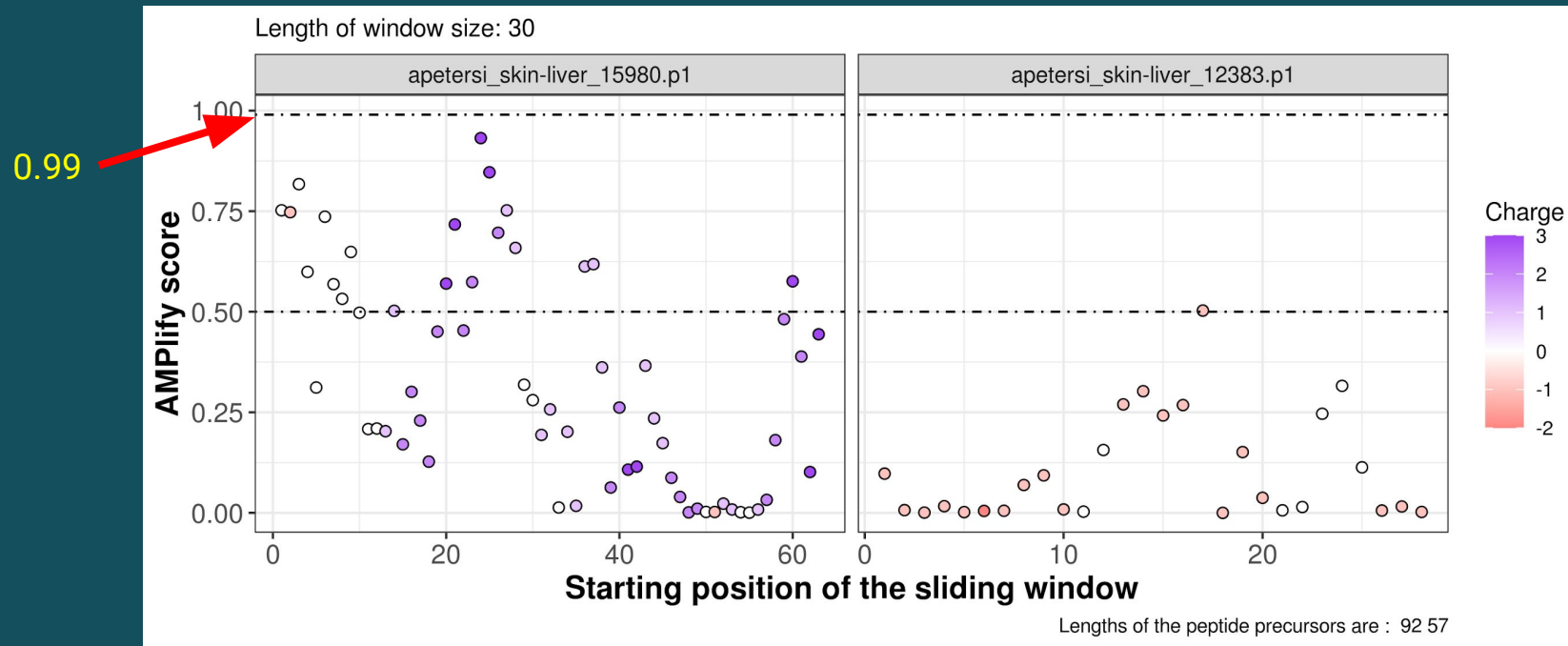
Negative Control: Cleaving on non-AMPs



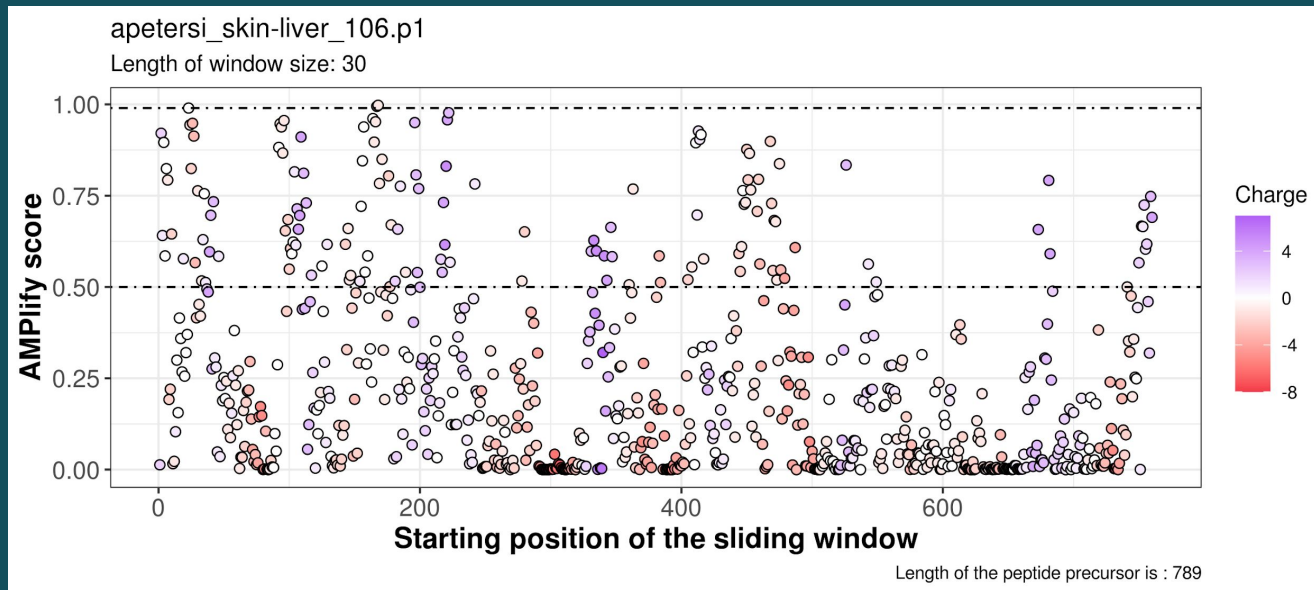
Summary

- ❖ Cleaving the known AMPs with various window sizes show that when the kmer size approaches the mature sequence length, the AMPLify score has less fluctuations
- ❖ The autocorrelation plot further demonstrates that consecutive pieces have a correlation in their AMPLify score
- ❖ Therefore, this method can help determine the mature sequence region of peptides more by showing plateaus

