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Erkan Caner Ozkat

has participated in 5th International Conference on Recent and Innovative Results in Engineering and Technology ICRIRET 2026 on August 25-26 in 2026 in Konya, Turkey.

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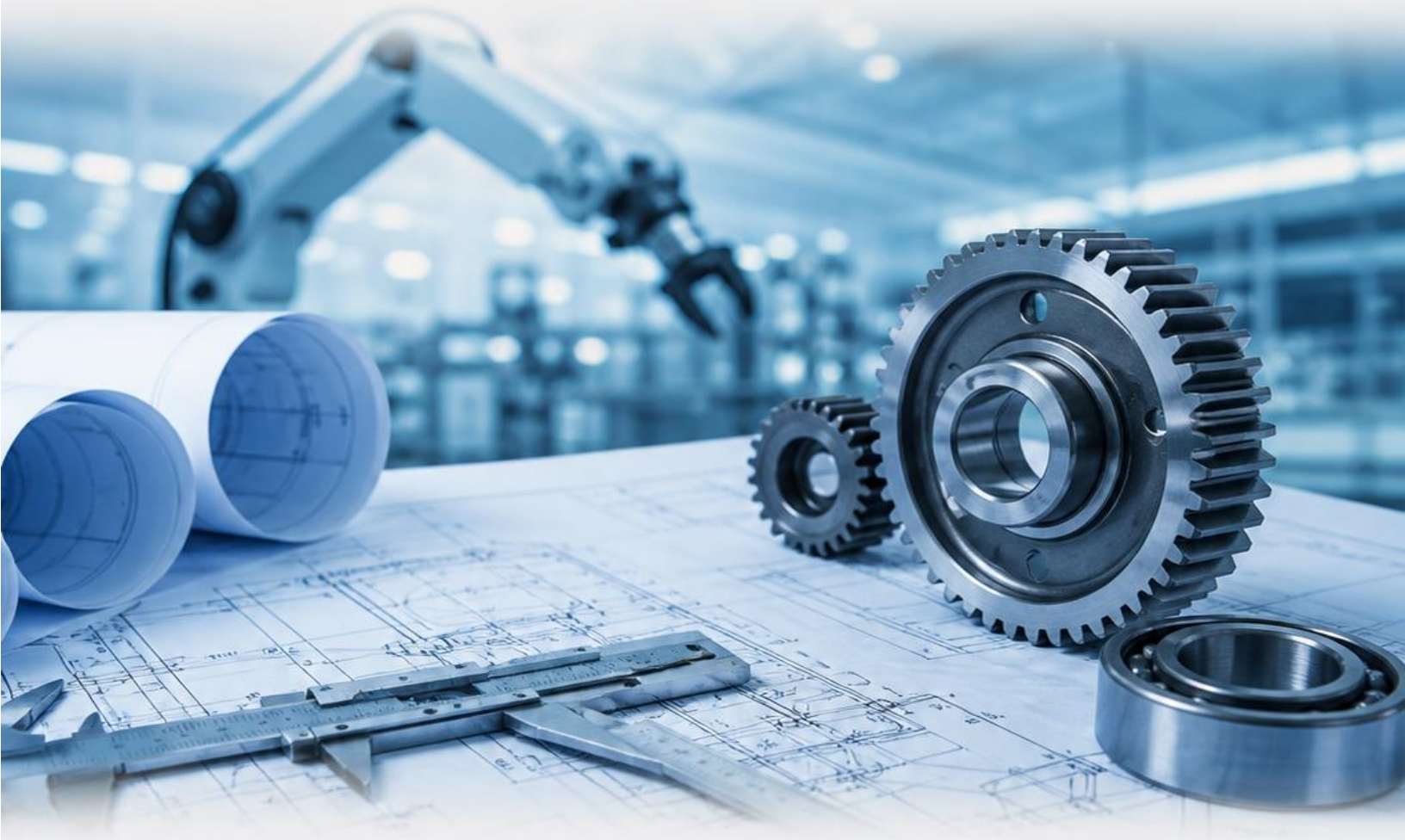

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INTRODUCTION

We had the great honor of 5th International Conference on Recent and Innovative Results in Engineering and Technology ICRIRET 2026. It was truly a great pleasure for us to greet a lot of participants from many different countries to attend ICRIRET 2026! We firmly believe that the conference will become an important international event in the field of cross-industry discussion about innovations in Academic Studies.

