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Crime scene

The forensic science of poisoning

Science Enhancement Programme

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The cover image shows a police investigation. A forensic scientist takes photos to record evidence at the scene. See the article on pages 1-3. (Image: Corepics)

Contents

Solving puzzles

On pages 1-3 of this issue of Catalyst, Tony Hargreaves looks at the field of forensic science. The word 'forensic' comes from the Latin word *forum*, a place where citizens met to debate important matters. So a forensic scientist prepares evidence to be debated in a court of law. Forensic scientists need to know a wide range of aspects of science: how poisons affect the body, how dead things decay, how bullets move, and so on. They need to be able to defend their conclusions in court, so they must be confident of their findings, or at least avoid overstating their ideas. If this is an area which could interest you, take a look at college courses in your area.

Somewhere you are unlikely to work is the surface of Mars. However, as Louisa Preston and Lewis Dartnell explain on pages 8-13, we can get ideas about what to look for on Mars by exploring some of the most extreme environments on Earth. It is a constant surprise to find microorganisms living in extremes of temperature, acidity, salinity and radiation.

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Investigating poisoning

Forensic science at work

Tony Hargreaves

Investigating serious crimes such as murder, rape and terrorism requires forensic science experts. These people work alongside the police to find the evidence that will bring the culprit to court. In this article, ***Tony Hargreaves*** *looks at criminal poisoning and shows how forensic science is used to solve serious murder cases.*

"23 cats killed in 3 years – poisoning suspect arrested"

It is not just people who are poisoned – it can be animals, both pets and wildlife. In many cases this is accidental poisoning, when chemicals such as pesticides are used incorrectly, but there have also been criminal cases of animal poisoning. When animals are poisoned investigators often focus on tracking down the 'bait station' and in one case a piece of chicken was found to contain a poison made from a common household product. To get a conviction it has to be proven that this was put down intentionally as bait.

Poisons, medicines and food

We tend to think that a poison is a special type of chemical substance that can kill a person, usually by swallowing it. But poisons are not special substances and many everyday substances can be poisonous if swallowed in large enough amounts. For example, if you take paracetamol for a headache it works perfectly well so long as you use the correct dosage. The headache disappears and no harm is done. However, if you take an overdose, the paracetamol can kill you. It is the size of the dose that makes the difference.

Food can also be a poison. For example, marzipan contains cyanide and this is what gives it its almond flavour. In normal amounts it is harmless because the amount of cyanide is so small. However, if you ate a kilogram of marzipan you would soon be dead from cyanide poisoning. In reality it would not be possible to ingest such a large amount as this would cause vomiting so there's no need to worry about a few extra slices of Christmas cake!

Obviously some substances are more poisonous than others. If you swallow a gram of sodium chloride (common salt) it will cause no harm. However, if you swallow a gram of sodium cyanide you will be dead within seconds.

In poisoning cases the substances used by the murderer are usually the more toxic chemicals so that a tiny amount may be put into a victim's food or drink without it being noticed.

How poisons work

A poison works by damaging the body's normal chemical reactions. For instance, respiration is an essential chemical reaction in the human body.

Forensic scientists at the scene of a murder

Key words

forensic science

poison

chemical analysis

careers

The new EU symbol for toxic substances

If a person inhales carbon monoxide this attaches to the haemoglobin and prevents it transporting oxygen from the lungs to the cells. Respiration stops and the person dies. Thus, carbon monoxide is regarded as a poisonous gas (see Box 1).

Box 1: Carbon monoxide

Carbon monoxide (CO) is a deadly gas which is produced during incomplete combustion of carbon-containing fuels. It is colourless, odourless, tasteless and difficult for people to detect. It can be produced in homes by gas or wood-burning heaters and cooking equipment, particularly if they are old. Symptoms of poisoning include headaches and dizziness at low levels of CO but it can cause death at high levels. Carbon monoxide detectors are available and are useful if CO is suspected of being produced by household equipment. Carbon monoxide poisoning is diagnosed by measuring the level of CO in the blood.

Most poisonings take place not by inhaling but through swallowing. Some poisons are absorbed through the skin, such as the chemical weapons that were used until quite recently in warfare.