Results

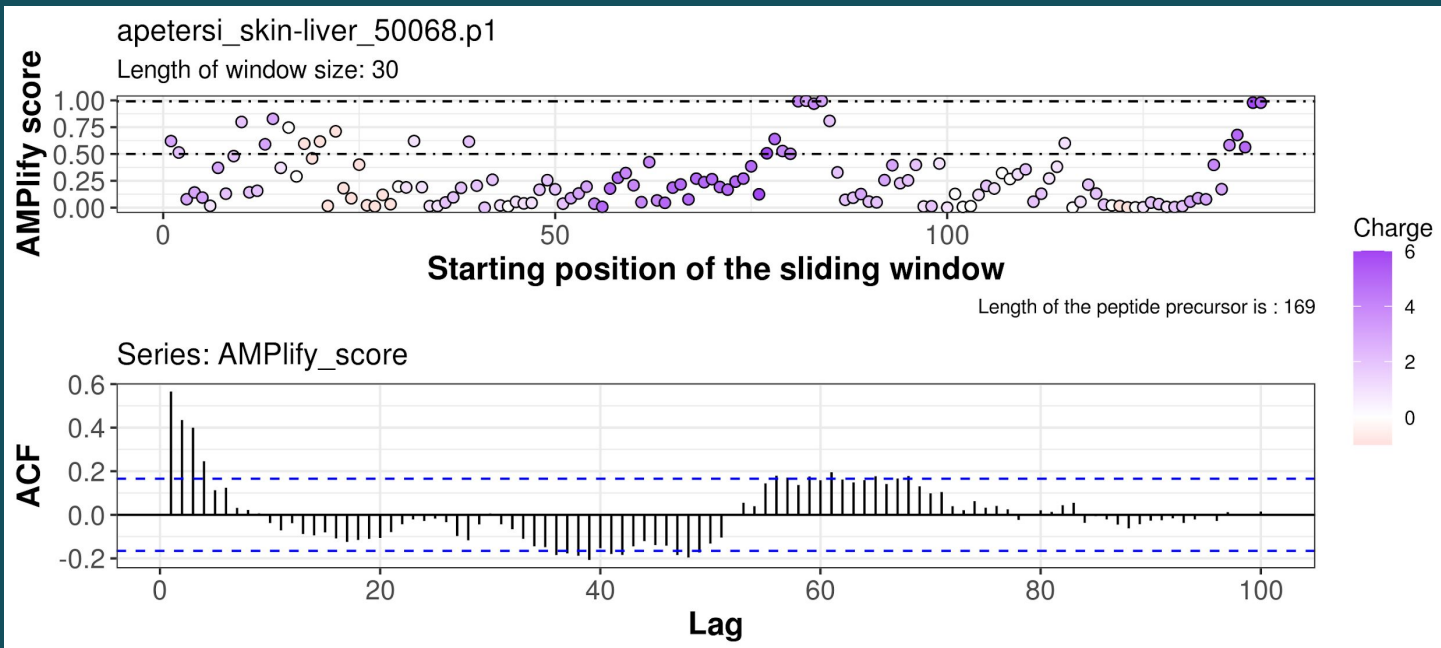


How AMPlify score varies across the peptide sequence



- ❖ At position 23, a neutral piece with sequence **GSTGFCWCLNENGEEIKGTRTAPGTKALTC** has a AMPlify score of 0.9898008000

Plots



Next steps...

- ❖ Determining the initial range of window sizes to cleave the peptides with
- ❖ How to determine this plateau more accurately
 - Working on making return maps that can identify the phase transition

Acknowledgements

Birol Lab

- ❖ Diana Lin
- ❖ Darcy Sutherland
- ❖ Lauren Coombe
- ❖ René Warren
- ❖ Inanc Birol

References