Three cooperating organizations supported the two-day conference. There were 127 papers accepted for presentation at ICRIRET 2026, contributed from different countries. We had plenary speeches and several well-known scientists and experts, to give invited talks at differentsessions.

The purpose of ICRIRET 2026 was to provide a forum for the participants to report and review innovative ideas, with up-to-date progress and developments, and discuss novel approaches to the application in the field of their own research areas and discuss challenges of doing science.

We sincerely hope that the exchange of ideas on doing research, science and improving education will help the participants, and international cooperation sharing the common interest will be enhanced.

On behalf the Organization Committee of ICRIRET 2026, we would like to heartily thank our cooperating organizations for all they have done for the conference. We would also like to thank the authors for theircontribution to the proceedings; the participants and friends of ICRIRET 2026, for their interest and efforts in helping us to make the conference possible; and the Editorial boards for their effective work and valuable advice, especially the ICRIRET 2026 secretariat and the ICRIRET 2026 staff, for their tireless efforts and outstanding services in preparing the conference and publishing the Proceedings.

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Sequence Homology Inflates Reported Accuracy in Antimicrobial Peptide Prediction: A Machine Learning and Deep Learning Benchmark

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Abstract – Machine learning is now routine for screening antimicrobial peptide candidates, and reported accuracies are high. This study asks how much of that accuracy depends on sequence homology between training and test data. A benchmark of 6288 mature peptides was assembled from curated UniProtKB annotation, using one extraction rule for both classes, so negative-set bias is excluded by construction. All 19,766,328 peptide pairs were aligned with local Smith-Waterman scoring. Partitions were drawn at 0.8 identity, the only threshold that clears a shuffled-sequence noise floor without chaining the set into one component. Twenty pipelines were then trained under two partitioning regimes with five seeds each. They comprise sixteen classical pipelines, three neural architectures and a control that only looks up the most similar training peptide. The peptide set proved strongly redundant, and a random partition left 62 per cent of test peptides with a close relative in training. Every trained pipeline lost accuracy when whole identity components were held out, by 0.039 in area under the curve on average, and the leading pipeline changed. The lookup control reached 0.958 under the random partition against 0.819 under the homology-aware one. Accuracy fell steadily as identity to the nearest training peptide decreased. Holding out whole greedy clusters, which is common practice, still left 87 per cent of held-out peptides with a close relative in training. Sequence-based peptide classifiers should therefore be partitioned by identity component, and test-to-training similarity should be reported alongside the headline metric.

Keywords – Antimicrobial Peptides, Data Leakage, Sequence Homology, Deep Learning, Benchmark Evaluation

I. INTRODUCTION

Bacterial antimicrobial resistance was associated with an estimated 4.95 million deaths in 2019 [1]. Antimicrobial peptides are short and mostly cationic sequences that act on microbial membranes [2]. They remain an attractive route to agents against resistant organisms, because they kill by physical disruption rather than through a single protein target [3]. Resistance therefore develops slowly against many of them. Laboratory screening of candidates is slow and costly, so computational triage has become a standard first step [4].

Sequence-based classifiers dominate that triage step. Early methods encoded a peptide as amino-acid or dipeptide composition, or as a block of biophysical descriptors. The encoded peptide was then passed to a random forest or a support vector machine [5]. Deep models followed, learning residue embeddings with convolutional and recurrent layers [6, 7]. The same architectures now drive genome mining and generative peptide design [8, 9]. Reported performance is uniformly high, and areas under the receiver operating characteristic curve above 0.95 are common.

Independent assessments do not support that reading. Web tools evaluated on unseen data lose a large part of their advertised accuracy [10]. A systematic comparison of published predictors found that their ranking depends on how the evaluation set was assembled [11]. One cause is already documented. The negative class in these benchmarks usually differs from the positive class in length and in composition,

which makes the task easier than it should be [12]. A second cause is generic to biological sequence data. Natural peptides fall into families whose members share ancestry [13]. A partition drawn at random therefore places close relatives on both sides of it. The same failure is well described for paired biological inputs [14] and for machine learning in genomics [15]. It is now treated as a leading source of irreproducible results [16].

