Project #8
Caspases, apoptosis and the degradome

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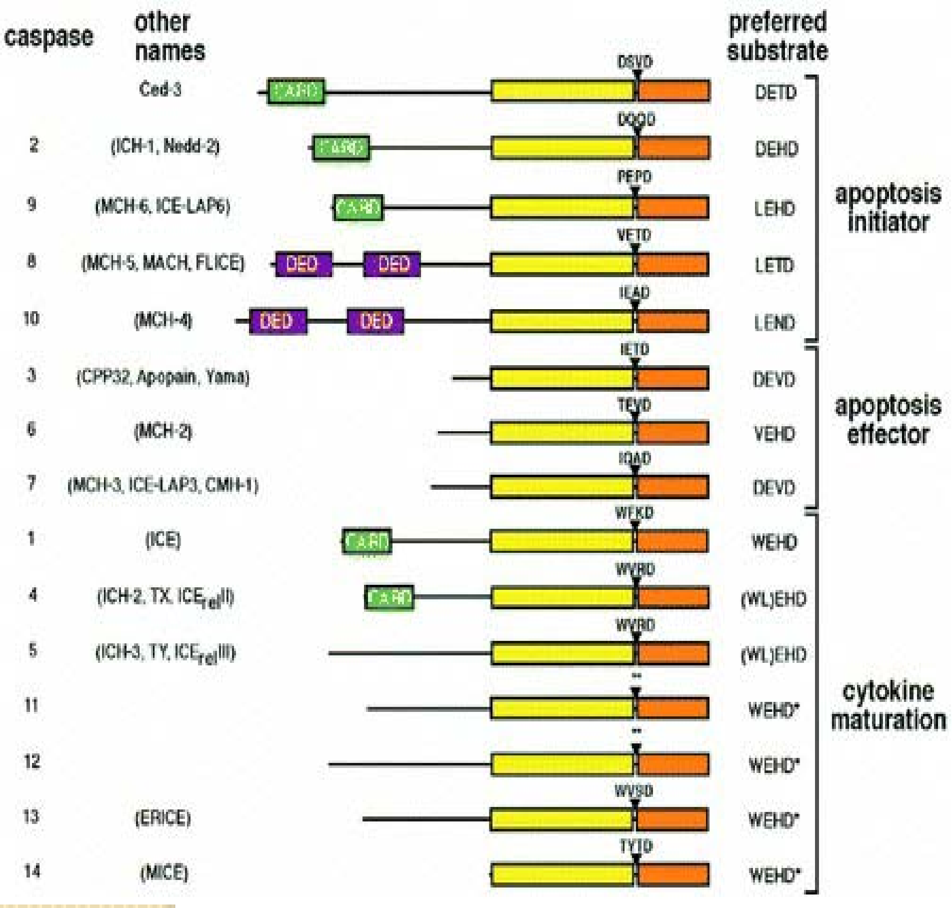
Introduction

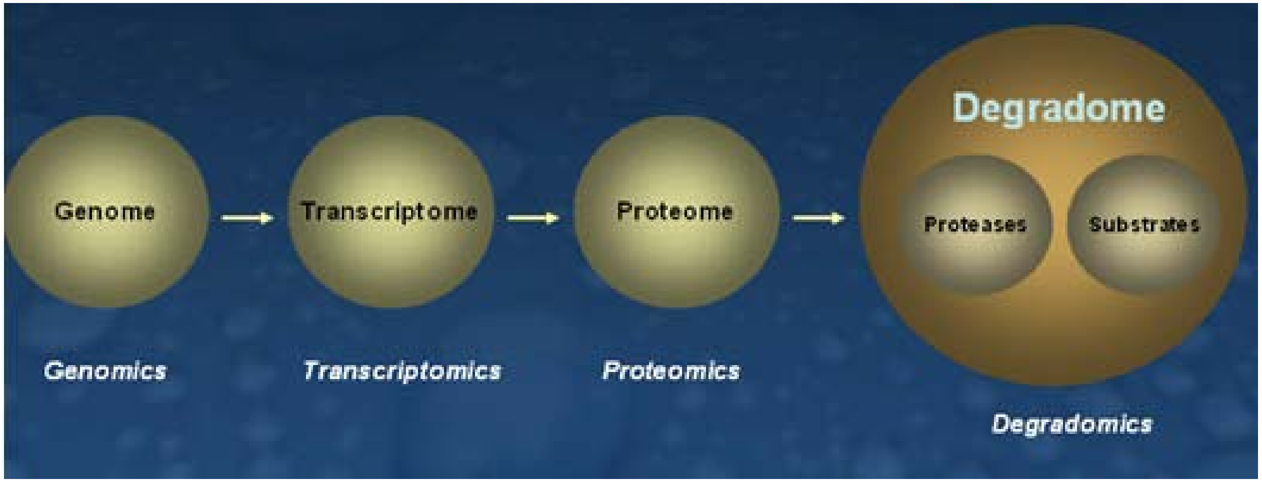
Caspase (Cysteinyl aspartate protease)



