



Synthetic Biology

WHAT IS IT?

Synthetic biology is a field of science that combines chemistry and biology to understand biological compounds, their function, and how they can be manipulated to treat disease or synthesise products. In one sense, it is like writing code for a computer program where your computer is a biological cell.

- *What are some important biological compounds that might be a focus for synthetic biologists?*

WHO'S DOING IT?

At Victoria University of Wellington's Ferrier Research Institute, a team of researchers from a range of different fields, led by Professor Emily Parker, are working on understanding and manufacturing useful natural compounds for a range of different purposes; and enzyme-catalysed reactions with the aim of finding new treatments for diseases and ways to produce valuable materials.

- *What is an enzyme?*
- *How important are they to the functioning of a cell?*

ENZYMES: THE LOCK AND KEY TO LIFE

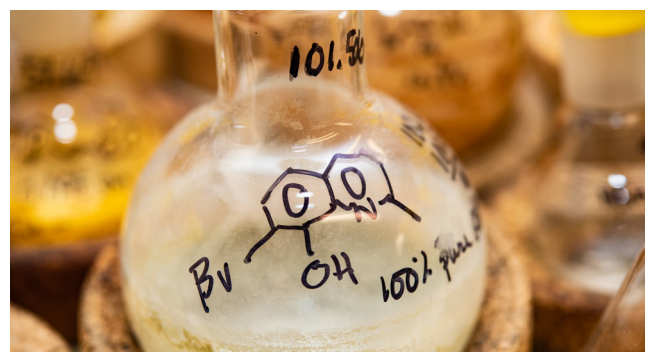
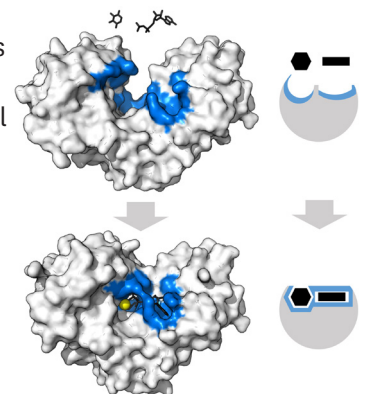
Enzymes are critical to life. They are proteins that catalyse reactions, speeding them up. Without enzymes, reactions wouldn't happen at a fast enough rate—if they happened at all—to carry out the processes that a cell needs to do to function. The enzyme, ATP Synthase produces adenosine triphosphate (ATP), the main energy transfer molecule of the cell. Without ATP synthase a cell would not be able to make ATP because the reaction is 'energetically unfavourable'. In other words, the reaction to make ATP requires energy whereas the reaction for ATP to split apart does

not. Cells use enzymes for everything. For example, a number of different enzymes are needed for cell division, and enzymes are responsible for producing the lipids that make up about half of the cell membrane.

- *What are other functions that enzymes carry out?*

HOW DO THEY WORK?

The main theory for how enzymes catalyse reactions is described as the 'lock and key' model. This model says that enzymes have an area called the 'active site' which is shaped in such a way as to only allow very specific molecules, called the 'substrate', to attach to it. The substrate binds to the active site, at which point they are either combined together to make a new compound, or broken up into smaller molecules. For example, proteases break down proteins into amino acids, and DNA polymerase puts together deoxyribonucleotides to make DNA molecules.



ACTIVITY – ENZYMES IN ACTION

This activity will show how enzymes can speed up natural processes, and highlight some potential applications.

You will need:

Jelly
Fresh pineapple or fresh kiwifruit
Tinned sliced pineapple

Instructions

1. Make up the jelly in three separate bowls, allow to set.
2. Place a slice of the fresh pineapple or fresh kiwifruit on top of one bowl of jelly, making sure that the fruit is in contact with the jelly.
3. Place a slice of the tinned pineapple on a different bowl of jelly, again making sure the fruit is in contact with the jelly.
4. Leave one bowl of jelly intact.

■ What do you predict will happen?

■ Why?

■ Why was one bowl of jelly left alone?



Discussion

The jelly with the fresh fruit should have liquefied, while the jelly with the tinned pineapple and the jelly with no fruit should be unaffected.

■ Why did this happen?

