4.6 Summary

We predicted AMPs based on models from different families across various taxa rather than using generalized AMP models. Thomas et. al. created a generalized AMP models to predict AMPs. One limitation of their models are not specific and not accurate. On the other hand, the specific models based on AMPs families is more robust and in addition to predicting AMPs with high accuracy, but also classifies them into specific subclasses such as cecropin, defensin, α-defensin etc.

A large-scale test on all of the currently sequenced and publicly available genomes would be useful to ascertain the robust of the methods used to create the webserver. Establishing the possibility that there are more AMPs through *in*-silico as compared to those discovered in laboratories, can provide an additional sense of direction for the wet lab scientist by testing a few predicted AMPs that have high confidence level for their activity.

The webserver will be useful to scan the ongoing genomes for potential AMPs in insects such as *Anopheles gambiae*, *Glossina morsitans*, *Phlebotomus logipalpis*, *Culex quinquefasciates* and *Anopheles funestus*. These insects are vectors that cause diseases such as trypanoiasis, leishmaniasis, yellow fever and malaria.

One limitation of this study is the lack of enough experimentally validated AMPS that hinders the creation of AMP family models. The other limitation is the small number of sequences used in the target and null databases. The *q*-value and PEP measures depends on the size of the database. The larger the number of sequences in the database that you search, the greater the number of false positives, hence more accurate statistical measure. In future the whole insect proteins from UniProt is intended to be used as the target database in order to generate the null sequences.

Chapter 5

Conclusion and future work

This chapter presents the usage of various computational methods to mine knowledge from the antimicrobial peptides (AMPs) dataset. The main objective of this thesis has been to create AMPs model in order to predict new AMPs. The main contributions of the thesis is broken into three subsections as well as their limitations. In the first subsection, the database of antimicrobial peptides is discussed. In the second subsection, the prediction of AMPs using support vector machines is presented. The section section is on the webserver. Finally, the direction for future work is presented.

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5.1 Research contribution and limitations

5.1.1 Antimicrobial peptide database

Databases are useful resource for mining and exploration of antimicrobial peptides, allowing users to query complex biological questions and analysis of data. In this thesis, a comprehensive database of antimicrobial peptides called DAMPD was created. DAMPD is a manually curated database populated with 1232 experimentally validated AMPs entries for both prokaryotic and eukaryotic sources. The procedure for creating the database involves data extraction using keywords and data curation.

The creation of DAMPD database was the first step towards a systematic analysis of AMPs. The DAMPD database was successfully developed and is freely accessible for academic and non-profit users at http://apps.sanbi.ac.za/dampd. The DAMPD database contains both search and analytical tools that

ease in search and analysis of biological query. In particular, classification of AMPs using profile hidden Markov model has been implemented. The profiles created can be used to classify new AMP families into known AMP families. HMM profiles were created for AMPs based on prior knowledge of the AMP families.

5.1.2 Classification of AMPs using support vector machines

Data modeling is usually a crucial step in data mining and yield ground for prediction purposes. The curated data in DAMPD was used to create AMPS models in various taxa. In chapter 3, an SVM-based machine learning approach coupled with optimization methods have been implemented to aid in classification of AMPs into their respective AMPs families. Global optimization methods such as grid search, pattern search and derivative-free simulated annealing were used to select the hyperparameters of SVM classifier. PS-SVM was the best hybrid method based on classification accuracy.

5.1.3 Creation of haemotophagous antimicrobial peptide predictor webserver

A webserver to predict haemotophagous insect AMPs into their respective families was created. The webserver is freely accessible at http://apps.sanbi.ac.za/Happ. This resource is useful to predict AMPs in ongoing genomes.

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Some of the future work include

- enriching the database with additional annotation such as information on promoter region and transcription factors for an AMP. The mode of action of AMPs will be added.
- using string kernels such as profile kernel, spectrum kernel and mismatch kernel instead of amino acid composition and physiochemical properties.
- incorporate feature selection in addition to parameter selection of SVM.
- predict AMPs once the genomes for haemotophagous insect are completed.
- use of modified pattern search method that uses perturbed coordinate directions rather than the spanning direction used in PS.