Andreas Strasser, et al, Annu. Rev. Biochem. 2000.

- Essential in cells for apoptosis, inflammation and other important cellular processes
- Can cleave substrates at specific tetrapeptide sites

Degradome



- Represent the complete set of proteases that are expressed at a specific time by a cell, tissue or organism
- The natural substrate repertoire of an enzyme in a cell, tissue or organism is termed as the protease degradome.

The importance of degradome

- Elucidating the roles of caspases & proteases in various cellular pathways;
- Characterization of individual protease degradomes can further clarify the roles and significance of each protease and their downstream proteolytic events at the systems level;
- The knowledge of protease degradomes will be useful for therapeutic research and drug discovery

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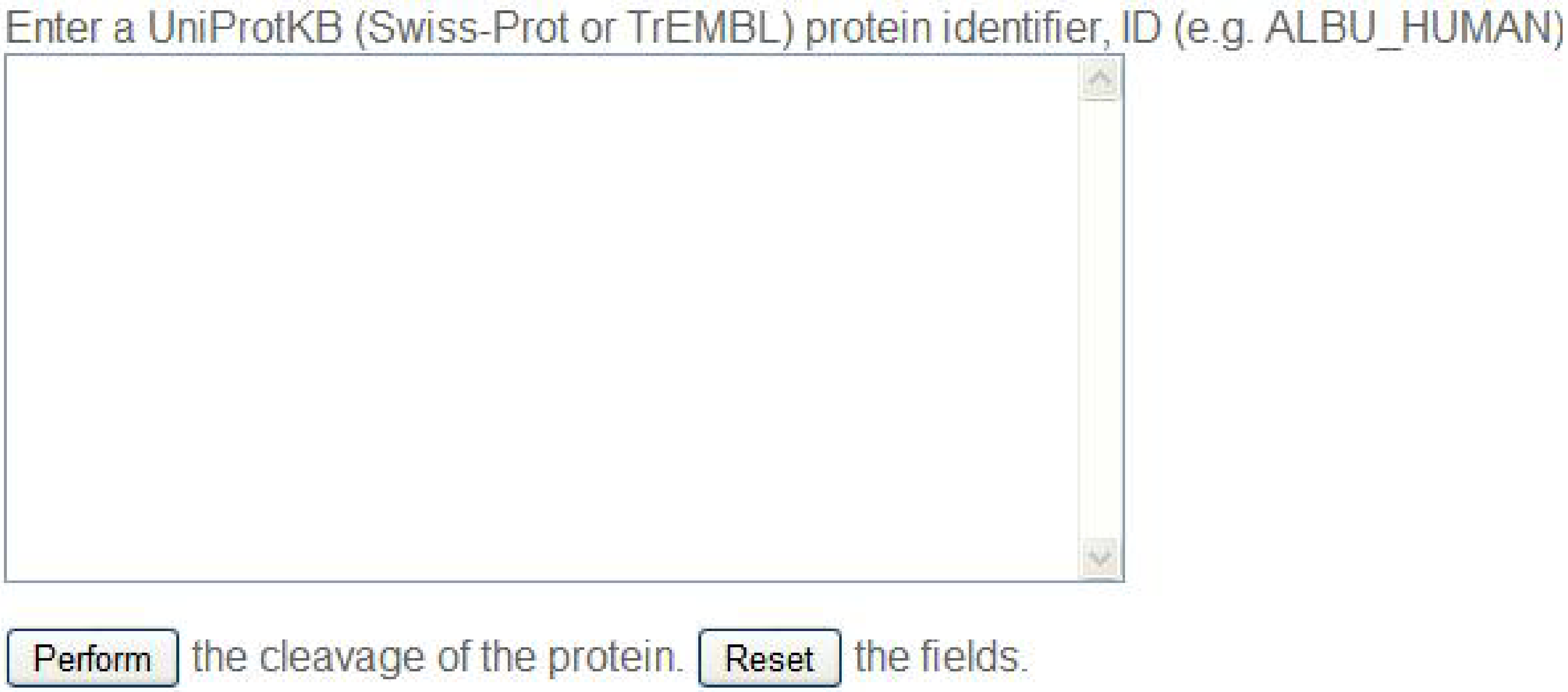
Computational tool