This paper measures how much reported accuracy survives when the second cause is removed. A peptide set is assembled from primary annotation by a rule that is identical for both classes. That isolates the homology question from the negative-selection question. Every pair of peptides is then aligned, and the redundancy of the set is quantified against a composition-matched null. Two partitions are drawn. The first is the usual random partition. The second removes whole identity clusters, so no test peptide has a close relative in training. Twenty prediction pipelines are trained under both partitions.

The study contributes five things. It provides a reproducible peptide benchmark built from a single primary source by a symmetric rule. It calibrates the identity threshold against shuffled sequences rather than adopting a convention. It shows that a partition drawn on greedy clusters still leaves close relatives on both sides, and quantifies that gap. It reports a like-for-like comparison of classical and deep pipelines under both regimes. It relates accuracy to the identity between each test peptide and its nearest training neighbour. The last step turns a binary argument about splitting into a measured curve.

II. Materials and Method

A. Peptide dataset

Peptides were taken from the reviewed section of UniProtKB, which carries manually curated sequence features [17]. Two pools were downloaded. The positive pool held entries annotated with the Antimicrobial keyword. The negative pool held entries that carry at least one annotated mature peptide, but none of the antimicrobial, antibiotic, antiviral, fungicide, toxin or bacteriolytic keywords. Membrane-lytic venom peptides are often antimicrobial without being annotated as such. The toxin keyword is excluded from the negative pool for that reason.

One row was extracted per annotated mature peptide feature, using the same rule in both pools. Features with uncertain boundaries were skipped. So were peptides outside five to one hundred residues, and peptides containing a non-standard amino-acid symbol. Sequences seen in more than one entry were collapsed to a single row. Thirty sequences that appeared under both labels were discarded as ambiguous. The class label is therefore a property of the parent entry, and the two classes are matched on annotation type. Table 1 describes the resulting set of 6288 peptides.

Table 1. Composition of the peptide benchmark.

Class	Peptides	Median length	Length IQR	Source organisms	UniProt entries
Antimicrobial	1846	25	18-33	432	1587
Non-antimicrobial	4442	20	11-31	842	3011

B. Sequence identity and clustering

All 19,766,328 pairs of peptides were aligned with the local Smith-Waterman algorithm [18]. Alignments were scored with BLOSUM62 [19], using a gap penalty of eleven for opening and one for extension. Identity is the number of identical aligned residues, divided by the length of the shorter peptide. A short local hit inside a long peptide therefore scores low. Dipeptide word filters lose almost all pruning power at the thresholds relevant to short peptides. The matrix was computed exhaustively for that reason, rather than approximated.

Two grouping rules were then applied to that matrix. The first is the greedy longest-first rule used by CD-HIT, which reports how much of the set is redundant [13]. The second is the transitive closure, in which every pair at or above the threshold is placed in one component. Only the closure supports a partition. Greedy clustering compares each sequence with cluster representatives alone, so two sequences in different greedy clusters can still be near-identical.

The threshold was calibrated rather than assumed. Each sequence was shuffled, which preserves its length and composition while destroying its ancestry. Pairs of shuffled sequences were then aligned in the

same way. Identity below 0.6 is not distinguishable from chance for peptides of this length. The shuffled null links 1.7 per cent of pairs at 0.4 and 0.08 per cent at 0.6. That places a floor under any usable threshold.

The closure then places a second constraint from above. At 0.6 identity the closure chains 96.6 per cent of the peptides into a single component, so no partition at that threshold is possible. At 0.7 the largest component still holds 49.2 per cent. At 0.8 it holds 1.8 per cent, and the set breaks into 2782 components. A partition at 0.6 must therefore hold out either 3.4 per cent of the peptides or 96.6 per cent of them. Neither is a usable test set. The homology-aware partition operates at 0.8, which is the least strict threshold that both clears the noise floor and admits a partition.

