# SUPPLEMENTARY INFORMATION

**A large-scale structural classification of antimicrobial peptides**

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ADAM is at http://bioinformatics.cs.ntou.edu.tw/ADAM.

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**Note:

The following descriptions of the terms are direct quotos from our previous study [1].

**Aliphatic Index:**

The aliphatic index, the relative volume of aliphatic residues in a peptide, is calculated as follows:

$$\mathrm{AI} = \mathrm{X}_{\mathrm{Ala}} + a * \mathrm{X}_{\mathrm{Val}} + b * (\mathrm{X}_{\mathrm{Leu}} + \mathrm{X}_{\mathrm{Ile}})$$

where *a* and *b* are the constants, which represent the relative volume of valine and leucine or isoleucine to alanine. $\mathrm{X}_{\mathrm{Ala}}$, $\mathrm{X}_{\mathrm{Val}}$, $\mathrm{X}_{\mathrm{Leu}}$, and $\mathrm{X}_{\mathrm{Ile}}$ are the fractions of alanine, valine, leucine and isoleucine multiplied by 100, respectively [2].

**Instability Index:**

The instability index, an estimate of peptide stability, is calculated as follows:

$$\mathrm{II} = \frac{10}{L}\sum_{\mathrm{i=1}}^{\mathrm{i=L-1}} DIWV(\mathrm{X_i}, \mathrm{X_{i+1}})$$

where *L* is the length of peptide and *DIWV* from the study by Guruprasad *et al.* is an instability weight value of a dipeptide starting at position i [3]. Peptides with II values greater than 40 are considered to be unstable.

**Hydropathicity:**

Grand average of hydropathicity index (GRAVY) is used to represent the hydrophobicity value of a peptide, which calculates the sum of the hydropathy values of all the amino acids divided by the sequence length. GRAVY was calculated using the hydropathy values from Kyte and Doolittle [4]. Positive GRAVY values indicate hydrophobic; negative values mean hydrophilic.

**Table S1. ADAM's Cluster AC_001 with 26 AMP structures associated with 207 unique AMP sequences.**

| PDB | | | | CATH | | | SCOP | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ID | Chain | #Seq | Pfam | Class | Architecture | Topology | Class | Fold | Superfamily | Family |
| 1AYJ | A | 126 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Plant defensins |
| 1BK8 | A | 2 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Plant defensins |
| 1FJN | A | 4 | Defensin_2 | NA | NA | NA | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Defensin MGD-1 |
| 1GPT | A | 2 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Plant defensins |
| 1I2U | A | 11 | Toxin_3 | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Insect defensins |
| 1ICA | A | 11 | Defensin_2 | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Insect defensins |
| 1JKZ | A | 1 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Plant defensins |
| 1L4V | A | 12 | Defensin_2 | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Insect defensins |
| 1MM0 | A | 1 | Toxin_37 | NA | NA | NA | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Insect defensins |
| 1MR4 | A | 5 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Plant defensins |
| 1MYN | A | 3 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Insect defensins |
| 1N4N | A | 2 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Plant defensins |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1OZZ | A | 18 | Toxin_3 | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Insect defensins |
| 1P00 | A | 9 | NA | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Insect defensins |
| 1P0A | A | 13 | Toxin_3 | Alpha Beta | 2-Layer Sandwich | Defensin A-like | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Insect defensins |
| 1TI5 | A | 3 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | NA | NA | NA | NA |
| 1ZFU | A | 2 | Defensin_2 | NA | NA | NA | NA | NA | NA | NA |
| 2A9H | E | 5 | Toxin_2 | NA | NA | NA | Small proteins | Knottins (small inhibitors, toxins, lectins) | Scorpion toxin-like | Short-chain scorpion toxins |
| 2B68 | A | 1 | Defensin_2 | NA | NA | NA | NA | NA | NA | NA |
| 2E2F | A | 3 | Antimicrobial_6 | NA | NA | NA | NA | NA | NA | NA |
| 2GL1 | A | 2 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | NA | NA | NA | NA |
| 2KGQ | A | 3 | NA | Alpha Beta | 2-Layer Sandwich | Defensin A-like | NA | NA | NA | NA |
| 2KSK | A | 2 | Gamma-thionin | NA | NA | NA | NA | NA | NA | NA |
| 2LJ7 | A | 2 | Gamma-thionin | Alpha Beta | 2-Layer Sandwich | Defensin A-like | NA | NA | NA | NA |
| 2LR5 | A | 1 | Defensin_2 | NA | NA | NA | NA | NA | NA | NA |
| 2LT8 | A | 1 | Defensin_2 | NA | NA | NA | NA | NA | NA | NA |