Some fruits, such as pineapple or kiwifruit, contain proteases, a type of enzyme that breaks down proteins into amino acids. Because jelly is made of gelatine, which is made from collagen, a common protein, the presence of the protease will break it down. This is also why eating too much pineapple or kiwifruit can cause your tongue to tingle or mouth to bleed as the protease digests your skin cells!

■ Why did the tinned pineapple not break the gelatine down?

Enzymes, like most proteins, can be denatured by high temperatures or changes in pH. When a protein is denatured it loses its shape and because shape is so important to the function of an enzyme, it becomes useless.

■ What are some potential applications of this enzyme?

■ Where would we anticipate finding protease in the human body?

This activity will show how enzymes are used by living cells to maintain natural functions.

You will need:

Pureed raw potato
Hydrogen peroxide – 10%
Detergent
Food colouring

Instructions

1. Pour 10ml of hydrogen into two test tubes.
2. Add five drops of detergent to each test tube.
3. Add three drops of food colouring to each test tube.
4. Add a small amount of pureed potato to one test tube and mark where the liquid comes up to.

■ What do you predict will happen?

■ Why?

Discussion

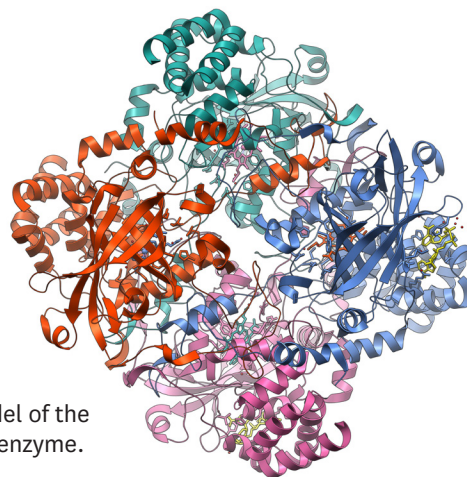
Bubbles should have appeared in the test tube containing the mixture of potato and hydrogen peroxide at a much higher rate than they did in the test tube containing only hydrogen peroxide.

■ Why did this happen?

Hydrogen peroxide is a natural by-product of a number of reactions that happen during cell metabolism. Because hydrogen peroxide is toxic, most cells will break it down into oxygen and water using another enzyme called catalase. Potato contains a relatively high concentration of catalase, though catalase is commercially extracted from animal livers.

Cells can also use hydrogen peroxide to kill invasive microbes, while using the catalase to protect themselves from its effects. Some bacteria have evolved their own catalase to deactivate the peroxide.

■ How could we use this information to protect ourselves from these bacteria?



A 3D model of the catalase enzyme.

FIGHTING DISEASE BY FIGHTING ENZYMES

Inhibiting enzymes

- *What are some ways we can prevent enzymes from working?*

Because enzymes are proteins, they can be denatured by high temperatures or changes in pH. However, using these methods to inhibit enzyme activity as a way of treating bacterial infections could also inhibit the enzymes of the patient.

The trick is in the 'lock and key' model. While the enzyme's active site is shaped to only allow a specific substrate to bind to it, other molecules can be shaped in a way that they can either completely or partially fit as well. Because they're occupying the space normally reserved for the substrate, the enzyme is effectively deactivated or inhibited.

By developing drugs that inhibit enzyme activity, researchers can disrupt the metabolism of cells and kill disease-causing microbes. Penicillin, the first antibiotic, works by inhibiting the enzyme that peptidoglycan, the molecule that makes up the cell wall of bacterial cells and is not present in human cells. This causes the bacterial cell wall to weaken and eventually burst.

- *What are the benefits to this particular type of treatment?*
- *What are some different examples of ways where inhibiting enzymes is used to treat disease?*
- *Based on what is understood about how enzymes work, how would their ability be inhibited?*

Targeting the Right Enzyme

Understanding the structure and function of an enzyme is critical to developing compounds that can inhibit their activity. One specific enzyme being investigated at the Ferrier Institute is Adenosine triphosphate phosphoribosyltransferase (ATP-PRT).