CASPASE SUBSTRATE CLEAVAGE PREDICTION METHODS

Peptide Cutter

- **PeptideCutter** predicts potential cleavage sites cleaved by proteases or chemicals in a given protein sequence.
- http://us.expasy.org/tools/peptidecutter



Enter a UniProtKB (Swiss-Prot or TrEMBL) protein identifier, ID (e.g. ALBU_HUMAN)

Perform the cleavage of the protein. Reset the fields.

CasPredictor

- Utilises the Caspase Cleavage Site searcher (CCSearcher) to point out potential substrate sites adjacent with PEST sequences. The algorithm is based on three parameters:
  - Substitution Index
  - Frequency Index
  - PEST index
- PEST sequences: P= proline, E= glutamate ,S=serine ,T= threonine.

- Predicted successfully more than 80% of the cleavage sites in experimentally verified caspase substrates.

- Not able to identify at least 20% of reported caspase substrates; some input data from recombinant proteins may not suitable in vivo.

Neural Networks

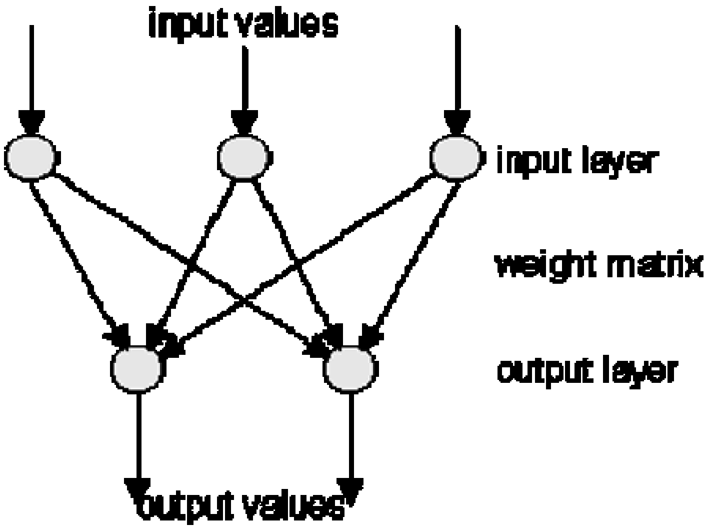
- Also known as Perceptron Network

- Sophisticated complex function incorporated in bioinformatic tools to solve complex problem and predict outcomes.

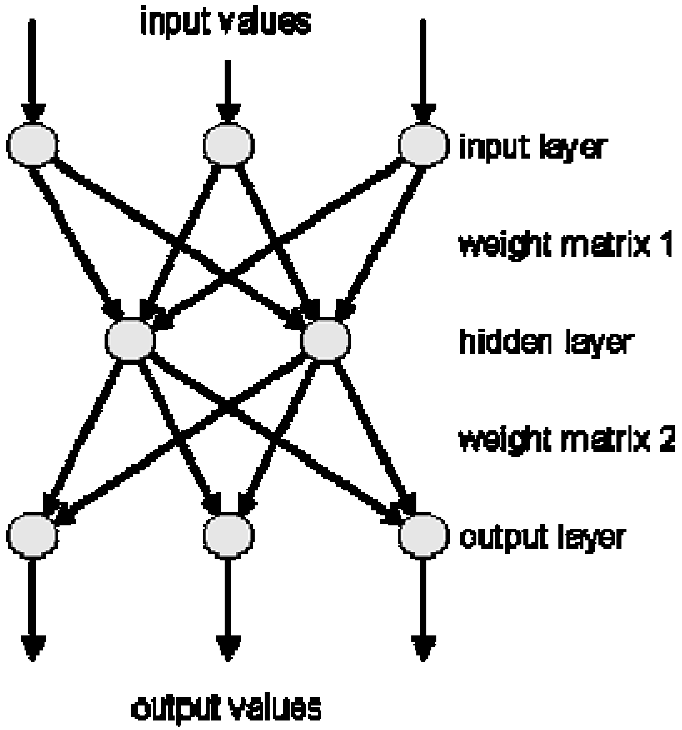
- Modelled after the physiological functioning of the brain