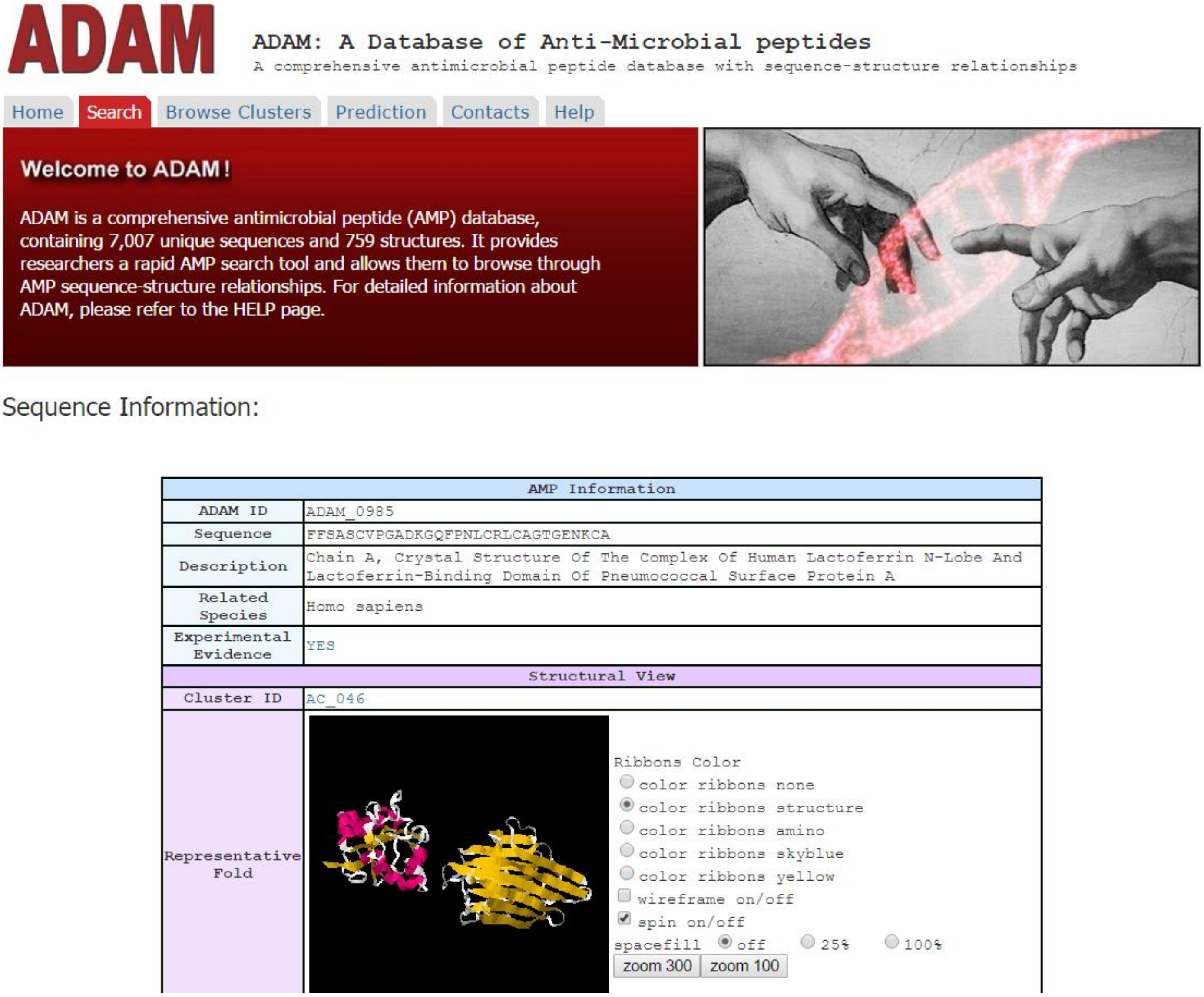
ADAM's Cluster AC_001 consists of 26 structures and 207 sequences. Among these 26 structures, six structures have neither CATH nor SCOP annotation. These with CATH or SCOP consistently fall into the same fold. Note: The homologous family field of CATH is not displayed, for all of them are empty here.