- *Based on the name of the enzyme, what is one molecule that makes up the substrate used by the enzyme?*

ATP-PRT catalyses the first step in the biosynthesis of histidine – an essential amino acid for the production of protein. Humans and other animals don't make histidine, so they need to get it from their food, while bacteria and plants can, and need, to synthesise it naturally to live.

- *Why do you think researchers have chosen ATP-PRT to investigate?*

Ferrier Research Institute scientists are using a combination of computer modelling and molecular biology experiments to understand the active sites of the enzyme to improve their ability to develop an effective inhibitor. Finding a molecule that inhibits the ability of bacteria and plants to synthesise histidine could lead to the development of new antibiotics and herbicides.

- *What are some other enzymes researchers are investigating with the hopes of developing new antibiotics, pesticides, herbicides or fungicides?*



A genetic sequencer

MICROBIAL FACTORIES

Biologists frequently find new compounds in nature that have exciting medical or commercial applications; however, these compounds might not be produced in large enough amounts to be viable, or could be difficult to extract.

■ *What are some potential solutions to this problem?*

There are two options available for producing natural compounds:

1. *Industrial synthesis e.g. Producing insulin in the lab using chemical methods.*
2. *Biosynthesis e.g. Genetically modifying bacteria or fungi to produce insulin or harvesting it from existing animals such as pigs.*

Synthetic biologists use the second option, biosynthesis, to make use of the biological machinery that already exists in the organism to produce natural compounds. This method lets them take advantage of the billions of years of evolution that have already developed an effective way to produce natural compounds.

■ *What are the important steps a synthetic biologist would need to take to genetically modify an organism to produce a new compound?*

Finding the Genetic Key

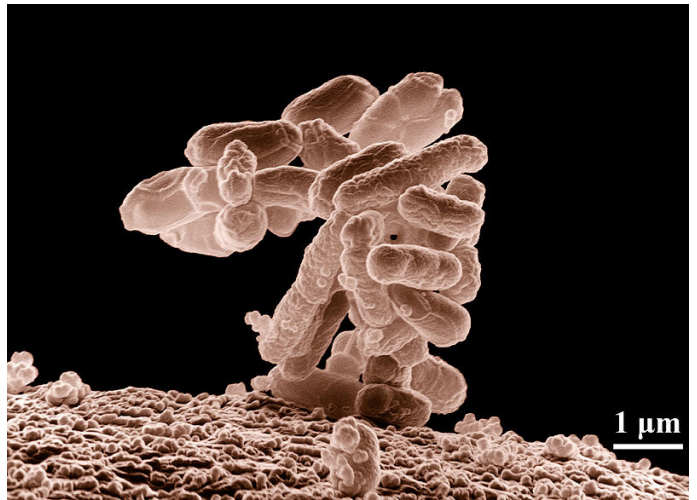
Once a compound has been discovered in an organism, researchers need to identify which gene or genes are responsible for its production.

■ *How can they do this?*

Researchers will not only need to identify the genetic factors, but also, they will need to figure out what materials are used to produce the compound they want. Not only that, but they will also need to understand whether there are any by-products that might affect the process.

At this stage, the synthetic biologists could simply use gene editing tools to transfer the genes responsible for the compound from the original organism into the genome of the organism they want to use to produce it (the 'host'). Alternatively, they could synthesise the genes completely in the lab without 'cutting' them out of the original genome and insert them into the host organism.

■ *Are there any benefits or drawbacks to either option?*



Escheria coli (E. coli) is frequently used to produce pharmaceutical products.



FIGHTING FLEAS WITH FUNGUS

Who?

A team of researchers from Victoria University of Wellington's Ferrier Research Institute, Callaghan Innovation, the University of Canterbury, and Massey University, led by Victoria University's Professor Emily Parker. The lead author of the article is Victoria University Postdoctoral Fellow, Dr. Kyle Van de Bittner.

What's the problem?

Nodulisporic acid A is a compound naturally produced by a species of fungus, *Hypoxylon pulvicidum*. It has been shown to be highly effective at treating fleas and ticks on cats and dogs; however, the fungus only produces nodulisporic acid A in small quantities, and the compound is so complex that researchers have not been able to produce it in the lab.

What did they use?