C. Partitioning regimes

Two regimes were compared, each drawn five times with different seeds. The random regime draws a stratified twenty per cent of peptides as the test set. That is the usual practice in this literature. The homology-aware regime instead draws whole identity components until the same share is reached. Components are chosen so that the class balance of the held-out side tracks the full set. No test peptide can then share 0.8 identity with any training peptide, because such a pair would lie in one component. An inner validation set of twenty per cent of the remainder is drawn under the same rule as the outer split. Early stopping and threshold selection therefore never see the leakage that the outer split excludes.

D. Representations and models

Sixteen classical pipelines cross four encodings with four learners. The encodings are amino-acid composition, dipeptide composition, a block of twenty-five biophysical descriptors, and the concatenation of the first and third. The descriptor block covers length, mass, net charge at pH 7.4 and isoelectric point. It also covers mean hydropathy [20] and the largest sliding-window hydrophobic moment at a helical angle of one hundred degrees [21]. The aliphatic index, the instability index and the Boman index [22] complete the physical block, together with predicted secondary-structure fractions and ten residue-group fractions. The learners are logistic regression and a support vector machine with a radial basis kernel. A random forest [23] and gradient boosted trees [24] complete the set, each with balanced class weights.

Three neural architectures read the residue sequence directly through a learned embedding. The first stacks two convolutions and pools over positions. The second is a bidirectional long short-term memory network [25]. The third combines a convolutional front end with a recurrent body and attention pooling. Batches are sorted by length and padded to their own longest member. Three quarters of a fixed-width tensor would otherwise be padding.

A twentieth pipeline is included as a control, and it fits nothing. For each test peptide it finds the most similar antimicrobial peptide in training. It also finds the most similar non-antimicrobial one. The peptide is scored by the difference between those two identities. Any accuracy the control reaches is attributable to sequence similarity alone. It therefore measures how far a benchmark can be solved by lookup.

E. Training and evaluation

Every pipeline saw the same training peptides and was scored on the same test peptides. Neural models were trained for at most thirty epochs with the Adam optimiser. The learning rate was 0.001, the batch size was 64 and the loss was class-weighted. Training stopped early after five epochs without improvement in validation area under the curve. Decision thresholds were selected on the validation peptides by maximising the Matthews correlation coefficient, which is the recommended summary for imbalanced problems [26]. Test results are reported by area under the curve, average precision, the Matthews coefficient, sensitivity and specificity.

The pipeline was implemented in Python 3.12 with Biopython [27], scikit-learn [28], XGBoost and PyTorch [29]. The complete grid of two regimes, five seeds and twenty pipelines gives 200 fitted models. It runs in about 8 minutes on eight cores. Aligning all peptide pairs takes about eleven minutes on the same machine.

III. Results

A. The peptide set is strongly redundant

The first result concerns the data rather than any model. At the calibrated threshold of 0.6, the 6288 peptides collapse into 1948 identity clusters. So 69 per cent of the set is redundant. Only 14 per cent of peptides are singletons, and the largest family holds 107 members. Redundancy does not disappear at stricter thresholds. Even at 0.9 identity, 47 per cent of peptides still share a cluster with another peptide. Figure 1 shows the length distribution, the distribution of pairwise identity, and the way both redundancy and linkage track the threshold.

That redundancy passes straight into a random partition. Averaged over five random splits, 62 per cent of test peptides have a training peptide at 0.8 identity or above. That figure rises to 95 per cent at 0.6 identity. The mean identity to the nearest training peptide is 0.83. Under the homology-aware splits no test peptide reaches 0.8 identity with any training peptide, and the mean nearest identity falls to 0.66. The two regimes therefore pose different questions. The first asks whether a model recognises relatives of peptides it has seen. The second asks whether it recognises peptides unlike anything it has seen.