**Frequently Asked Questions:**

**Q:** How does ADAM provide AMP sequence-structure or structure-sequence relationships?

**A:** The goal of ADAM is to provide an easy access to link AMP sequences to structures and vice versa on a comprehensive AMP dataset. We assume that one AMP sequence can be mapped to at most one AMP structure and one AMP structural fold can consist of many structures.

To examine AMP sequence-structure relationship, ADAM offers users to search information directly based on AMP sequences. The other search options include ADAM ID, name or keyword, sequence length, taxonomy, links to other AMP databases, and Pfam family as well as PDB ID and ADAM cluster ID. A sample result page is as shown in Supplementary Fig. S1. Through this information page, users can find out what kind of structure the AMP sequence has and which structural fold it belongs to.


ADAM
ADAM: A Database of Anti-Microbial peptides
A comprehensive antimicrobial peptide database with sequence-structure relationships
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Welcome to ADAM!

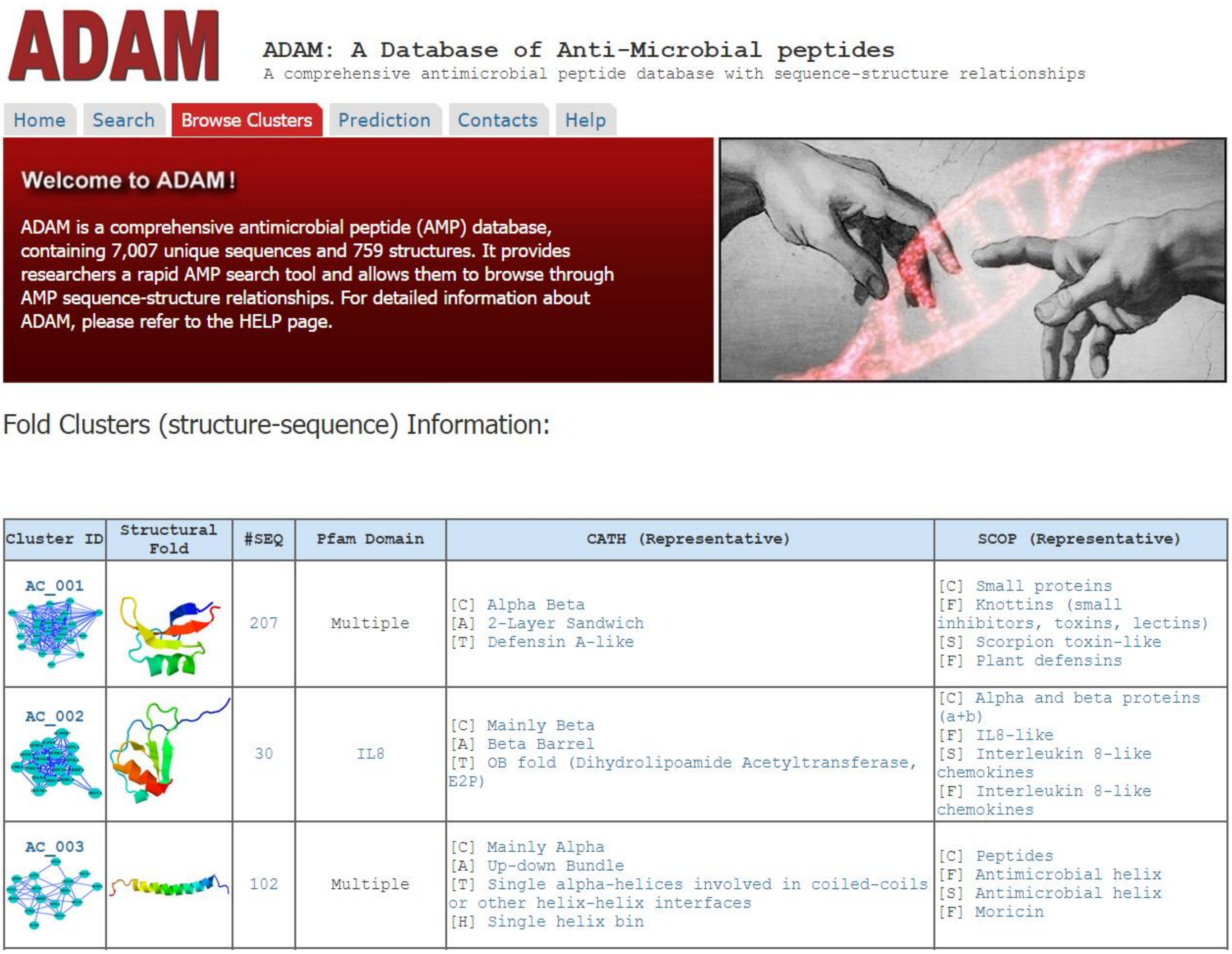
ADAM is a comprehensive antimicrobial peptide (AMP) database, containing 7,007 unique sequences and 759 structures. It provides researchers a rapid AMP search tool and allows them to browse through AMP sequence-structure relationships. For detailed information about ADAM, please refer to the HELP page.

