Examining the Relationship Between Biophysical Properties of Antimicrobial Peptides (AMPs) and their Drug Efficacies

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Abstract

Antibiotic resistance is a growing global health threat. One consequence is that patients with cystic fibrosis (CF) are prone to developing antibiotic resistant lung infections caused by multiple strains of bacteria, including *Pseudomonas aeruginosa*. Due to the limited number of treatment options for patients with chronic antibiotic resistant infections, there is a need for finding new antibiotics that allow for effective eradication of bacterial infections, such as those in the CF lung. Many antimicrobial peptides (AMPs) have been annotated in databases and are considered as potential alternatives for current antibiotics. However, in many instances, the suitability of AMPs as drug molecules has not been extensively explored. Here, we propose that certain molecular properties of AMPs favor high antibiotic efficacy. **Using information from AMP databases, we combined statistical analyses and machine learning techniques to identify relationships between various biophysical properties of AMPs and their drug efficacies.** Analyses suggest that net charge and maximum average hydrophobic moment are the most important properties in determining if a peptide is useful against *P. aeruginosa* infections. Maximum average hydrophobic residue, average alpha helix propensity score, hydrophobic proportion, and peptide length still contribute to this determination but to lesser degrees. Cation-pi interactions, on the other hand, do not appear to factor into this decision at all. Based on these properties, our current work is focused on designing and experimentally testing new peptides that may have activity against *P. aeruginosa*.

Methods - Computations

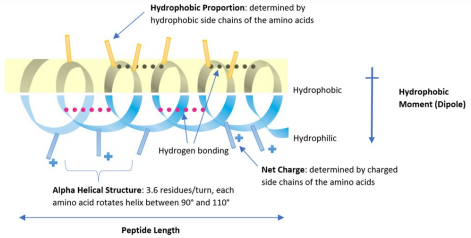


Fig 1. Schematic of molecular properties likely to be of biological significance

1. Peptide Length

2. Net Charge

$$= Lys + Arg\ - Asp\ - Glu$$

3. Hydrophobic Proportion[2]

$$= \frac{Cys+Phe+Ile+Leu+Met+Pro+Val+Trp+Tyr}{Peptide\ Length}$$

An amino acid is defined as hydrophobic if its value exceeds 0.70 in Fauchere & Pliska's scale

4. Maximum Average Hydrophobic Moment[1],[2]

$$< \mu_{max} > = \frac{\sqrt{\left[\sum_i H_i \cos(i\delta)\right]^2 + \left[\sum_i H_i \sin(i\delta)\right]^2}}{11}$$

δ represents the angle b/w the amino acid side chains (90-110°), i represents the residue number in position i in an 11 amino acid window, H_i represents the ith amino acid's hydrophobicity in Fauchere & Pliska's scale

5. Maximum Average Hydrophobicity[1],[2]

$$< H_{max} > = \frac{\sum_i H_i}{11}$$

H_i represents the ith amino acid's hydrophobicity in Fauchere & Pliska's scale

6. Average Alpha Helix Propensity Score[3],[4]

$$< \alpha > = \frac{\sum_i e^{Hx_i}}{Peptide\ Length}$$

Hx_i represents the ith amino acid's helix propensity in Pace-Schols scale

7. Cation Pi Interactions[5]

$$Trp \longrightarrow Arg\ (i, i+4)$$

Results

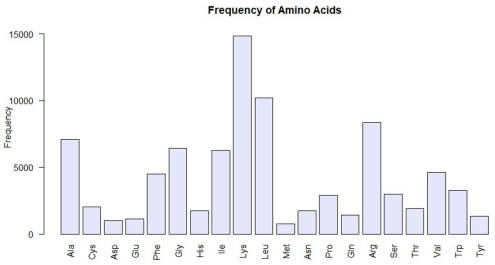


Fig 2. Distribution of amino acid residues that make up the subset of n = 4,218 AMPs from the DBAASP. We obtained this subset by filtering the DBAASP based on length and known activity against *P. aeruginosa*. These amino acid frequencies were then used to assign a probability of occurrence for each amino acid when generating new, synthetic peptide sequences.

