SAVING THE WORLD

WITH ENZYMES

ENZYMES ARE ESSENTIAL IN ALL ASPECTS OF LIFE.

BIOCHEMICAL SOCIETY

DESIGNING YOUR GOLD CREST PROJECT

This pack includes background information and examples of saving the world with enzymes. It also provides examples of lab-based practicals and computer-based research projects to help you design your own Gold CREST Award project.

To achieve your Gold CREST Award you are required to meet a set of criteria. This resource sheet is designed to help you work towards achieving your award.

You are expected to design, plan and carry out an investigation with a clear set of aims involving a **minimum of 70 hours of work**.

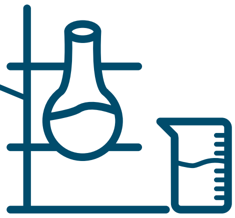
This project should be self-directed and contribute something unique to the scientific community. You are expected to organise your time effectively and demonstrate a high level of understanding of the topic and a clear strategy to solve the problem you are addressing.

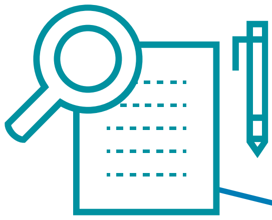
Your report will include background information, progress of your investigation, results/findings, conclusions and reflection. You need to present your findings in an understandable manner using an appropriate medium.

Look at the **CREST Awards site** for more information about the required criteria.

DESIGNING YOUR GOLD CREST PROJECT

If you plan to design and implement a lab based practical have a look at our examples: Project example **1** **'Breaking down with natural enzymes'** and example **2** **'Testing biological detergents'.**

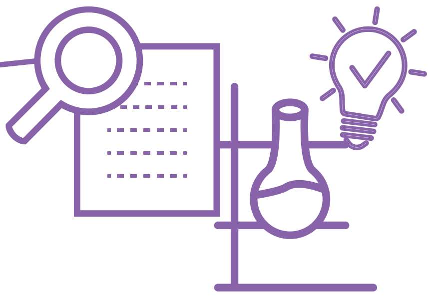




If you want to design an investigative research project have a look at example **3** **'Designing your own biofuel'** and example **4** **'Drug development'.**

You can use any of the examples included as starting points for your own projects or just as an example of designing your own.

You can combine any of these examples to create a project that involves different areas of enzymes in Biochemistry using physical experiments as well as web-based research projects.



SAVING THE WORLD WITH ENZYMES

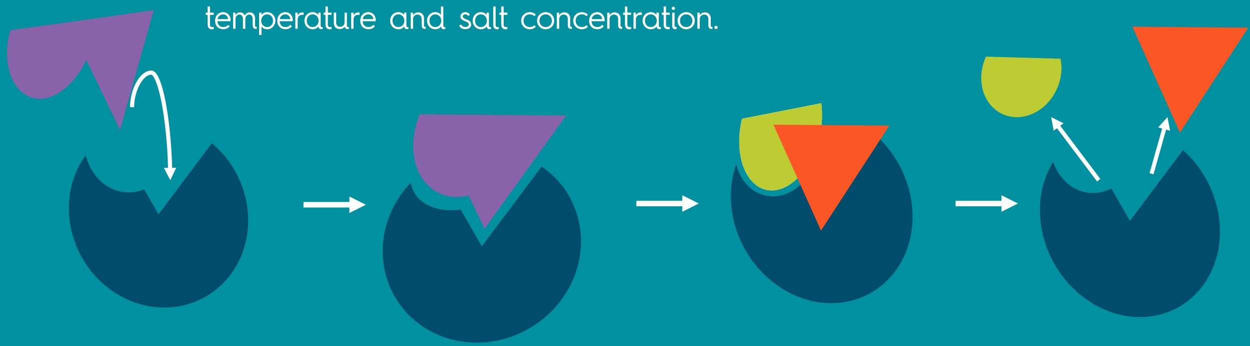
WHAT ARE ENZYMES?

Enzymes are proteins that speed up, or **catalyse**, chemical reactions via a mechanism known as the Induced Fit model. These molecules can make reactions up to 1 trillion times faster. That's the difference between **1500** years and **5** seconds! Enzymes work with other non-protein molecules, **co-factors**, to convert substrates into products. Co-factors are commonly metal ions e.g. Mg^{2+} or co-enzymes e.g. NAD which can accept hydrogen atoms to form NADH. The 2018 Nobel Prize for chemistry was awarded to **Frances H. Arnold** for developing a method for directing the evolution of enzymes making them more active for use in pharmaceuticals and renewable fuels.