Appendix A

Supplementary material for Chapter 2

ClustalW results page

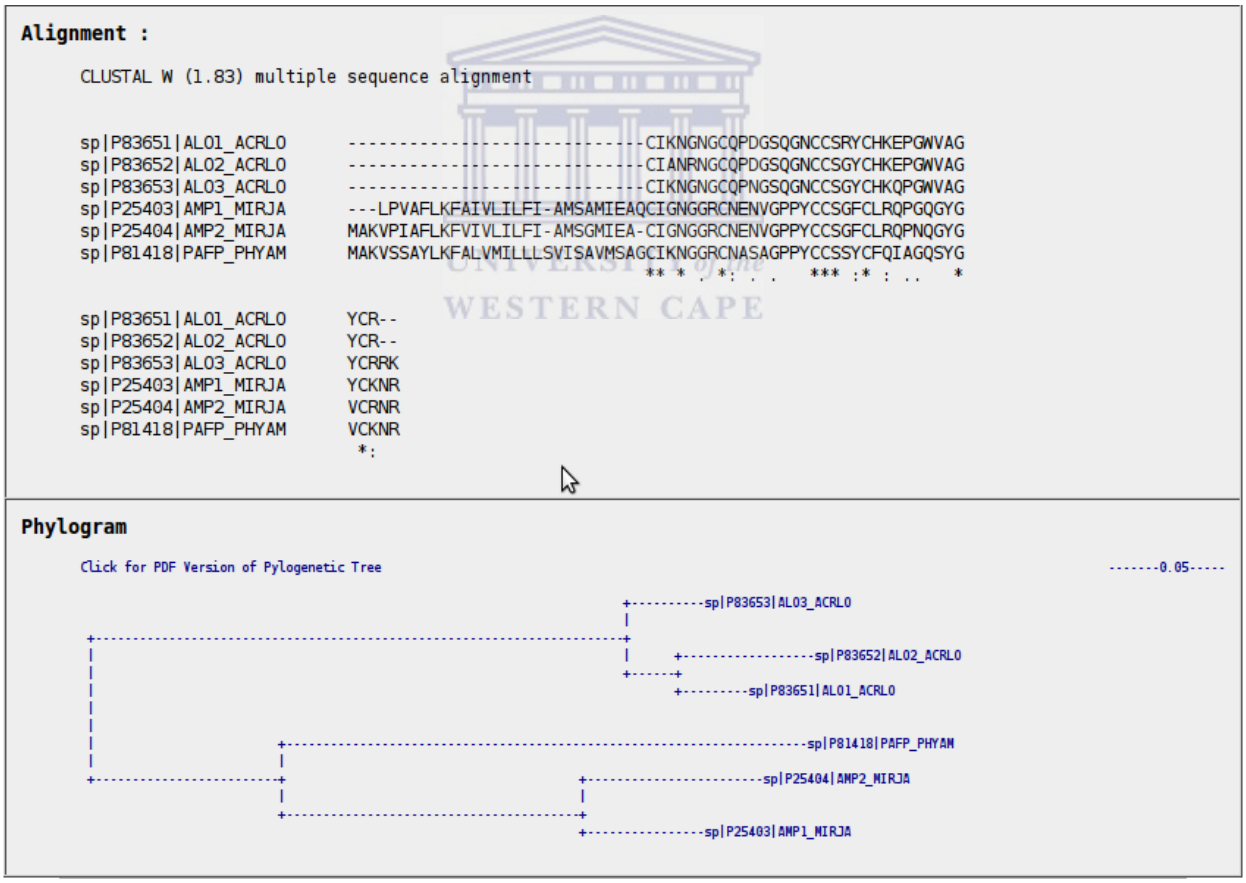

Figure A.1: ClustalW results of AMP family page

HMMER results page

Alignment

```
hmmsearch - search a sequence database with a profile HMM
HMMER 2.3.2 (Oct 2003)
Copyright (C) 1992-2003 HHMI/Washington University School of Medicine
Freely distributed under the GNU General Public License (GPL)
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
HMM file:                 /var/www/dampd/tmp/12225_030411.hmm [12225_030411]
Sequence database:        /var/www/dampd/tmp/12225_030411.query
per-sequence score cutoff:  [none]
per-domain score cutoff:    [none]
per-sequence Eval cutoff:   <= 10
per-domain Eval cutoff:     [none]
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Query HMM:   12225_030411
Accession:   [none]
Description: [none]
  [HMM has been calibrated; E-values are empirical estimates]

Scores for complete sequences (score includes all domains):