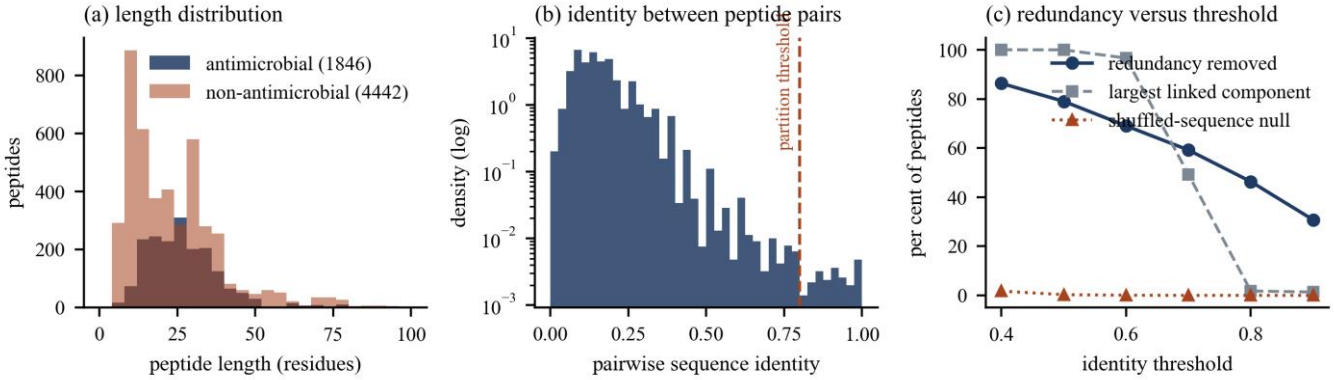

Fig. 1 Composition and redundancy of the peptide benchmark. Panel (c) shows redundancy, the share of peptides in the largest linked component, and the rate at which shuffled sequences of the same composition are linked.

B. Cluster-disjoint splitting is not homology-free

A partition drawn on greedy clusters does not deliver what it promises, and this is worth separating from the main result. Greedy clusters at 0.6 identity were held out whole, so no cluster appears on both sides. Even so, 87 per cent of held-out peptides still had a training peptide at 0.6 identity or above. The mean identity to the nearest training peptide was 0.67. Holding out greedy clusters at 0.8 leaves 23 per cent of test peptides with a relative at that level. Components remove both figures to zero by construction, which is why the benchmark uses them.

The reason is structural rather than incidental. Greedy assignment compares each sequence with cluster representatives alone. Two peptides that match no common representative can still be near-identical to each other, and they are then placed in different clusters. Any study that splits on clusters from a greedy tool inherits this gap. Reporting the achieved identity between test and training sequences would expose it immediately.

C. Accuracy under the two partitioning regimes

Table 2 reports every pipeline under both regimes. Figure 2 shows the same values as paired points. Under the random regime the best pipeline is DPC/RF, at an area under the curve of 0.970. Under the homology-aware regime the leader is HYBRID/RF, at 0.936. Averaged over the nineteen trained pipelines, the change of regime costs 0.039. The loss is present for every pipeline, and ranges from 0.008 to 0.138. The pooled paired test over all pipelines and seeds gives $p < 0.0001$.

The lookup control makes the mechanism explicit. That pipeline fits nothing, and only compares a test peptide with its most similar training neighbours. It still reaches 0.958 under the random regime. The best trained pipeline on the same splits reaches 0.970. Under the homology-aware regime the same control

falls to 0.819. Sequence similarity alone therefore accounts for most of what a random split measures. It accounts for much less of what a homology-aware split measures.

Model choice matters less than the partition. Within the sixteen classical pipelines, the regime explains 24.3 per cent of the variance in the area under the curve. The encoding explains 1.8 per cent and the learner 54.9 per cent. The seed contributes 6.5 per cent. The best deep architecture reaches 0.966 under the random regime and 0.923 under the homology-aware one. The best classical pipeline reaches 0.970 and 0.936. Deep models therefore fall behind their classical counterparts once relatives are removed.

Table 2. Test AUROC under both regimes, as the mean and standard deviation of five seeds. Delta is the accuracy lost when relatives are removed from training.