NOBEL

HOW DO ENZYMES WORK? THE INDUCED FIT MODEL.

Enzymes reactions are highly specific to their substrate, however, these proteins are sensitive to changes in their environment including pH, temperature and salt concentration.



Substrate binds to the active site

Enzyme changes shape as substrate binds

Enzyme/Substrate converts to Enzyme/Product complex

Product released from enzyme

ENZYMES IN DISEASE

Enzymes are found at every part of multi-step reactions, so faulty or missing enzymes can be detrimental to the pathways they are involved in and have subsequent negative effects on essential human systems. *Galactosemia* (symptoms include vomiting, liver enlargement, jaundice) is caused by deficiency in sugar galactose breakdown. *Cystic Fibrosis* (affecting the lungs, pancreas, liver, kidneys and intestines) is caused by DNase deficiency. ***Think about other diseases caused by faulty or absent enzymes. How do these enzymes become faulty?***

CLASSES OF ENZYMES

Enzymes are classed based on their activity. These include **transferases**, **oxidoreductases**, **hydrolases**, **lyases**, **isomerases** and **ligases**. ***What do these types of enzymes do?***

HOW WE USE ENZYMES

Enzyme tests are commonly used as disease markers by looking for abnormal levels in the blood. For example, the amount of the enzyme **Creatine Phosophokinase** (CPK) in the blood changes after a heart attack. In healthy individuals the levels of this enzyme in the blood are low, but the concentration increases when the protein 'leaks' from the heart during an attack.

Enzymes high specificity makes them good for targeting drug and antibiotic development. As shown with the Induced Fit model, enzymes bind particular substrates. This means they can be targeted specifically so as not to impact or disrupt other processes and proteins in the body. For example **Rennet**, enzyme tablets used in cheese production, cause liquid milk to solidify. Chymosin, a major component, is the enzyme in Rennet which is extracted from calf's stomachs and targets and digests milk.

Bio-detergents have been developed to use natural enzymes to clean clothes. These enzymes breakdown stains attached to clothing without affecting the material. Biofuels, for example biodiesel, provide renewable sources of energy from waste. Bioplastics are derived from biomass sources, such as food waste and vegetable oils. ***Can you identify enzymes required for converting waste into fuel or plastic?***

SAVING THE WORLD WITH ENZYMES

THERE ARE MORE THAN

3000

UNIQUE ENZYMES IN THE HUMAN BODY.

BIOPLASTICS MAKE UP

0.2%

OF THE GLOBAL POLYMER MARKET

BIOPLASTICS ARE A TYPE OF BIODEGRADABLE PLASTIC DERIVED FROM BIOLOGICAL SUBSTANCES. THEY ARE MADE USING MICROORGANISMS THAT PROCESS BASE MATERIALS INCLUDING VEGETABLE OILS, CELLULOSE, STARCHES AND ACIDS.

SOME HUMAN ENZYMES INCLUDE DIGESTIVE ENZYMES SUCH AS PEPSIN AND TRYPSIN IN THE GUT THAT DIGEST PROTEINS. LIPASE SECRETED BY THE PANCREAS BREAKS DOWN FATS AND AMYLASE IN THE SALIVA BREAK DOWN CARBOHYDRATES.

THE ENZYME BROMELAIN IN PINEAPPLE HELPS DIGESTION AND TREATING MUSCLE INJURIES. THIS IS THE COMPONENT THAT HURTS PEOPLE'S TONGUES WHEN THEY EAT PINEAPPLE! THIS IS BECAUSE BROMELAIN BREAKS DOWN PROTEINS, SO WHEN YOU EAT IT, IT ATTACKS YOUR TONGUE, LIPS AND CHEEKS. ONCE SWALLOWED, THE ENZYMES IN THE PINEAPPLE ARE DENATURED IN YOUR STOMACH ACID AND NO LONGER WORK.

BIOLOGICAL DETERGENTS CONTAIN CERTAIN ENZYMES THAT ARE EFFECTIVE AT CLEANING STAINS. THEY SERVE AS A 'BOOSTER' TECHNOLOGY TO BREAK DOWN THE COMPONENTS OF STAINS. THIS MEANS THEY FUNCTION IN DIFFERENT CONDITIONS TO NON-BIOLOGICAL DETERGENTS.

