

22BIO211: Intelligence of Biological Systems - 2

NON RIBOSOMAL PROTEINS

Dr. Manjusha Nair M
Amrita School of Computing, Amritapuri
Email : manjushanair@am.amrita.edu
Contact No: 9447745519

The problem to address

- No 30-mer in the *Bacillus brevis* genome encoding Tyrocidine B1
- How could a bacterium produce a peptide that is not encoded by the bacterium's genome?
- Where is Tyrocidine encoded in the *Bacillus brevis* genome?

Non Ribosomal Proteins (NRP)

- Central Dogma of Molecular Biology implies that all peptides must be encoded by the genome.
- Nobel laureate Edward Tatum in 1963, devised an ingenious experiment- inhibited the ribosomes
 - *All proteins production stopped – except for tyrocidines and gramicidins!*
 - *Tatum to hypothesize that some yet unknown non-ribosomal mechanism must assemble these peptides.*
- In 1969, Fritz Lipmann (another Nobel laureate) demonstrated that tyrocidines and gramicidins are non-ribosomal peptides (NRPs), synthesized not by the ribosome, but by a giant protein called **NRP synthetase**.

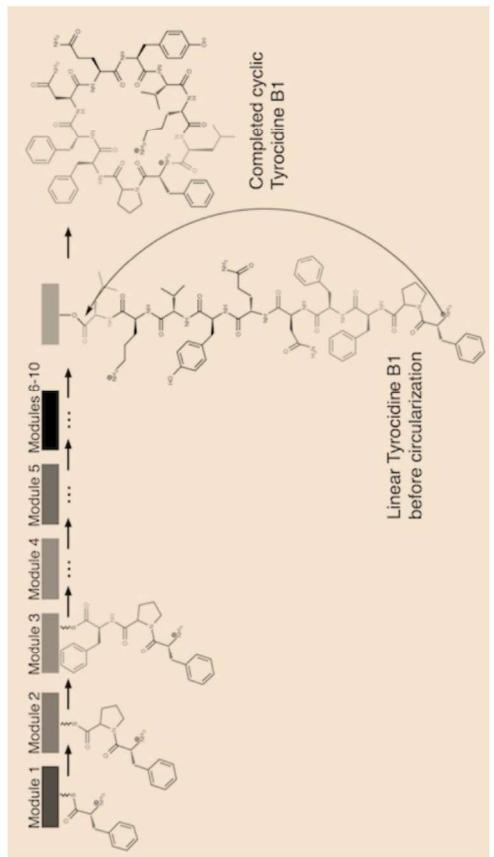
Non-Ribosomal Proteins (NRP)

- NRP synthetase enzyme

- *This enzyme pieces together antibiotic peptides without any reliance on RNA or the genetic code!*
- *Every NRP synthetase assembles peptides by growing them one amino acid at a time*

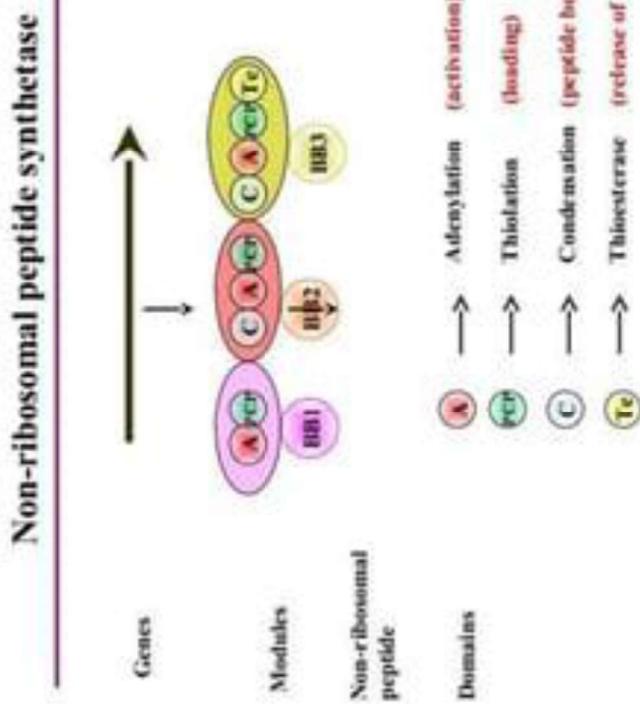
Each of ten different modules (shown by different colors in the figure) adds a single amino acid to the peptide, which in the figure is one of many tyrocidines produced by *Bacillus brevis*.