- Bioinformatic tools using Neural Network Learning includes:
  - Proteasomal Cleavage prediction site: NetChop 3.0
  - Signal Peptide Cleavage prediction site: SignalP

Neural Network



Single-layered Perceptron



Multi-layered Perceptron

Support Vector Machines (SVM)

- Supervised learning algorithm used in bioinformatic tools to predict outcomes with high confidence.
- Construct classification groups using margins

Input Training Examples

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Analysis and Pattern Recognition

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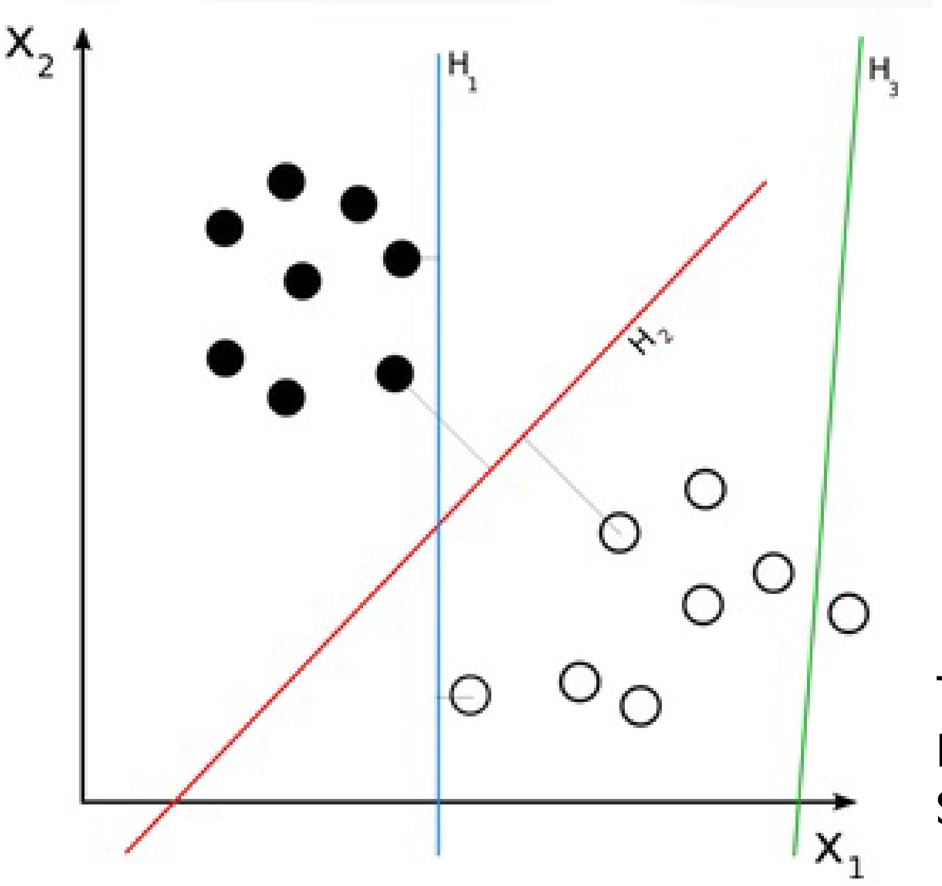
Form Classifications Ready for predictions