Sequence Information:

| AMP Information | |
|---|---|
| ADAM ID | ADAM_0985 |
| Sequence | FFSASCVPGADKGQFPNLCRLCAGTGENKCA |
| Description | Chain A, Crystal Structure Of The Complex Of Human Lactoferrin N-Lobe And Lactoferrin-Binding Domain Of Pneumococcal Surface Protein A |
| Related Species | Homo sapiens |
| Experimental Evidence | YES |
| **Structural View** | |
| Cluster ID | AC_046 |
| Representative Fold | Ribbons Color<br>○ color ribbons none<br>● color ribbons structure<br>○ color ribbons amino<br>○ color ribbons skyblue<br>○ color ribbons yellow<br>□ wireframe on/off<br>☑ spin on/off<br>spacefill ● off ○ 25% ○ 100%<br>zoom 300 zoom 100 |

**Figure S1. Basic AMP information provided by ADAM.** Here is a partial page of the basic information. Links on this page allow users to explore the relationship from AMP sequence to structure.

ADAM provides three structural-fold approaches to examine AMP structure-sequence relationships: (1) AMP fold clusters by TM-score, (2) CATH, and (3) SCOP. The default method is AMP fold clusters because not every PDB structure is annotated by CATH or SCOP and the similarities of any two structures can be measured by TM-score. As described previously, a graph-based clustering approach using TM-score is utilized to build the AMP fold clusters [5]. In this graph, the vertices represent the AMP structures and an edge between two vertices exists if the two AMP structures are similar, which is determined by TM-score. Thus the structures within the same cluster share the same structural fold, indicated by TM-score. ADAM lists all the associated AMP sequences within the same fold directly from the browsing page under the detail button (Supplementary Fig. S2). Besides, ADAM allows users to browse the relationships through CATH or SCOP. The hyperlinks underneath the CATH or SCOP annotation in the browsing page would display the AMP structures under the particular classification at any of the four levels of CATH (class, architecture, topology, and homologous superfamily) or SCOP (class, fold, superfamily, and family). All the three approaches can view an AMP structure or an AMP structural fold with the associated AMP sequences.



ADAM: A Database of Anti-Microbial peptides

A comprehensive antimicrobial peptide database with sequence-structure relationships

Welcome to ADAM!

ADAM is a comprehensive antimicrobial peptide (AMP) database, containing 7,007 unique sequences and 759 structures. It provides researchers a rapid AMP search tool and allows them to browse through AMP sequence-structure relationships. For detailed information about ADAM, please refer to the HELP page.