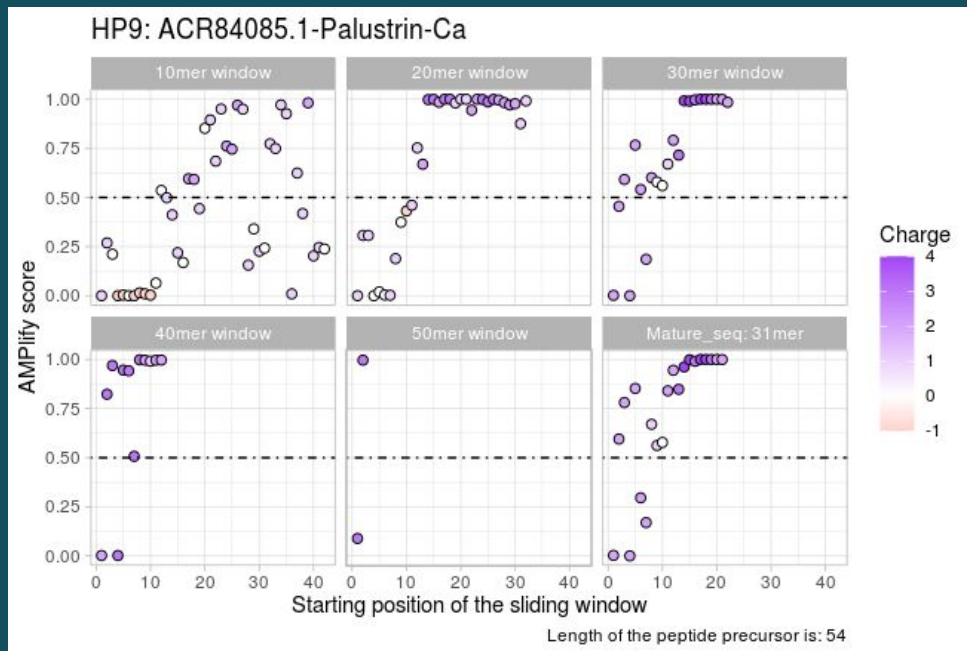
1. Duckert, P., Brunak, S., & Blom, N. (2004). Prediction of proprotein convertase cleavage sites. *Protein Engineering, Design And Selection*, 17(1), 107-112. doi: 10.1093/protein/gzh013
2. Orfanoudaki, G., Markaki, M., Chatzi, K., Tsamardinos, I., & Economou, A. (2017). MatureP: prediction of secreted proteins with exclusive information from their mature regions. *Scientific Reports*, 7(1). doi: 10.1038/s41598-017-03557-4

Supplementary slides!

- Has more plots

EXTRA SLIDE!

HP9: ACR84085.1-Palustrin-Ca



HP2: AFR43665.1-Ranatuerin-2PRc