Sequence          Description                        Score    E-value  N
--------          -----------                        -----    ------- ---
sp|Q62715|DEF2_RAT Neutrophil antibiotic peptide NP-2  186.0    1.1e-56   1

Parsed for domains:
Sequence          Domain  seq-f seq-t    hmm-f hmm-t      score  E-value
--------          ------- ----- -----    ----- -----      -----  -------
sp|Q62715|DEF2_RAT   1/1       1    94 []     1    92 []   186.0  1.1e-56
```

Figure A.2: Classification results of a query sequence using α-defensin HMM profile.

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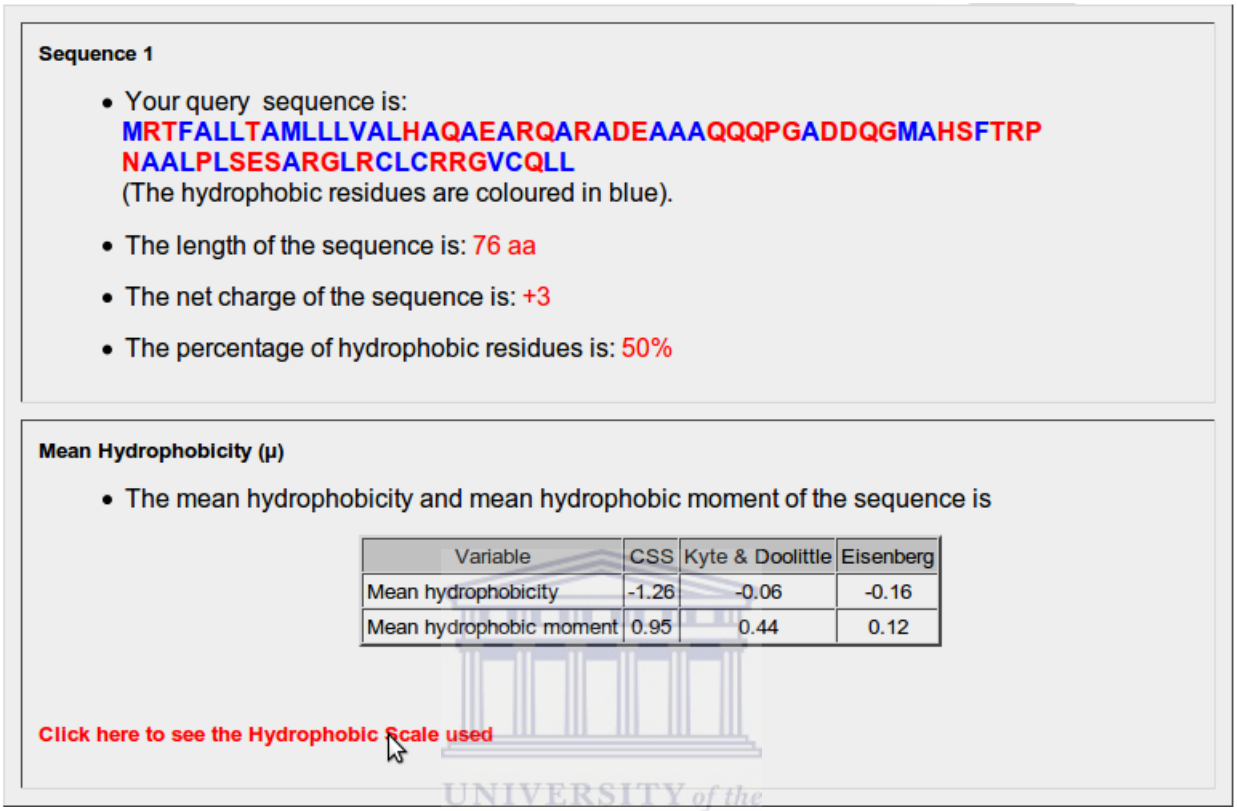
Hydrocalculator results page



Sequence 1

- Your query sequence is:
  MRTFALLTAMLLLVALHAQAEARQARADEAAAQQQPGADDQGMAHSFTRP
  NAALPLSESARGLRCLCRRGVCQLL
  (The hydrophobic residues are coloured in blue).
- The length of the sequence is: 76 aa
- The net charge of the sequence is: +3
- The percentage of hydrophobic residues is: 50%