Fold Clusters (structure-sequence) Information:

| Cluster ID | Structural Fold | #SEQ | Pfam Domain | CATH (Representative) | SCOP (Representative) |
|---|---|---|---|---|---|
| AC_001 | | 207 | Multiple | [C] Alpha Beta<br>[A] 2-Layer Sandwich<br>[T] Defensin A-like | [C] Small proteins<br>[F] Knottins (small inhibitors, toxins, lectins)<br>[S] Scorpion toxin-like<br>[F] Plant defensins |
| AC_002 | | 30 | IL8 | [C] Mainly Beta<br>[A] Beta Barrel<br>[T] OB fold (Dihydrolipoamide Acetyltransferase, E2P) | [C] Alpha and beta proteins (a+b)<br>[F] IL8-like<br>[S] Interleukin 8-like chemokines<br>[F] Interleukin 8-like chemokines |
| AC_003 | | 102 | Multiple | [C] Mainly Alpha<br>[A] Up-down Bundle<br>[T] Single alpha-helices involved in coiled-coils or other helix-helix interfaces<br>[H] Single helix bin | [C] Peptides<br>[F] Antimicrobial helix<br>[S] Antimicrobial helix<br>[F] Moricin |

**Figure S2. AMP structure-sequence relationships displayed in ADAM's browsing page.** Three approaches to view the relationships in ADAM are AMP fold clusters (Cluster ID), CATH, and SCOP. The hypertexts of Cluster ID, CATH, and SCOP would list the PDB structures, the number of the AMP sequences, Pfam domains, and fold annotation. Besides, the hypertext of the number of the sequences would list all the associated AMP sequences.

**Q:** Why did ADAM apply AMP fold clusters to annotate the AMP structures in addition to CATH and SCOP?

**A:** The main reason why ADAM applied AMP fold clusters by TM-score is that ADAM needs an effective way to classify all of the AMP structures. There are several options to conduct this analysis. CATH and SCOP are our first choice. However, not every PDB structure is annotated by CATH or SCOP. Over a third of these structures have neither CATH nor SCOP annotation. TM-score provides a relatively rapid and reliable method to measure the similarity of any two PDB structures.

**Q:** ADAM focuses on AMP structural folds, but can ADAM display AMP structure-sequence relationship through AMP secondary structure?

**A:** Yes, ADAM can. By default, ADAM displays the relationships through fold cluster, CATH, and SCOP. In fact, the first level of CATH (class) and SCOP (class) refers to the secondary structure. The hyperlinks are available to visualize the comprehensive AMP relationship by secondary structure.

**Supplementary information concerning AMP Prediction tools:**

Built on the AMP sequences of ADAM, two prediction tools are available on ADAM (http://bioinformatics.cs.ntou.edu.tw/adam/tool.html) to identify potential AMP sequences. Support vector machines (SVM) and hidden Markov models (HMM) are used separately to build these tools. The SVM predictor is based on AMP composition and the HMM predictor utilizes the Pfam domains.

### SVM

Our SVM model was trained on the AMP sequences of ADAM, using amino acid composition as the learning features (Supplementary Fig. S3). LIBSVM (http://www.csie.ntu.edu.tw/~cjlin/libsvm/) [6] was chosen to build our SVM prediction model. A radial basis function kernel was applied to the SVM model, whose optimal cost and gamma parameters for the kernel were determined by LIBSVM.