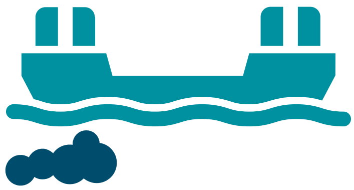
ENZYMES ARE USED IN FOOD PRODUCTION. PROTEASES FROM ANIMALS AND PLANTS WHICH REPRESENT 60% OF ALL INDUSTRIAL ENZYMES, ARE USED IN BREWING, MEAT TENDERISATION AND MAKING MILK CURD. MICROBIAL LIPASES ARE USED IN CHEESE FLAVOUR PRODUCTION. AMYLASE ENZYMES ARE USED IN IMPROVING BREAD QUALITY, BAKING AND CLARIFYING FRUIT JUICE.

THERE ARE MANY MORE EXAMPLES.

SAVING THE WORLD WITH ENZYMES

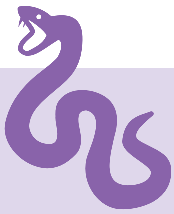
BIOREMEDIATION **IS THE PROCESS OF TREATING A CONTAMINATED MEDIUM, FOR EXAMPLE THE SEWAGE SYSTEM, A DIRTY WATER SUPPLY, SOIL MATERIAL, OR EVEN IN OIL SPILLS. THIS IS ACHIEVED BY STIMULATING THE GROWTH OF MICROORGANISMS TO DEGRADE THE TARGET POLLUTANTS. IT CAN BE CHEAPER THAN OTHER METHODS OF WASTE DISPOSAL! BIOREMEDIATION REQUIRES OXYGEN AS AN ELECTRON ACCEPTOR FOR THE OXIDATION OF THE POLLUTANTS. THIS THEREFORE IS KNOWN AS AEROBIC BIOREMEDIATION.**

THE USE OF THIS TECHNIQUE AROSE IN 1989, AFTER THE EXXON VALDEZ TANKER RAN INTO A ROCK IN ALASKA AND SPILLED 53.1 MILLION GALLONS OF OIL. BY 2005, THERE WERE 79 BACTERIAL CLASSES RECORDED FOR USE IN BIOREMEDIATION.



ENZYMES FROM ANIMALS, INCLUDING SNAKES AND LEECHES, ARE OFTEN USED IN PHARMACEUTICALS. THIS HIGH CATALYTIC EFFICIENCY, THERMAL STABILITY AND RESISTANCE TO PROTEOLYSIS (DEGRADATION OF PROTEINS) MAKE THEM USEFUL TO BIOCHEMISTS.

THE ENZYME HIRUDIN IN LEECH SALIVA PREVENTS BLOOD FROM CLOTTING, HELPING THE LEECH SUCK AS MUCH BLOOD AS IT NEEDS. IT IS USED AS A BLOOD ANTI-COAGULANT DRUG.



SNAKE VENOM IS MODIFIED SALIVA THAT CONTAINS ZOOTOXINS WITH MORE THAN 20 DIFFERENT ENZYMES. THEY FACILITATE THE IMMOBILISATION AND DIGESTION OF PREY BY DAMPENING THEIR NERVE IMPULSES, MITOCHONDRIA AND BLOOD CELLS. SNAKE VENOM IS USED IN DRUGS TO FIGHT BRONCHIAL ASTHMA, PARKINSON'S AND ALZHEIMER'S DISEASE AND PAIN DISORDERS DUE TO ITS TARGETING OF NERVE RECEPTORS. THE VENOM CAN 'DAMPEN' NERVE SIGNALS TO REDUCE PAIN.

TANNINS ARE A GROUP OF PROTEINS THAT ARE USED TO INHIBIT ENZYMES, PARTICULARLY THOSE INVOLVED IN DIGESTION. VEGETABLE TANNINS EXTRACTED FROM BARK TREES ARE USED IN THE LEATHER TANNING INDUSTRY.

XYLANASE IS A NATURALLY OCCURRING ENZYME PRODUCED BY BACTERIA, FUNGI, INSECTS, CRUSTACEANS SUCH AS SNAILS AND SEEDS. THE ENZYME IS USED IN THE PAPER PRODUCTION INDUSTRY, PARTICULARLY IN PULP BLEACHING.