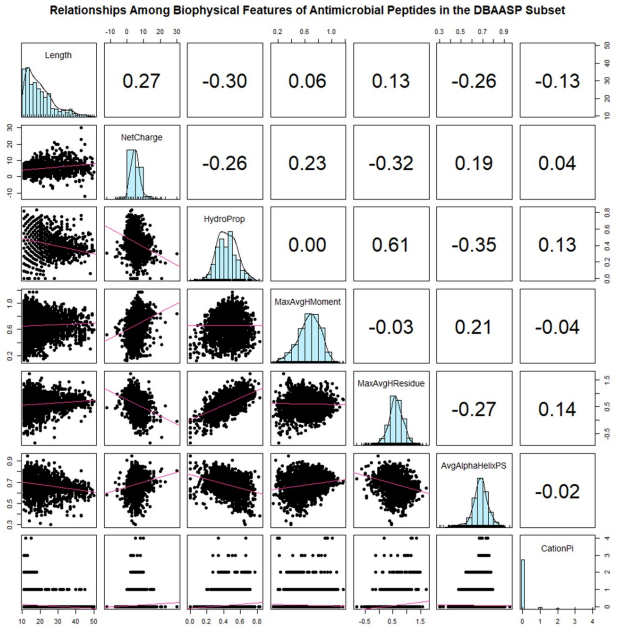
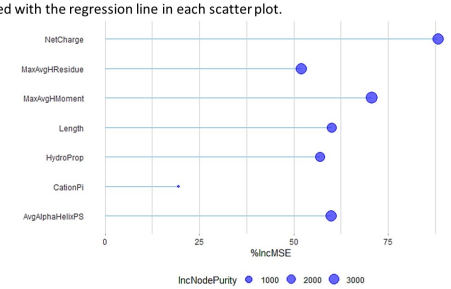


Fig 3. Scatterplot Matrix. The diagonal panels provide histograms for each molecular property, the panels below the diagonal depict bivariate scatter plots, and the panels above the diagonal report the Pearson correlation coefficients associated with the regression line in each scatter plot.

Fig 4. Random Forest. The importance of a biophysical property is determined by measuring the increase in mean square error and the increase in node purity when that property is randomly permuted. Larger increases are indications of more important properties.



Discussion and Conclusions

- Since none of the correlation coefficients displayed in the scatterplot matrix exceeds 0.75, we can conclude that none of the molecular properties is strongly linearly correlated with each other.
- The feature importance attribute of the random forest regression model indicates that net charge and maximum average hydrophobic moment are the most distinctive biophysical properties influencing peptide effectiveness.
  - The presence of cation-pi interactions, on the other hand, is unlikely to affect peptide effectiveness.
- Based on our learnings from the bioinformatic analyses of the DBAASP, we designed 50,000 new, synthetic peptide sequences.
  - We used the random forest regression model to generate predictions for their log_2(MIC) values.
  - From the 50,000 computer generated peptide sequences, we identified a set of eight sequences predicted to be non-hemolytic and predicted to have strong antimicrobial activities:

Sequence	Predicted log_2(MIC)
AKRTQRFPRWKKCLRLGFVGCKGNILKAA	-0.175
RVRKGAGTSRKLKIVKNLGRHIVWFKGIP	0.105
IRKYRPGLFAKFHKLLKNRKIGGKNL	0.141
MKSKPTVIMRYRFWRVGKL	0.307
RQACGTKAKARLRKPRCLTIGRRVVRKFSKWR	0.329
KMVAKKVKIKRCKRKVHKLPGFGSISIL	0.335
LIAKYGKHAKFKAKKKPQGISGVPKRFYKALWIG	0.432
PRRIKTGAAKRKPKLSKKWNQKLLKKLTPFGW	0.464

Future Directions

In future studies, we seek to:

- Assess the accuracy of the random forest predictive tool
- Confirm the antimicrobial activity of the eight synthetic peptides experimentally by conducting Minimum Inhibitory Concentration (MIC) assays
- Confirm that the eight synthetic peptides are indeed non-hemolytic

References

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