- *In a final step, the peptide is circularized.*



Non-Ribosomal Proteins (NRP)

- Most NRPs have cyclic or branched structures
- Bacteria and fungi are the most common NRPs producers.
- Each NRP Synthetase can synthesize only one type of peptide.
- NRPs contain 100s of different amino acids (not only 20).

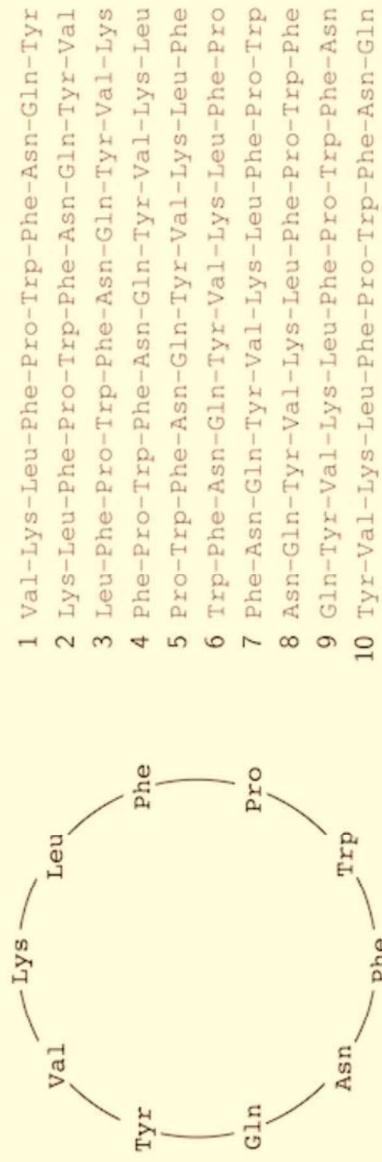


Applications of NRPs

- Pharmaceutical applications
 - *They have been optimized by eons of evolution as "molecular bullets" that bacteria and fungi use to kill their enemies.*
 - *antibacterial drugs*
 - *Antiviral or antifungal drugs*
 - *Anti-tumor agents and immunosuppressors*
- Others are used by bacteria to communicate with other cells.

Cyclic Peptides

- Tyrocidines and Gramicidins are cyclic peptides.



- Tyrocidine B1 has ten different linear representations
- We should run the Peptide Encoding Problem on every one of these sequences to find potential 30-mers coding for Tyrocidine B1.
- Yet, we find no 30-mer in the *Bacillus brevis* genome encoding Tyrocidine B1!