EXAMPLE PROJECT IDEAS

ENZYMES

BREAKING DOWN USING NATURAL ENZYMES

An example of a Lab-based practical you can do Testing the activity of natural enzymes.

1. Extract enzymes from natural products e.g. proteases from a pineapple or kiwi or catalase from a raw potato. Mash up the product and collect the juice - these are your enzymes. Make sure to use the enzymes immediately as the longer they are left the less active the enzyme will become.

2. Test the activity of the enzyme using a control substrate e.g. jelly, bread or any other substrates you can find. Incubate the enzyme and substrate for a set amount of time and temperature (keep this constant). Observe and monitor what happens to the substrate.

3. Repeat using enzymes from different natural products and keep the amount you use the same so you can accurately compare. Are there variations, which natural enzymes break down the substrate and which do not? Look at **http://www.rsc.org/learn-chemistry/resource/res00000815/enzymes-and-jellies?cmpid=CMP00000908** for further guidance.

4. Vary the conditions of the enzyme reactions with the substrate. Test the effect of temperature by putting the reaction in the fridge or by a radiator; you can use a thermometer to monitor the exact temperature. Change the pH by making the reaction acidic adding Coca-Cola or basic by adding bicarbonate soda; change the time of the reaction. Make sure to only change one variable per reaction and maintain the other variables e.g. if you change the temperature keep a neutral pH.

5. Assess how these variables affect the enzyme activity: do the enzymes require very specific conditions or do they work well under a variety of conditions?

6. Find other examples of natural enzymes and carry out research on where you can find them. For example look at phosphatase enzymes from plants or catechol oxidase (from bananas). Identify the specific conditions your enzymes work best in.

7. You could test different substrates, for example bread. Assess whether different enzymes favor different substrates. Why do some enzymes break some things and not others?

Your research should give you a catalogue of natural enzymes that you will have tested, the substrates of these enzymes and their optimum activity conditions. For more ideas on how to design your enzyme experiment, use the links here **www.brighthub.com/education/k-12/articles/57976.aspx** or **http://www.nuffieldfoundation.org/practical-biology/enzyme-catalysed-reactions.**

ENZYMES

BIOLOGICAL DETERGENTS VS. NON-BIOLOGICAL DETERGENTS

An example of a Lab-based practical you can do

Enzymes in washing detergents are becoming increasingly popular. Think about why this may be. Carry out a comparative study comparing the original non-biological detergents to the newer biological detergents (you will be able to find a selection in any local supermarket, perhaps see if the same company does a bio and a non-bio detergent to compare. Some common examples include Ariel, Persil, Surf but there are many more). You could compare between brands or within a brand or you could choose a few different detergents.

Once you have chosen your detergents, start by creating your stains. Stains you could do may include mud, juice, tomato sauce or fruit. Think about the stains you are using and the enzymes that would be required to remove the stains. Some stains may be tougher to remove, why is this? You may want to test a few different stains, for example oily fat stains would be broken down by lipases, protein stains such as from eggs would require proteases and a mixed stain would require more action to break it down. Think about what enzymes are required to break down each stain you make. The stains need to be as equal in size and intensity to ensure a fair experiment, so use the same staining implement, leave the stains to dry before continuing.

Incubate the stained rags with the different detergents - don't forget to add water (you are trying to replicate what goes on in the washing machine so some movement would be good too). Can you observe any differences among the detergents and between the different stains? How will you measure this difference?

Try with different conditions including pH, temperature and time - for example, changing the temperature of water you use. What are the favoured conditions of the detergents? Do these differ between bio-detergents and non-bio detergents?

You can also compare your detergents and conditions by their ability to break down hard boiled eggs. Check out this link for further ideas on this **https://www.thenakedscientists.com/get-naked/experiments/dissolving-eggs-power-enzymes**.

Based on your observations, what is your opinion of these different detergents?

ENZYMES

DESIGNING YOUR OWN BIOFUEL

An example of an investigative research-based project you can do.

Where does your kitchen waste go? When you put waste in the different coloured bins or for recycling, it gets taken away but most people don't really know what happens to it. Here is your chance to find out. Do some research into current waste disposal: what systems are in place to break down the waste, how do previous systems compare to systems used today? A lot of research and current techniques use biological methods to dispose of waste: carry out a search on these techniques to understand the processes involved.