The synthetic biologists used a tool called MIDAS (Modular Idempotent DNA Assembly System). MIDAS lets scientists assemble new genes by constructing them from a library of DNA parts, allowing them to quickly and easily test genes.

More information on MIDAS:

<https://www.ncbi.nlm.nih.gov/pubmed/29620866>

- *How do you think they inserted the assembled genes into the genome of the fungus?*
- *What are some advantages to using MIDAS over other techniques?*

What was their solution?

The two limiting factors were the rate of production of the compound and its complexity. *H. pulvicidum* had already solved the problem of the complexity of the compound, so all the researchers needed to do was to find a way to increase its production.

The researchers first analysed the genome of *H. pulvicidum* and identified 13 potential genes in a gene cluster that were associated with making nodulisporic acids. Using MIDAS, they constructed a

series of plasmids made up of different combinations of these genes and inserted them into the genome of *Penicillium paxilli*, a fungus often used by researchers as a model for investigating how compounds like nodulisporic acid are made.

- *The researchers also tested the individual genes but why wasn't this enough? Why did they need to test combinations of genes as well?*

The researchers were able to test their results by analysing the different compounds produced by each combination of genes.

- *What tests would be used to identify any new products resulting from the new gene sequences?*

Using this method, they identified four genes that were responsible for the production of nodulisporic acid F, a critical first step in the biosynthesis of nodulisporic acid A. They also discovered that *H. pulvicidum* was missing a gene responsible for an important enzyme to the biosynthesis of nodulisporic acid F, which may explain why it only produced a small amount of the target compound. This gene was present in *P. paxilli*, solving the second challenge facing the researchers – producing enough of the compound for it to be useful.