Sequencing Antibiotics

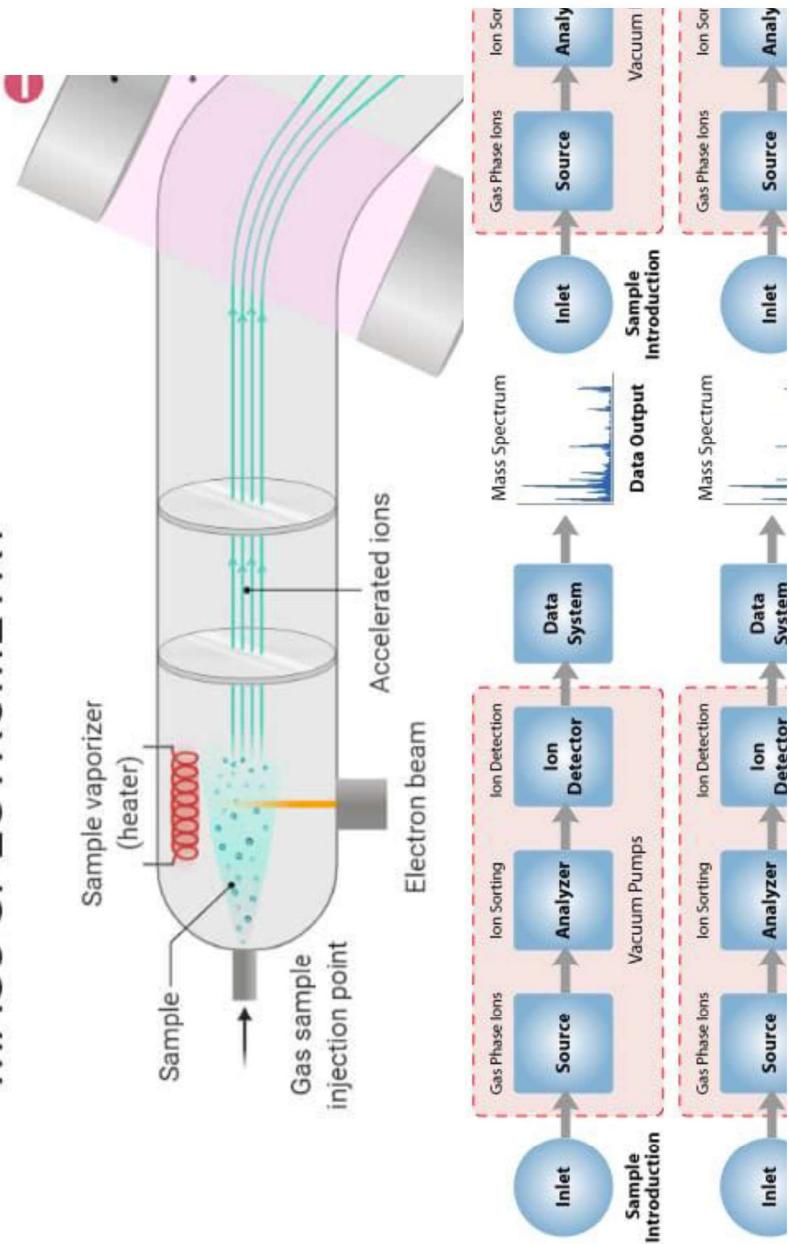
- Sequencing peptides of antibiotics is difficult because
 - NRPs do not adhere to the Central Dogma
 - many NRPs (including tyrocidines and gramicidins) are cyclic.
- Thus, the standard tools for sequencing linear peptides, are not applicable to NRP analysis.
 - One Solution
 - Mass spectrometry
 - shatters molecules into pieces and then weighs the resulting fragments.

Mass Spectrometry

- The mass spectrometer measures the mass of a molecule in daltons (Da);
 - 1 Da is approximately equal to the mass of a single nuclear particle (i.e., a proton or neutron).
 - 1 Da is not exactly equal to the mass of a proton/neutron, and we may need to account for different naturally occurring isotopes of each atom when weighing a molecule
- Integer Mass
 - amino acid glycine - Chemical formula C_2H_3ON
 - $2 \cdot 12 + 3 \cdot 1 + 1 \cdot 16 + 1 \cdot 14 = 57.$
 - amino acids typically have non-integer masses (e.g., glycine has total integer mass equal to approximately 57.02 Da)