Principles of SVM

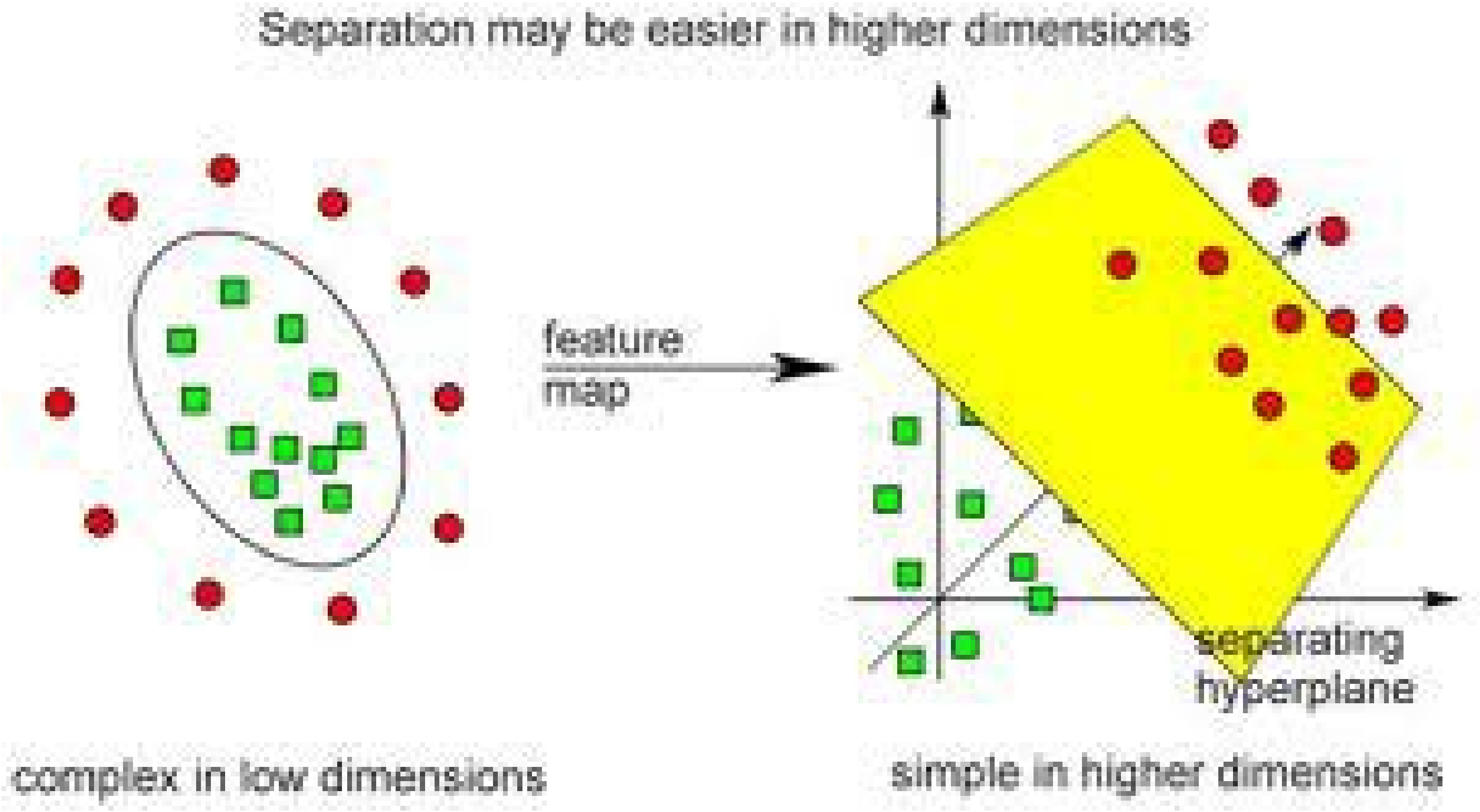
- Optimal Margin Classifier
  - Maximise distance between classifications



