Analyzing pH-dependent Dynamics of Antimicrobial Peptides through Physics-based and Machine Learning Methods

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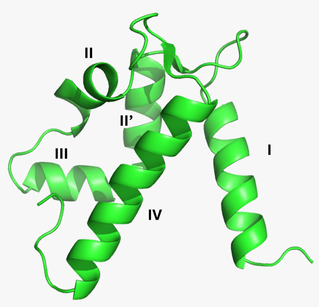
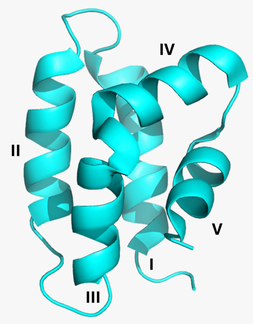
1 Introduction

- **Antimicrobial peptides (AMPs)** are **small proteins found in the innate immune system of many organisms.** A natural inhibitor of many bacteria, interest in understanding and designing AMPs has grown.
- Here, we investigate the dynamics of two AMPs: Human Psoriasin (PDB ID: 2psr) [1] and a parasitic protein, Ameobaphore A (PDB ID: 1of9) [2] which share similarities in their structure and are through to interact with host membranes using similar mechanisms
- Previous studies have shown that these AMPs form a pore in bacterial membrane, allowing essential nutrients to exit the cell and killing the organism. This activity is enhanced under acidic conditions.

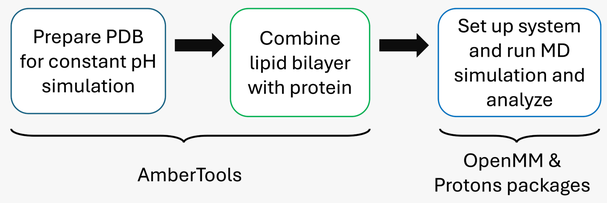


2psr



1of9

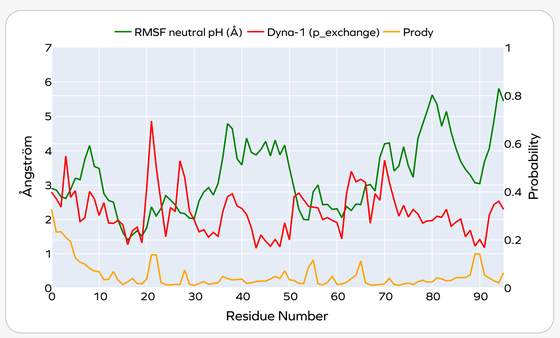
2 Methodology



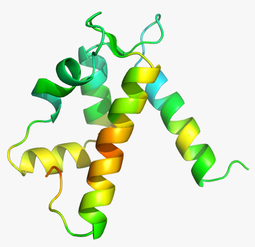
- Three different methods were compared for their ability to predict protein dynamics: **Molecular Dynamics (MD)** simulations, **Dyna-1** [4], and **ProDy** [5].
- MD simulations take a physics-based approach to simulating protein dynamics, utilizing principles in classical dynamics to update a system of proteins over time. We additionally incorporated an external package (Protons [3]) to **allow for an environment with a constant pH over time.**
- Dyna-1 is a deep-learning method trained on NMR structures, some of which belong to the AMP family.
- ProDy is another physics-based framework that utilizes distances between pairs of residues to predict fluctuations.

3 Results

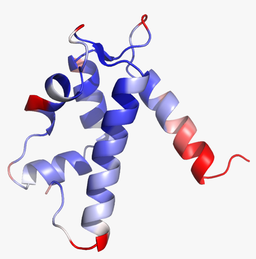
Human Psoriasin (PDB ID: 2psr)