Pipeline	AUROC random	AUROC homology-aware	Delta	MCC random	MCC homology
NN-identity	0.958 ± 0.003	0.819 ± 0.030	0.138	0.783	0.506
HYBRID/RF	0.968 ± 0.007	0.936 ± 0.013	0.033	0.817	0.746
HYBRID/XGB	0.968 ± 0.009	0.933 ± 0.015	0.035	0.836	0.731
HYBRID/SVM	0.956 ± 0.011	0.929 ± 0.014	0.027	0.806	0.742
PHYSCHEM/RF	0.962 ± 0.008	0.926 ± 0.017	0.037	0.814	0.732
AAC/RF	0.966 ± 0.008	0.923 ± 0.010	0.042	0.796	0.688
PHYSCHEM/XGB	0.963 ± 0.009	0.923 ± 0.017	0.040	0.825	0.706
PHYSCHEM/SVM	0.947 ± 0.015	0.919 ± 0.018	0.028	0.792	0.715
AAC/XGB	0.963 ± 0.007	0.917 ± 0.019	0.045	0.793	0.681
AAC/SVM	0.948 ± 0.010	0.915 ± 0.016	0.033	0.760	0.677
DPC/RF	0.970 ± 0.004	0.911 ± 0.018	0.059	0.828	0.705
DPC/SVM	0.961 ± 0.008	0.904 ± 0.020	0.057	0.811	0.683
DPC/XGB	0.960 ± 0.003	0.901 ± 0.020	0.059	0.802	0.683
HYBRID/LR	0.889 ± 0.016	0.881 ± 0.023	0.008	0.634	0.635
PHYSCHEM/LR	0.884 ± 0.016	0.876 ± 0.023	0.009	0.632	0.647
AAC/LR	0.866 ± 0.014	0.856 ± 0.012	0.010	0.593	0.588
DPC/LR	0.916 ± 0.005	0.851 ± 0.021	0.065	0.680	0.549
CNN-BiLSTM	0.962 ± 0.008	0.923 ± 0.013	0.039	0.811	0.711
BiLSTM	0.956 ± 0.013	0.912 ± 0.022	0.044	0.795	0.697
CNN	0.966 ± 0.008	0.903 ± 0.016	0.063	0.818	0.675