Two
Dimensional
Scenario

Principles of SVM

- Optimal Margin Classifier



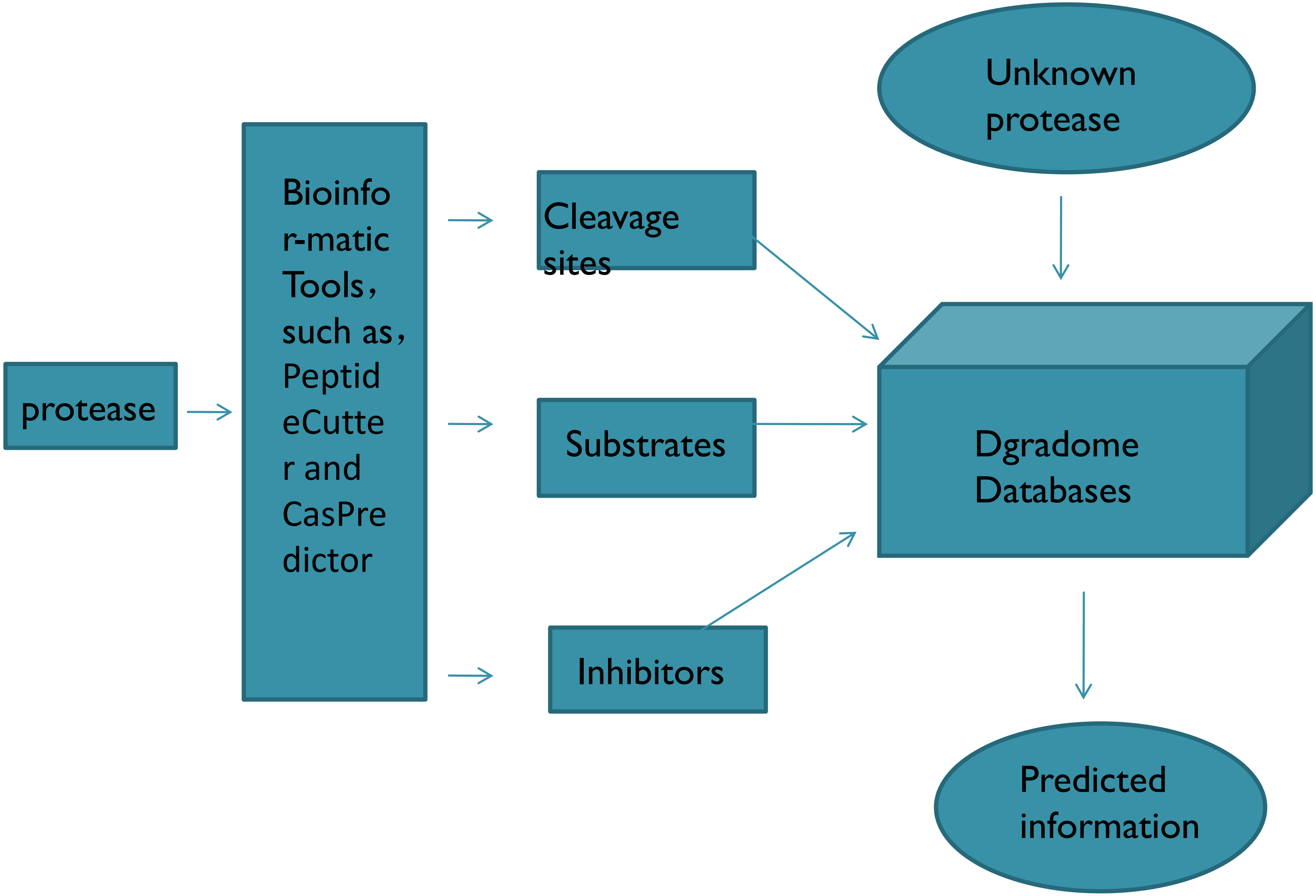
Degradome

How can bioinformatics tools help in degradome research?

- Manage database
- Predicts substrates of known protease
- Identifies unknown cleavage sites of known substrates
- Facilitates discovery of unidentified proteins

How can bioinformatics tools help in degradome research?

- Simple software
    - PeptideCutter
    - PEPS
- Databases & algorithms
    - CasPredictor
- Complex /Expert systems
    - Neural Networks
    - Support Vector Machines (SVM) method



protease

Bioinfor-matic Tools，such as，PeptideCutter and CasPredictor

Cleavage sites

Substrates

Inhibitors

Unknown protease

Dgradome Databases

Predicted information

Conclusion

SUMMARY

- The prediction methods are useful tools for research and Predict novel targets which are not yet studied

- Cost effective and relatively rapid process for predicting potential substrates.

- High throughput allows generation and screening of large volumes of data

LIMITATIONS

- Reliability of bioinformatic tools depends on the present findings and also the algorithm written by programmer.

- Predicted substrates using bioinformatic tools may not be physiologically relevant and still requires experimental validations.

- Users of bioinformatic tools needs to have a certain degree of biological expertise and IT knowledge.

CONCLUSION

- Bioinformatics is a necessary tool in research and has great potential if used correctly
- Results produced from bioinformatic tools should be taken with caution
- Very useful for speeding up discovery and knowledge mining
- May help provide a clearer and more complete picture once the research field is mature enough