Mean Hydrophobicity (μ)

- The mean hydrophobicity and mean hydrophobic moment of the sequence is

Variable	CSS	Kyte & Doolittle	Eisenberg
Mean hydrophobicity	-1.26	-0.06	-0.16
Mean hydrophobic moment	0.95	0.44	0.12

Click here to see the Hydrophobic Scale used

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Figure A.3: Hydrocalculator results of α-defensin sequence

SignalP results page

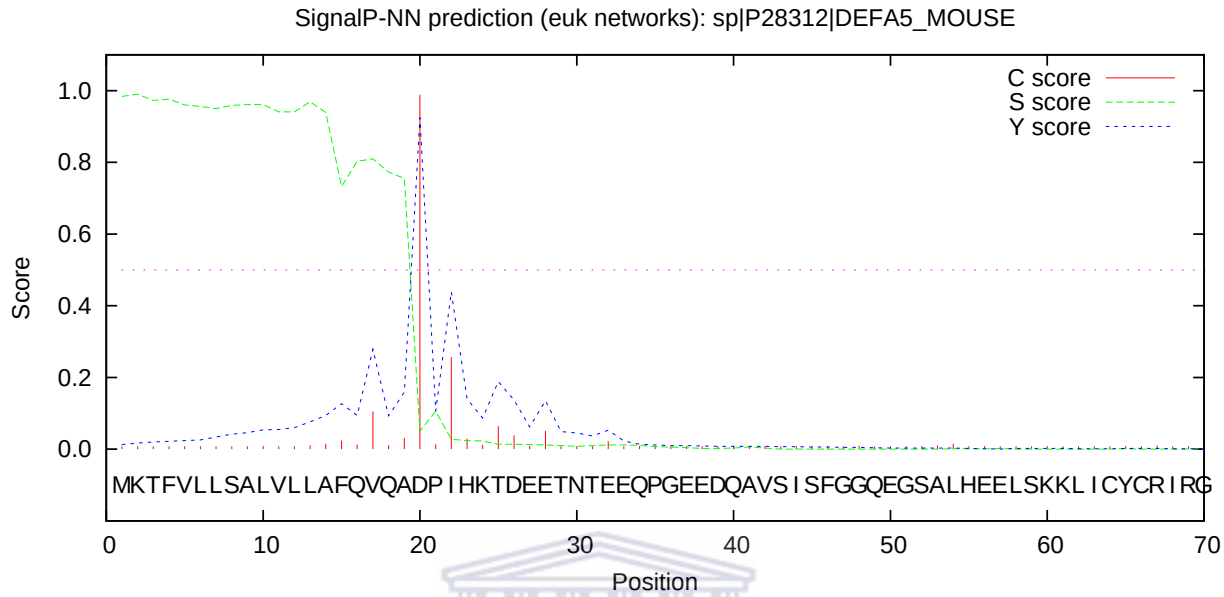

Figure A.4: SignalP results of α-defensin sequence

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Appendix B

Supplementary material for Chapter 3

Pattern search method

This example illustrates how the previous Algorithm 1 works in $\mathbb{R}^2$. In Figure B.1, x^k is the current iterate at the k^{th} iteration and is represented by the dotted circle $\odot$. The solid circle $\bullet$ indicates the position of the trial point $p^i \in P^k$ to be examined, where $i = 1, \cdots, r$. The small open circle $\circ$ and the circled asterisk $\circledast$ represent unsuccessful and successful trial points respectively of the POLL step. The POLL step begins by evaluating the function value of the trial point $p^i \in P^k$, point by point, where $i = 1, \cdots, 4$, as shown in Figure B.1. In Figure 2.2(a), the PS method computes the trial point p^1 by a step of size Δ^k. It computes the function value at p^1. If $f(p^1) > f(x^k)$ then it examines the next trial point p^2 as shown in Figure 2.2(b). If it is not successful at p^2, i.e., $f(p^2) > f(x^k)$ then it computes p_3 as shown in Figure 2.2(c). If p^3 is still unsuccessful then the process is repeated until all the trial points in P^k are examined, i.e., until p^4 is computed as shown in Figure 2.2(d). If all the points in the POLL set P^k (i.e., p^1, p^2, p^3 and p^4) are not successful then the step size is reduced by half as shown in Figure 2.2(e), i.e., the next POLL step begins at $x^{k+1} = x^k$ with $\Delta^{k+1} = \frac{1}{2}\Delta^k$. On the other hand, suppose that the trial point p^2 is successful, i.e., $f(p^2) < f(x^k)$ as shown in Figure 2.2(f), then the whole POLL step process starts anew at $x^{k+1} = p^2$ with enlarged step size, i.e., $\Delta^{k+1} = 2\Delta^k$ as shown in Figure 2.2(h). A similar cycle as shown in (a), (b), (c) and (d) of Figure 2.2 will be repeated (if necessary) for the new POLL at x^{k+1}.

