NON-RIBOSOMAL PEPTIDES

HOW DO WE SEQUENCE PEPTIDES THAT DON’T DEPEND ON mRNA?

Tyrocidine structure via wikipedia
WHAT’S DIFFERENT BETWEEN NRPS AND TRADITIONAL PEPTIDES?

• Linear peptides traditionally transcribed via ribosome from mRNA

• NRPs produced by non-ribosomal peptide synthetases (no mRNA)
  • Adds one amino acid at a time
  • Produces linear peptide that then circularizes
HOW WAS THE CYCLIC PEPTIDE SEQUENCED?

Use the weights of the fragmented peptides to reconstruct the sequence

• How?
  • We know the weights of each amino acid
  • Can create a function to mimic gas chromatography
    • GC: input - peptide, output - spectrum of the weights of all fragments

Masses of all 20 amino acids:

| G | A | S | P | V | T | C | I | L | N | D | K | Q | E | M | H | F | R | Y | W |
| 57| 71| 87| 97| 99| 101| 103| 113| 113| 114| 115| 128| 128| 129| 131| 137| 147| 156| 163| 186 |
HOW WAS THE CYCLIC PEPTIDE SEQUENCED?

Theoretical spectrum: mass of every possible subpeptide, plus 0 and the mass of the peptide.
HOW WAS THE CYCLIC PEPTIDE SEQUENCED?

• Cyclopeptide Sequencing Problem: Reconstruct a cyclic peptide from its theoretical spectrum

• GC mimicking function gives us the theoretical spectrum we can use as input

```
CYCLOPEPTIDSEQUENCING(Spectrum)
    Peptides ← a set containing only the empty peptide
    while Peptides is nonempty
        Peptides ← EXPAND(Peptides)
        for each peptide Peptide in Peptides:
            if MASS(Peptide) = PARENTMASS(Spectrum)
                if CYCLOSPECTRUM(Peptide) = Spectrum
                    output Peptide
                    remove Peptide from Peptides
                else if Peptide is not consistent with Spectrum
                    remove Peptide from Peptides
```
Cyclopeptide Sequencing Problem:

• Create a list, Peptides, which starts out empty

• EXPAND function adds on each of 20 amino acids to each “branch” in Peptides

• Check the theoretical spectrum of branches in Peptides against the known spectrum (input)

• If they match, return the peptide!

CyclopeptideSequencing([0, 113, 114, 128, 129, 227, 242, 242, 257, 355, 356, 370, 371, 484])

→ Returns “NIEQ” ... Why?

CyclopeptideSequencing([0, 113, 114, 128, 129, 227, 242, 242, 257, 355, 356, 370, 371, 484])

→ Returns “NIEQ” … Why?

If we look back at the amino acid weights...

The function works through the list of amino acids in this order, so I matches before L does and is thus the first output

N         I/L         Q

This gets us the right amino acids, but they are still in the wrong order...
NQEL = Input
NIEQ = Output
Same sequence, different direction

Why?
Not really sure…
WHAT DO THESE RESULTS MEAN?

• That as long as pairs of amino acids with the same mass are kept in mind...

• and as long the direction of the output read doesn’t matter:

This approach to answering the question of how to sequence a non-ribosomal peptide works!

• Can also be used for other ribosomal peptides if the GC spectrum of the peptide is known and the linear versions of functions are used