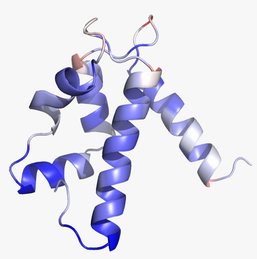
Neutral MD

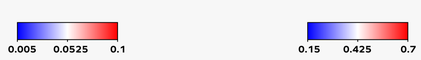


ProDy

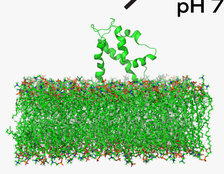


Dyna-1



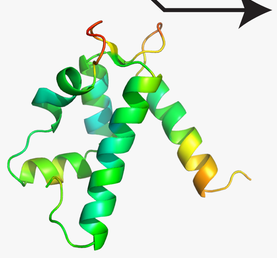


pH 7.4

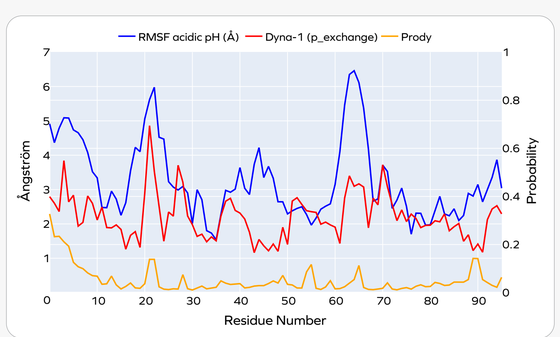


Starting Frame

pH 5.4

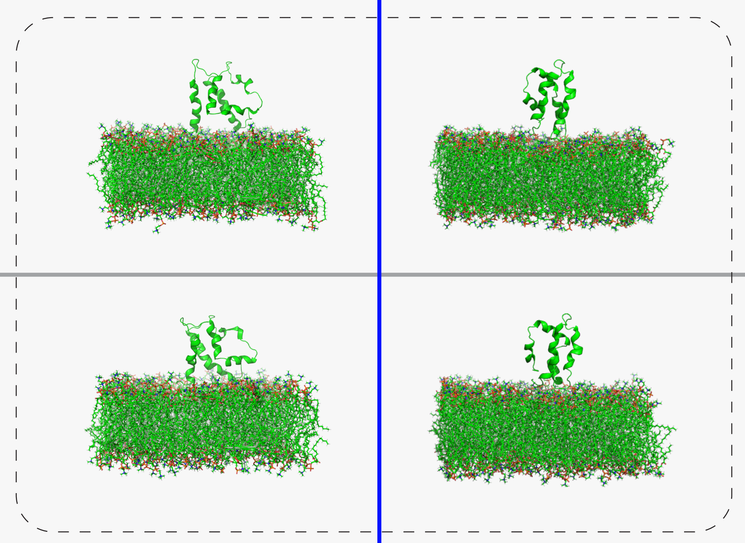


Acidic MD



Observation: Increased Activity in Acidic pH Condition

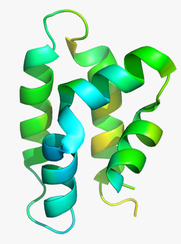
Neutral

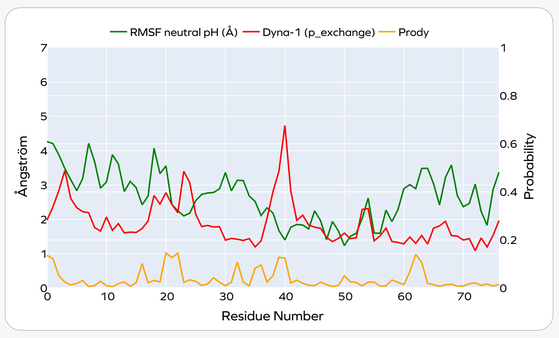


Acidic

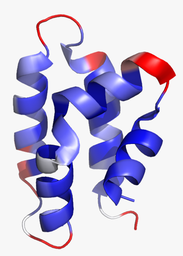
Ameobaphore A (PDB ID: 1of9)

Neutral MD

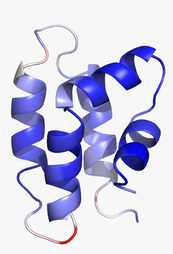


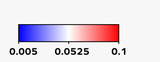


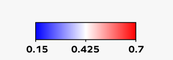
ProDy



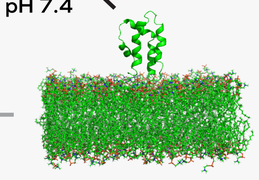
Dyna-1





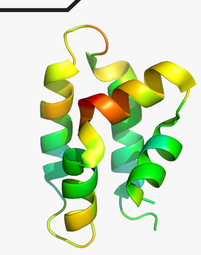


pH 7.4

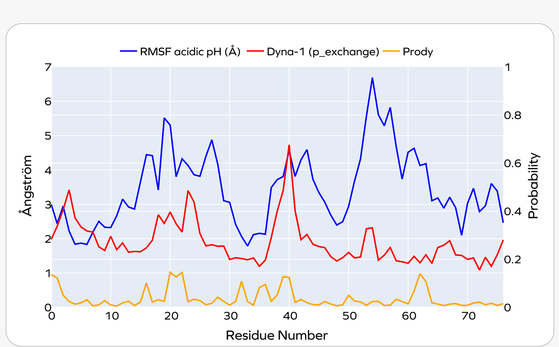


Starting Frame

pH 5.4



Acidic MD



4 Conclusions

- Overall, **Dyna-1 and ProDy are able to predict the flexible regions of the protein, but they tend to only perform well on loops**. Dyna-1 is trained on NMR data of various proteins, allowing it to learn various conformations of a sequence, but lacks information about how pH influences dynamics.
- ProDy only considers distances between residues, therefore lack prior information on the conformations various sequences can take. Despite this limitation, **ProDy is still able to predict protein dynamics given a static structure, although with lower variance in fluctuations compared to MD and Dyna-1.**
- Our MD simulations had the additional ability to consider the effects of pH over time, which provided insight into how the dynamics of the AMPs change when more favorable conditions are present. Supporting prior experimental work [1,2], **our MD analysis showed both AMPs have increased activity under acidic conditions.** While we were not able to see pore formation in the membrane with these simulations, we expect to see this process with a longer simulation time.

References

[1] Brodersen, D. E., Nyborg, J., & Kjeldgaard, M. (1999). Zinc-binding site of an S100 protein revealed. Two crystal structures of Ca2+-bound human psoriasin (S100A7) in the Zn2+-loaded and Zn2+-free states. Biochemistry, 38(6)
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[4] Wayment-Steele et al. (2025). Learning millisecond protein dynamics from what is missing in NMR spectra. bioRxiv, 2025-03.
[5] Bakan, A., Meireles, L. M., & Bahar, I. (2011). ProDy: protein dynamics inferred from theory and experiments. Bioinformatics, 27(11)

Acknowledgements

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