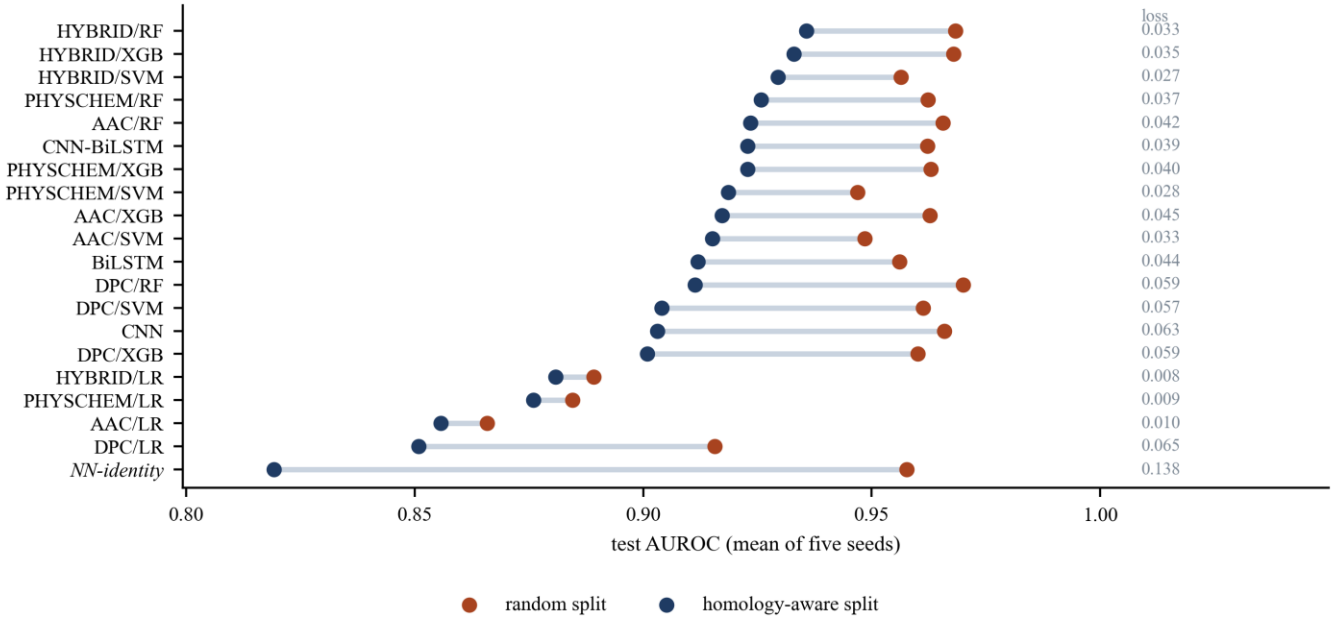

Fig. 2 Every pipeline under both partitioning regimes. The number beside each pair is the accuracy lost when close relatives are removed from the training set.

D. *Accuracy tracks similarity to the training set*

The comparison of regimes hides a continuous relationship, which Figure 3 makes visible. Test peptides from the random splits were binned by their identity to the nearest training peptide. Accuracy was then recomputed inside each bin. For the leading pipeline the area under the curve is 0.992 in the highest identity band, at 0.9 and above. It falls to 0.831 for peptides below 0.6 identity, a gap of 0.161. Panel (b) explains why the headline number sits close to the first figure. Under a random split, 43 per cent of test peptides fall in the highest identity band alone.

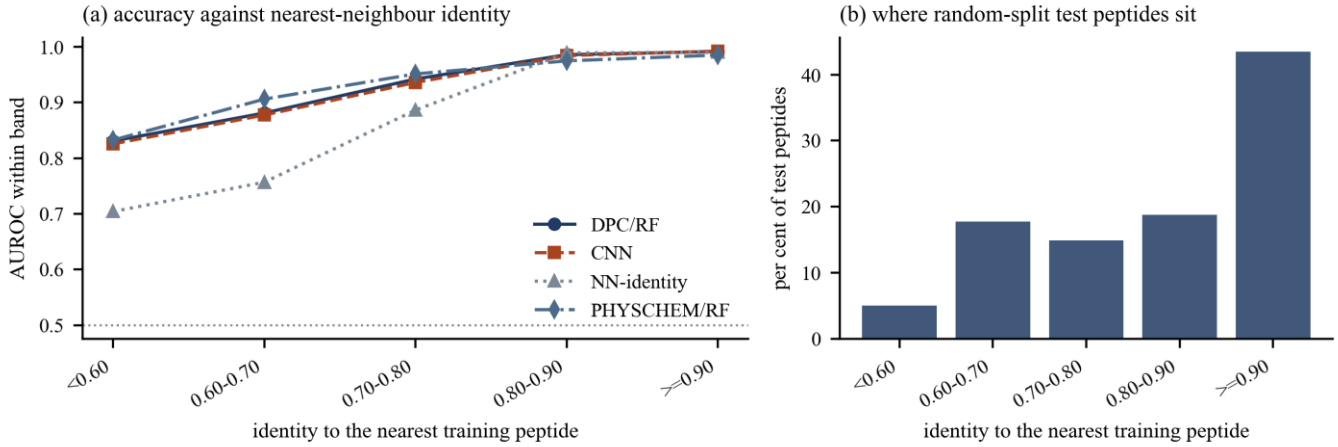

Fig. 3 Accuracy against the identity between each test peptide and its nearest training peptide, pooled over the five random splits.

E. *What separates the two classes*

The biophysical picture agrees with established descriptions of membrane-active peptides. That agreement is a useful check that the benchmark is not an artefact. Table 3 lists the descriptors that separate the classes most strongly. Net charge leads, followed by charge density, isoelectric point and the hydrophobic moment. Antimicrobial peptides here carry a mean net charge of 2.45 at pH 7.4. The negative class carries -0.25. No single descriptor is sufficient on its own, and the strongest reaches an area under the curve of only 0.783.

Table 3. The eight biophysical descriptors that separate the classes most strongly, ranked by rank-biserial effect size. All p values are Holm-corrected.