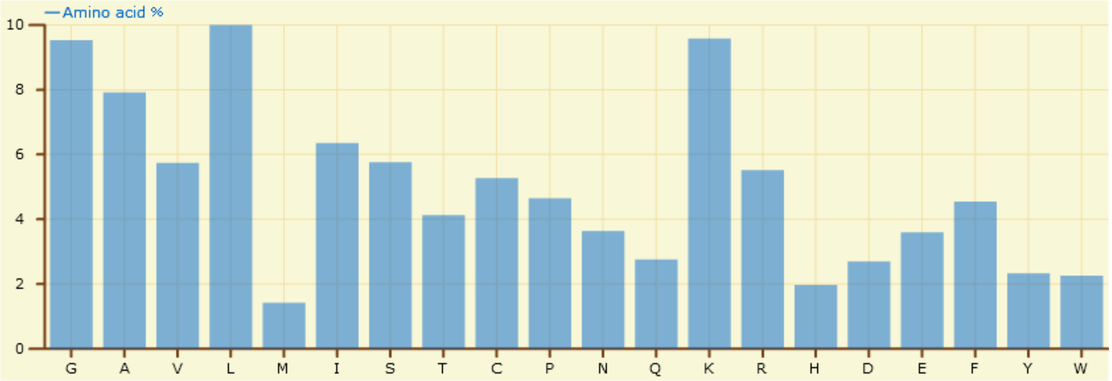


**Figure S3. Average amino acid composition of the AMPs in ADAM.**

### HMM

Our HMM predictor was built on two kinds of HMM profiles: (1). The Pfam domains found in the AMP sequences of ADAM. (2). Additional HMM profiles for the AMP sequences without any Pfam domains in ADAM. Here a conserved domain analysis was performed on the twelve AMP databases using the Pfam 27.0 database [7]. It is known that the Pfam domains cover nearly 80% of all the proteins, but the Pfam coverage among the twelve AMP databases was only around 40% ~ 70% (Supplementary Fig. S4). HIPdb has the least Pfam coverage, only around 17.2%. Relative low coverage indicates that AMPs are still waiting to be explored. In our analysis, 236 Pfam families are found in the ADAM sequences. For the ADAM sequences without any Pfam families, 30 repeating patterns were collected to form the additional HMM profiles. Our HMM predictor utilizes the two kinds of HMM profiles to identify potential AMPs though sequence homolog.

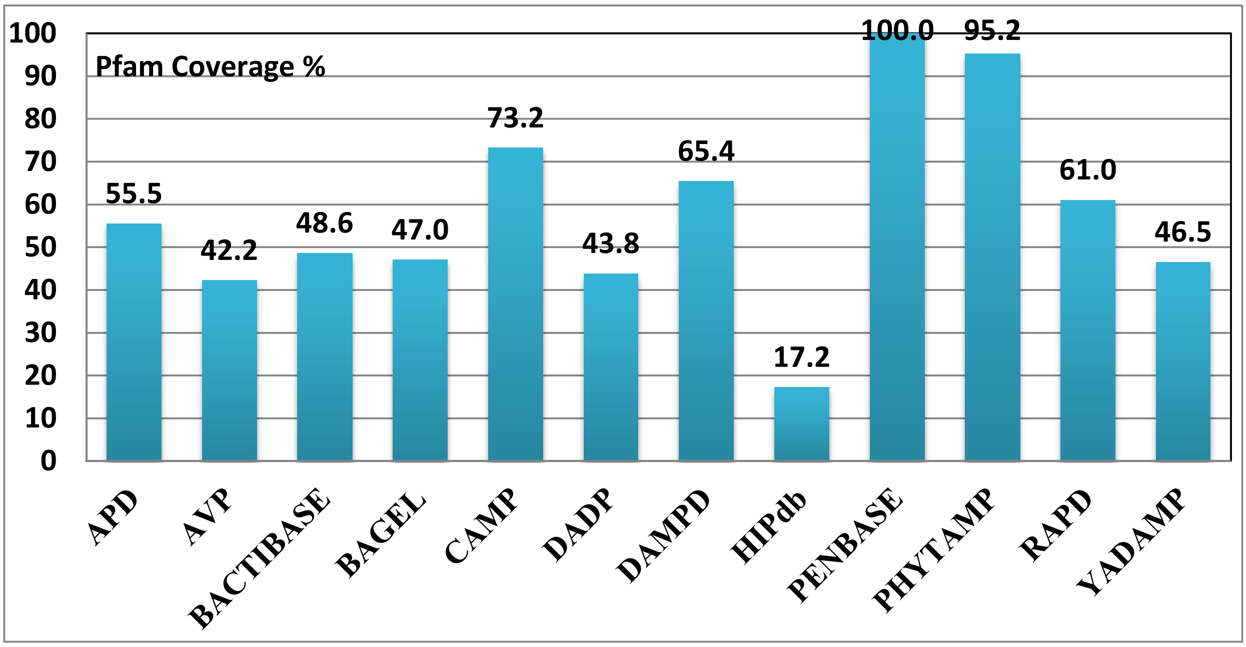


**Figure S4. Pfam coverage of the twelve AMP databases.**

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