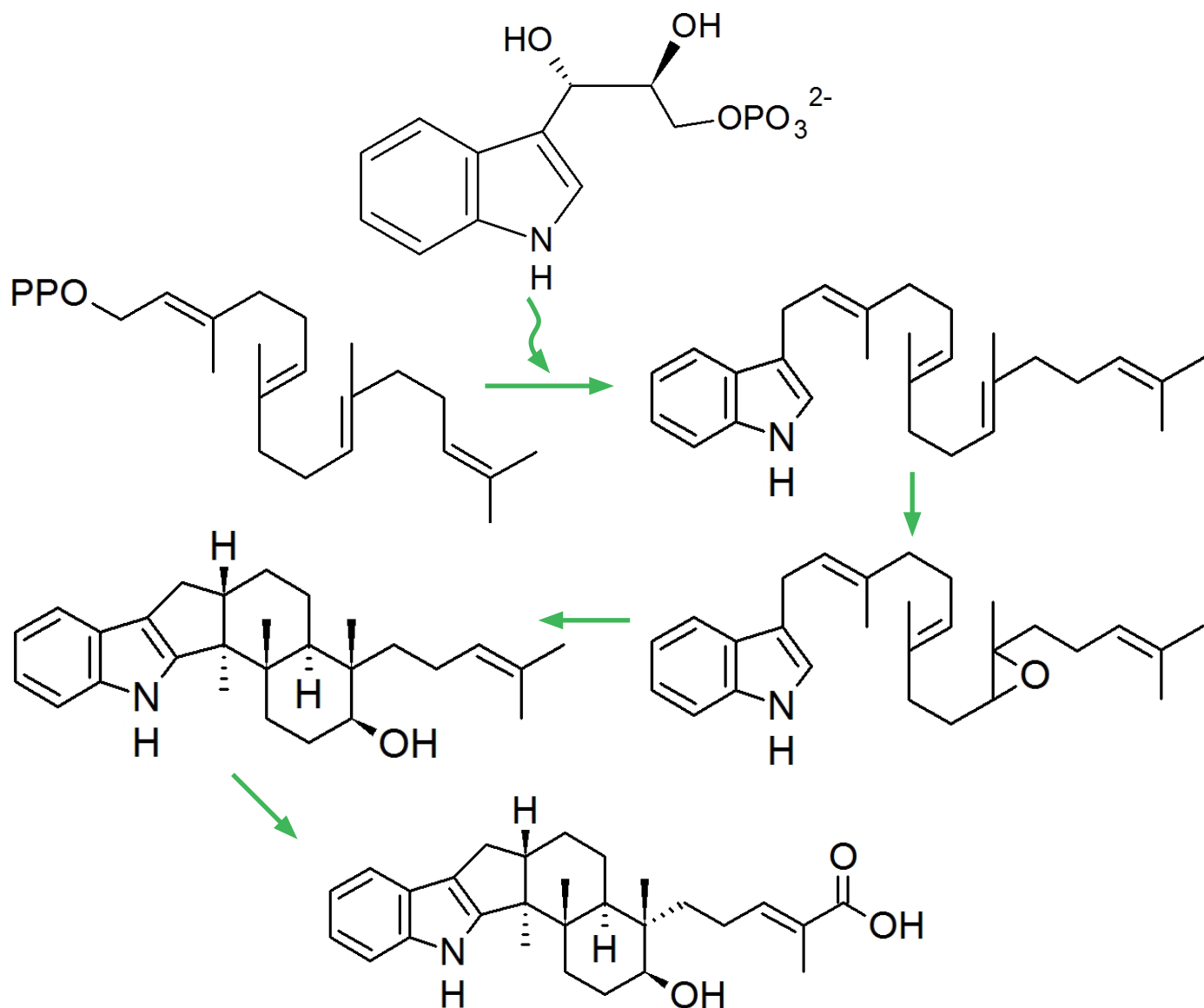


Hypoxylon pulvicidum

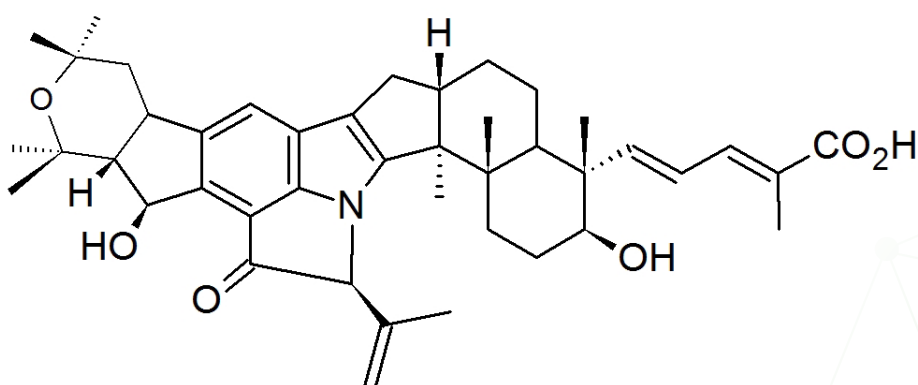


BIOSYNTHESISING NODULISPORIC ACID F

Below are the last four steps in the biosynthesis of Nodulisporic Acid F. Each step (indicated by an arrow) is catalysed by a different enzyme.



- Which functional groups can you identify in these compounds?
- What changes are made at each step?
- How are these changes being made within the cell? What is/are responsible?
- Below is nodulisporic acid A, the target compound. What are the main differences between it and nodulisporic acid F?



GLOSSARY

Enzyme:	Proteins that act as catalysts. They speed up reactions without being used up. Enzymes always have the suffix -ase, e.g. synthase
Substrate:	A molecule that binds to an enzyme's active site.
Protease:	An enzyme that breaks down proteins.
Catalase:	A common enzyme that catalyses the breaking down of hydrogen peroxide into water and oxygen. Protects the cell from peroxide, a product of normal metabolic reactions.
Protein Denaturation:	The alteration of the shape of a protein that results in it no longer carrying out its original function. Can take place through the application of heat, acid, alkali, or mechanical stress.
Enzyme Inhibition:	When an enzyme's ability to catalyse reactions is reduced or stopped entirely. Can be reversible or irreversible depending on the mode of inhibition.
Biosynthesis:	Using living organisms or cells to produce complex organic molecules through enzyme-catalysis.
Industrial Synthesis:	Producing organic molecules using technological processes.
Gene Cluster:	A group of two or more genes within an organisms genome that have a shared general function and are relatively close to each other within the DNA sequence.
Plasmid:	A small DNA molecule that is not part of chromosomal DNA. Often circular.