Descriptor	Antimicrobial	Non-antimicrobial	Effect size	Univariate AUROC	p (Holm)
net charge	2.45	-0.25	0.57	0.783	< 0.0001
charge density	0.09	-0.01	0.52	0.761	< 0.0001
isoelectric point	9.12	7.13	0.46	0.731	< 0.0001
frac hydrophobic	0.46	0.37	0.44	0.718	< 0.0001
aliphatic index	101.88	67.84	0.43	0.713	< 0.0001
hydrophobic moment	0.55	0.43	0.40	0.701	< 0.0001
turn fraction	0.25	0.33	-0.39	0.306	< 0.0001
gravy	0.18	-0.37	0.38	0.691	< 0.0001

IV. Discussion

The central finding is that the partition, not the model, decides what a benchmark of this kind reports. Nineteen trained pipelines all lose accuracy when close relatives are removed from training. They span composition counts, biophysical descriptors, tree ensembles and three neural architectures. The loss averages 0.039, and it is larger than any difference between the pipelines themselves. A control that fits no parameters reaches 0.958 under a random split. That is close to the trained models. Much of what a random split rewards is therefore retrieval rather than generalisation.

The size of the effect deserves a fair statement. The best pipeline still reaches 0.936 under the homology-aware partition, so recognition does not collapse. Cationic charge and amphipathicity are genuine physical correlates of membrane activity, and a model can learn them without memorising

families. Three observations nevertheless stand. Every pipeline loses accuracy, the leading pipeline changes identity, and rank agreement between the regimes is only 0.46. Within a single random split, accuracy ranges from 0.831 to 0.992 depending on how close the nearest training peptide is.

This complements the known negative-selection problem in the field rather than repeating it [12]. That work showed that benchmarks are easier than they look, because the negative class is drawn from a different population. Here both classes were drawn by an identical rule from the same annotation type. That route to inflation is closed by construction. The inflation measured here survives the control, and comes instead from relatedness within each class. The two effects are independent and can act together.

A second practical point concerns how such partitions are built. Holding out whole clusters from a greedy tool is common practice and feels sufficient. It is not. Greedy assignment compares sequences with cluster representatives alone, so relatives can be split across clusters. Here that left 87 per cent of held-out peptides with a relative above the nominal threshold. The transitive closure removes the gap, at the cost of chaining if the threshold is set too low. The two constraints together leave a narrow window of usable thresholds, and that window should be reported.

The dose-response curve gives practical guidance. Accuracy is high for peptides with a close relative in training. It falls steadily as that relative becomes more distant. A predictor is therefore trustworthy in proportion to the similarity between query and training set. That is a statement about applicability domain rather than about the model. Reporting the identity between test peptides and the training set would let readers judge which regime a published number belongs to. Such reporting costs nothing and is easy to automate.

Three limitations should be stated. First, the benchmark uses curated annotation, so it inherits the biases of what has been studied and deposited. Peptide families of medical interest are over-represented, and databases of measured activity would answer a complementary question [30]. Second, activity is treated as binary. The quantity of interest is a minimum inhibitory concentration against a named organism. Third, the neural models here are compact and trained from scratch on a few thousand sequences. Larger pretrained protein language models may transfer better to distant peptides. Testing that claim under a homology-aware partition is the natural next step.

V. Conclusion

This study asked how much reported accuracy in antimicrobial peptide classification depends on sequence homology between training and test data. A benchmark of 6288 mature peptides was assembled from curated annotation, by a rule identical for both classes. Every pair was aligned, and the clustering threshold was calibrated against composition-matched shuffled sequences. Twenty pipelines were then trained under a random partition, and under a partition that removes whole identity clusters.

Three results follow. The peptide set is strongly redundant, and a random split leaves 62 per cent of test peptides with a close relative in training. Every pipeline loses accuracy under the homology-aware partition, by 0.039 on average. That loss exceeds the spread between pipelines. A lookup control that fits nothing reaches 0.958 under the random partition, so similarity alone explains much of the apparent skill.

The practical recommendation is narrow and testable. Papers reporting sequence-based peptide classifiers should partition by identity cluster. They should state the threshold and how it was chosen. They should also report the identity between test sequences and the training set. Doing so lowers the headline number, and makes it mean what readers assume it means.

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