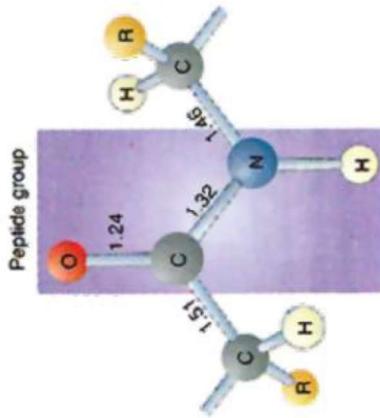
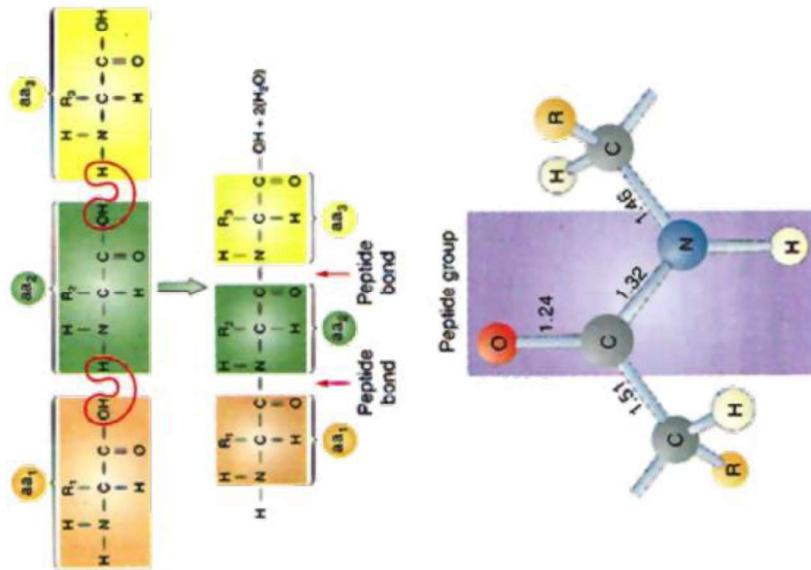
Mass Spectrometry

MASS SPECTROMETRY



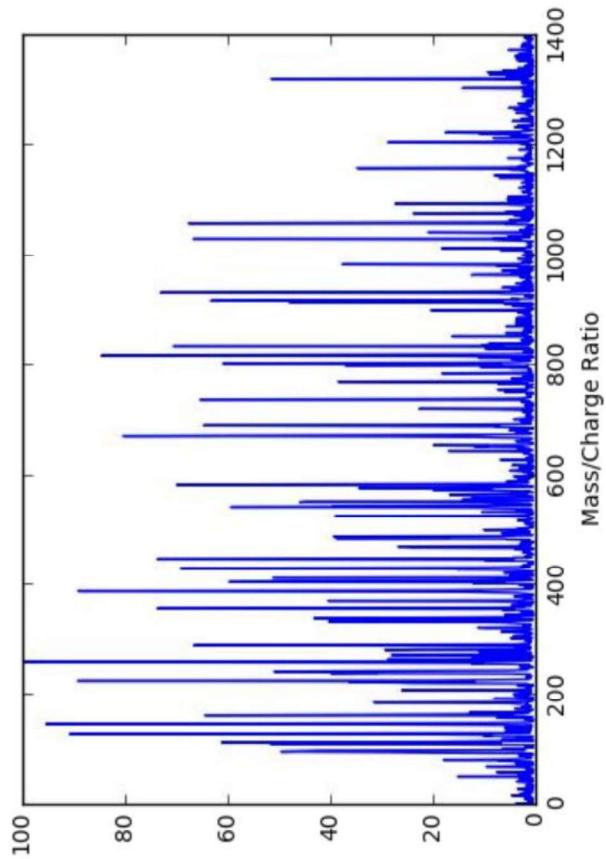
Structure of a Peptide Chain

- Peptides are chains of amino acids that are joined by peptide bonds
- These bonds reduce the weight of each amino acid by one H₂O molecule
- The result is called a residue
- A Mass Spectrograph can precisely measure the molecular weight (and charge and abundance) of any peptide chain
- Since the molecular weight of each of the possible 20 residues is known precisely, one can ask the question, which combination of residues would give a particular weight?



Mass Spectrum

- Peaks appear at frequently occurring mass locations
 - Y-axis indicates the relative abundance, sometimes called relative intensity
 - The peaks roughly correspond to our mass numbers



Summary

- The problem to Address
- Non-Ribosomal Proteins (NRPs)
- Applications of NRPs
- Cyclic Peptides
- Mass Spectrometry
- Structure of Peptide Chain
- Mass Spectrum