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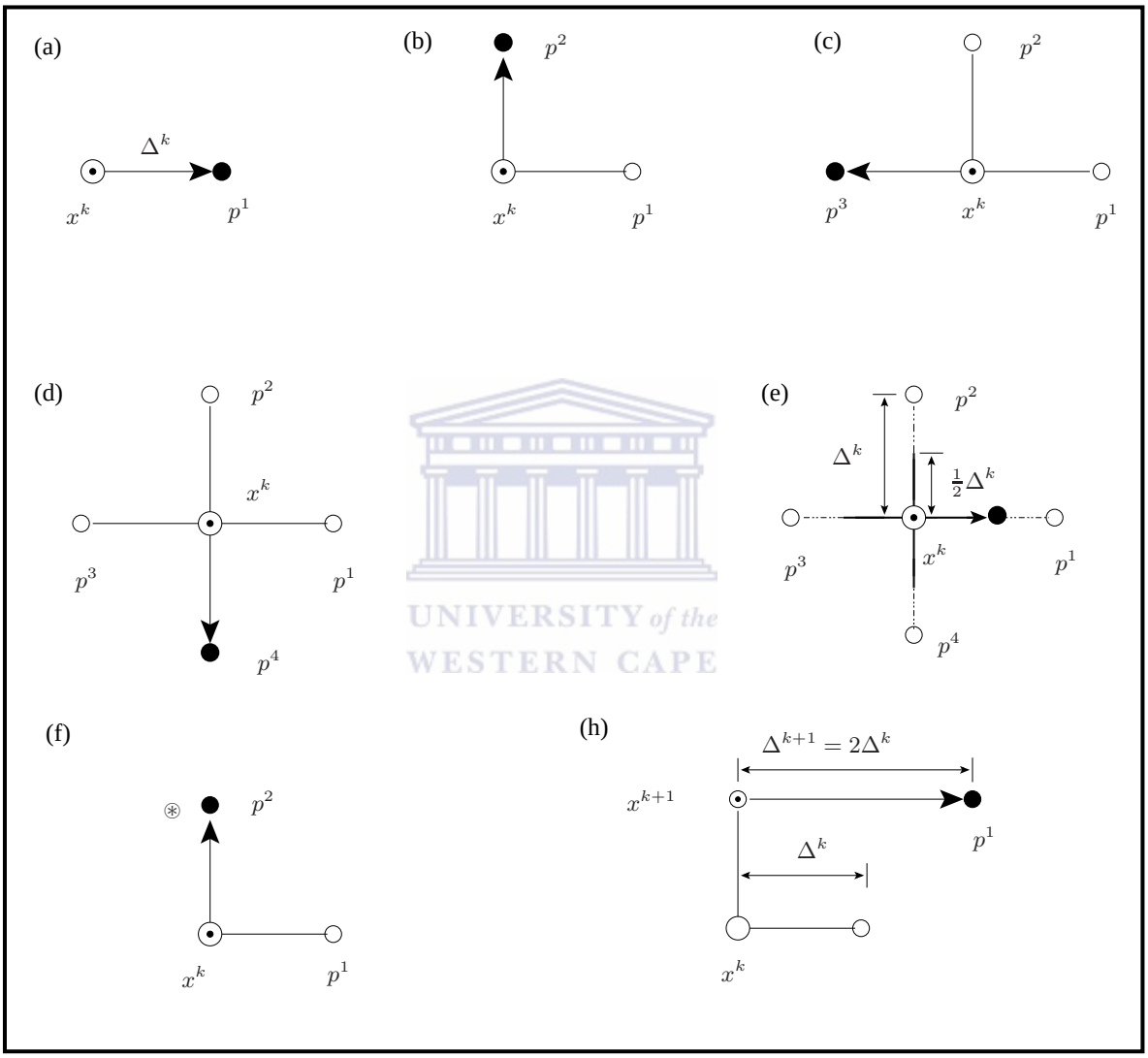

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Figure B.1: Figures (a)-(h) shows how the POLL steps works in the PS method.