Design a system to break down your everyday household waste. Observe the types of things you throw away: what do they consist of? If you were to dispose of them at home, what resources would you need to achieve this. Do some research to find out what the enzymes are that break down these compounds - not everything may be degradable. With the products you get, what opportunities are there to recycle these and reuse them within the household?

You could even test the activity of some natural enzymes at breaking down your household waste. Try extracting the natural enzymes from fruit, such as pineapple and kiwis, or even raw potatoes. Mash up the fruit and collect the juice. Incubate the waste material with your enzymes and observe what happens. Think about the processes that are occurring. Would you expect to see the product breaking down? Use this as evidence to support your waste-disposal system designs. Don't forget to consider the risks of your 'home-waste disposal system': are there likely to be any toxic side products? How would you control these?

Compare organic waste (food waste) to synthetic waste (paper or plastic). Do the enzymes break these down? Think about and predict what the products of your broken down substrates would be.

One use of broken down waste is in making bioplastic and biofuel. Research the process required to make these. Are the products you get from your own disposal system suitable to make biofuel or bioplastic? You will need to look into the components in a biofuel and bioplastic to do this.

If you would like to have a look about how biofuel is made at home check out the website: **makebiofuel.co.uk/make-biodiesel-at-home/**. Making biofuels is a tricky business so if you are interested in trying it out make sure you get permission from a supervisor.

EXAMPLE PROJECT IDEAS ENZYMES

DRUG DEVELOPMENT

An example of an investigative research-based project you can do.

Enzymes are used in all aspects of medicine; in diagnosis, treatment and prevention. Think about some examples of enzymes found in medicine and where they come from - look at why the enzyme is used, the specific role it has, how it functions as well as the species the enzyme comes from. One example includes the treatment of inflammation associated with surgery wounds. Proteolytic enzymes are used in medicine to do this, they include bromelain, pancreatin, trypsin and chymotrypsin.

Use online tools to find out information about these enzymes used in medicine. You can use the Protein Data Bank (**http://www.rcsb.org/)** and UniProt, an online protein database (**www.uniprot.org**) to search for proteins. Use this resource to determine the protein structure and function. You can also look at the 3D models and pictures of your proteins here **http://proteopedia.org/wiki/index.php/Main_Page**

Enzymes are often built from structures called domains that have similar functions. Conserved domains are regions of a protein that are usually found across different species and in different proteins but retain a specific activity and are usually essential. Identify any conserved domains within the protein (these should be highlighted in the databases; you can do further searches on the names of the domains to get further information). Identify domains responsible for its activity, for example what does the active site do and what substrates does it bind to?

Use these domains to find similar enzymes that work in the same way. Are these other proteins involved in drug development? Do they have similar structure or DNA sequence, or are they similar in the functions they have? To look at specific DNA sequences that could be found conserved across your chosen proteins, use the Gene3D databank (**www.cathdb.info**). Think about what animals have them and the types of disease they could be used to treat. Perhaps some of these enzymes work in combination with other enzymes. Are these enzymes of a similar type or are they completely different? The table shows some information you may build up about your enzymes

Name	Drug	Model	Role	Activity	Substrate	Conserved domains	Drug combinations	Related enzymes
Enzyme 1								
Enzyme 2								

Imagine you have found an exciting new enzyme that could be used in drug development - do some research. What experimental tests and criteria would be needed to make your new drug and get it accepted to be used by doctors? How hard is it to get drugs developed and accepted? Can you identify potential problems for medical professionals and hospitals when they rely on these systems to treat patients?

RISK ASSESSMENT

The activities described in this booklet are considered low risk.

Equipment to be used:
* Vegetable masher
* Bowls or container boxes

Potential hazards:
* Cuts and minor injuries

Standard handling procedures:
* Care with manual handling of any sharp edges (adult supervision is recommended).

Chemicals to be used and produced:
* Juice/vegetable extracts
* Washing detergents
* Side products from enzymatic/chemical digestion of organic materials

Potential hazards:
* Eye splatter/splashes
* Skin irritation

Standard handling procedures:
* Wearing of gloves, eye protection (i.e. safety glasses/goggles) and lab coat or long-sleeved apron.

USEFUL LINKS

CHECK OUT USEFUL NOTES
www.bbc.co.uk/bitesize

CHECK OUT VIDEOS AT
www.livescience.com

ESSAYS IN BIOCHEMISTRY - ENZYMES
http://essays.biochemistry.org/content/59

Author: Tabitha Jenkins, March 2019