Grid search method

A grid search tries values of each parameter across the specified search range using geometric steps. Grid searches are computationally expensive because the model must be evaluated at many points within the grid for each parameter. For example, if a grid search is used with 20 search intervals and the svm three parameters (c, σ) then the model must be evaluated at 20×20=400 grid points. If cross-validation is used for each model evaluation, the number of actual SVM calculations would be further multiplied by the number of cross-validation folds. For large models, this approach may be computationally infeasible.

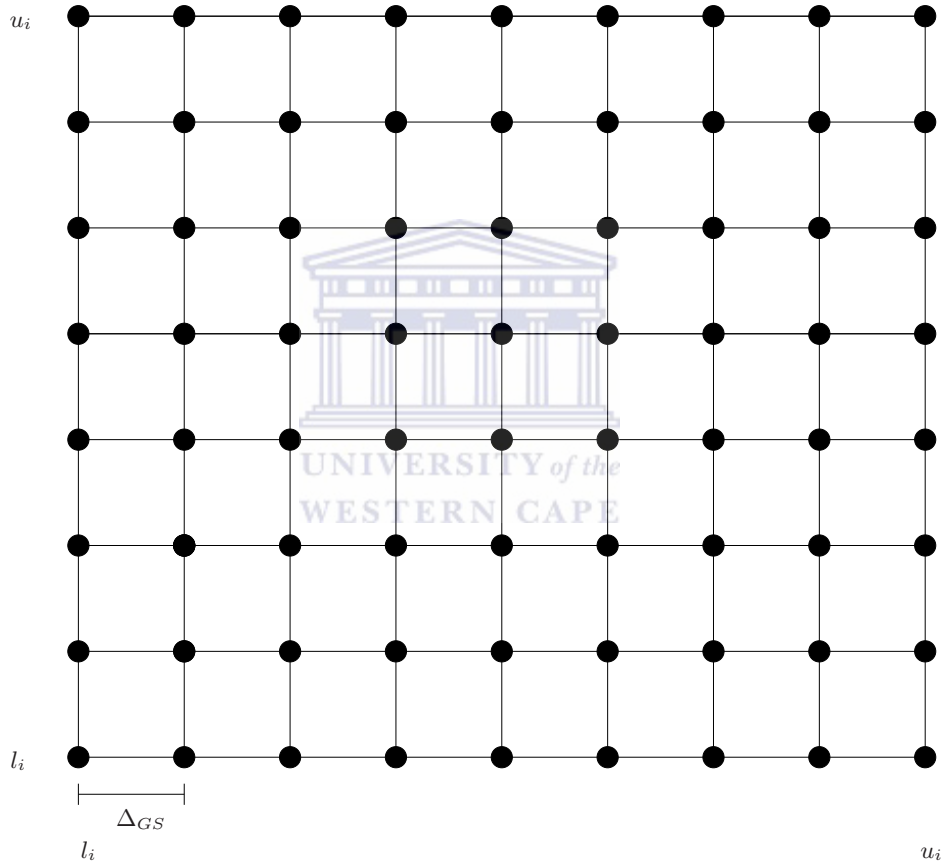

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Figure B.2: Figure shows how the Grid Search works in a two dimensional optimization problem

Negative sets

The negative set was downloaded from UniProt using the keywords below:

- actinopterygii (((golgi or cytoplasm or endoplasmic reticulum or mitochondria) NOT antimicrobial) AND length:[0 TO 100]) AND taxonomy:"Actinopterygii [7898]"
- amphibian (((golgi or cytoplasm or endoplasmic reticulum or mitochondria) NOT antimicrobial) AND length:[0 TO 100]) AND taxonomy:"Amphibia [8292]"
- arachnida (((golgi or cytoplasm or endoplasmic reticulum or mitochondria) NOT antimicrobial) AND length:[0 TO 100]) AND taxonomy:"Arachnida [6854]"
- bacteria (((golgi or cytoplasm or endoplasmic reticulum or mitochondria) NOT antimicrobial) AND length:[0 TO 100]) AND taxonomy:"Eubacterium [1730]"
- crustacea (((golgi or cytoplasm or endoplasmic reticulum or mitochondria) NOT antimicrobial) AND length:[0 TO 100]) AND taxonomy:"Crustacea [6657]"
- insecta (((golgi or cytoplasm or endoplasmic reticulum or mitochondria) NOT antimicrobial) AND length:[0 TO 100]) AND taxonomy:"Insecta [50557]"
- mammalia (((golgi or cytoplasm or endoplasmic reticulum or mitochondria) NOT antimicrobial) AND length:[0 TO 70]) AND taxonomy:"Mammalia [40674]"
- plant (((golgi or cytoplasm or endoplasmic reticulum or mitochondria) NOT antimicrobial) AND length:[0 TO 100]) AND taxonomy:"Viridiplantae [33090]"

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Features indices

- **EISD840101 Consensus normalized hydrophobicity scale Kawashima et al. (2008)**

  A: 0.25, R: -1.76, N: -0.64, D: -0.72, C: 0.04, Q: -0.69, E: -0.62, G: 0.16, H: -0.40, I: 0.73, L: 0.53, K: -1.10, M: 0.26, F: 0.61, P: -0.07, S: -0.26, T: -0.18, W: 0.37, Y: 0.02, V: 0.54

- **HOPT810101 Hydrophilicity value Kawashima et al. (2008)**

  A: -0.5, R: 3.0, N: 0.2, D: 3.0, C: -1.0, Q: 0.2, E: 3.0, G: 0.0, H: -0.5, I: -1.8, L: -1.8, K: 3.0, M: -1.3, F: -2.5, P: 0.0, S: 0.3, T: -0.4, W: -3.4, Y: -2.3, V: -1.5

- **VELV850101 Electron-ion interaction potential Kawashima et al. (2008)**

  A: .03731, R: .09593, N: .00359, D: .12630, C: .08292, Q: .07606, E: .00580, G: .00499, H: .02415, I: .00000, L: .00000, K: .03710, M: .08226, F: .09460, P: .01979, S: .08292, T: .09408, W: .05481, Y: .05159, V: .00569

- **ZIMJ680101 Hydrophobicity Kawashima et al. (2008)**

  A: 0.83, R: 0.83, N: 0.09, D: 0.64, C: 1.48, Q: 0.00, E: 0.65, G: 0.10, H: 1.10, I: 3.07, L: 2.52, K: 1.60, M: 1.40, F: 2.75, P: 2.70, S: 0.14, T: 0.54, W: 0.31, Y: 2.97, V: 1.79

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- **ZIMJ680102 Bulkiness Kawashima et al. (2008)**

  A: 11.50, R: 14.28, N: 12.82, D: 11.68, C: 13.46, Q: 14.45, E: 13.57, G: 3.40, H: 13.69, I: 21.40, L: 21.40, K: 15.71, M: 16.25, F: 19.80, P: 17.43, S: 9.47, T: 15.77, W: 21.67, Y: 18.03, V: 21.57

- **ZIMJ680103 Polarity Kawashima et al. (2008)**

  A: 0.00, R: 52.00, N: 3.38, D: 49.70, C: 1.48, Q: 3.53, E: 49.90, G: 0.00, H: 51.60, I: 0.13, L: 0.13, K: 49.50, M: 1.43, F: 0.35, P: 1.58, S: 1.67, T: 1.66, W: 2.10, Y: 1.61, V: 0.13

- **JURD980101 Modified Kyte-Doolittle hydrophobicity scale Kawashima et al. (2008)**

  A: 1.10, R: -5.10, N: -3.50, D: -3.60, C: 2.50, Q: -3.68, E: -3.20, G: -0.64, H: -3.20, I: 4.50, L: 3.80, K: -4.11, M: 1.90, F: 2.80, P: -1.90, S: -0.50, T: -0.70, W: -0.46, Y: -1.3, V: 4.2

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