The crime scene

Where a body is found in suspicious circumstances, it is the responsibility of the forensic scientist to find the evidence that will lead the police to the culprit. If it is a case of suspected poisoning there may be some of the poison left at the crime scene and there will certainly be residues of it in the dead body.

Analysing a few specks of suspicious white powder from a crime scene is usually quite straightforward and it can often be identified within a matter of hours. Poison that is inside the body is more difficult to analyse - see Box 2. Usually the forensic scientist has to cut open the body and remove some of the organs. These are then prepared by dissolving a sample of the organ. The prepared solution is then analysed in a modern analytical instrument.

If it was thought, for example, that the deceased had died from swallowing arsenic then the stomach would be removed so that its contents could be dissolved in acid. The solution produced is then placed into an instrument called an Atomic Absorption Spectrophotometer (AAS) – see Box 3.

The instrument passes the prepared solution into a flame. Certain metals produce characteristic colours as you may have seen in flame tests in the school lab. The AAS analyses the light from the flame. This enables the substance to be identified and the amount present to be detected.

A flame test on copper sulfate gives a characteristic coloured flame.

Box 2: A notorious poisoner

Marie Lafarge, a Frenchwoman, was convicted of poisoning her husband with arsenic in 1840. This was the first case in which someone was convicted mainly upon forensic analysis results. She had purchased arsenic from the apothecary saying that it was to be used to kill the rats that infested her house. Her husband, Charles Lafarge, fell ill and was seen by a doctor. His condition was thought to be due to cholera, a disease that was common at that time. Later, suspicions were aroused when residues of white powder were found in a glass he had drunk from. Soon Charles was dead and samples from his body were taken for analysis using tests which showed that he had ingested arsenic. Marie was brought before the court, found guilty and sentenced to life imprisonment.

Box 3: Atomic Absorption Spectrometers

A solution of the suspected poison, such as arsenic, is passed into a high temperature flame where it emits light. The light is analysed by the instrument and the amount of arsenic measured. The instrument is also used to analyse samples for a wide range of metals.

A taste of poison

In the days before these modern scientific instruments, the forensic scientist had to use basic chemical tests. However, not many chemical methods were available and so in some cases the scientist had to taste the actual body fluids to identify the poison. This was done by comparing the taste of a body fluid with standard solutions of known poisons. Needless to say this was an unpleasant task. It was especially obnoxious when the body was not fresh but had been decomposing for a few months.

Working as a forensic scientist

To be a forensic scientist you need to have a science degree, usually in chemistry, biology or a related subject such as biochemistry or pharmacology. An assistant forensic scientist would need good GCSE passes and A-levels including at least one science A-level. In general most of the forensic science work involves chemistry, particularly analytical chemistry.

Forensic scientists analyse a wide range of evidence including human remains, firearms, explosives, DNA, body fluids, wildlife, fingerprints and impression marks. In addition to routine analysis, forensic science also involves research to enable the police to keep one step ahead of the criminal.

A forensic science technician examines a handgun for fingerprints and traces of blood.

Tony Hargreaves is a part time university lecturer with an interest in the applications of chemistry. He teaches chemistry, forensic science and maths.

Look here!

More information about careers in forensic science:
http://tinyurl.com/ars5kst

How animal poisoning is investigated:
http://tinyurl.com/alow7bz

Stefania Hartley

Mount Etna
Europe's biggest volcano

gnuckx

Mount Etna is known as in Sicilian dialect Mungibeddu, 'The Mountain'. This eruption occurred on 30 July 2011.

Key words
volcano
geology
igneous rock
tectonic plates

*Where can you find pristine rock which has never seen the sun, a fertile soil for a vineyard, and ski slopes with a sea view? **Stefania Hartley** tells us about Mount Etna on the Italian island of Sicily.*

Etna has impressive credentials. It is the biggest and tallest active volcano in Europe, over 3000m in height, and one of the most active volcanoes in the world. It is a candidate for UNESCO's World Heritage list.

Etna has been designated a Decade Volcano by the International Association of Volcanology and Chemistry of the Earth's Interior. 'Decade volcanoes' are 16 volcanoes deemed worthy of particular study in light of their history of large, destructive eruptions and their proximity to populated areas.

Leandro

Panoramic view of snow-capped Etna rising out of the clouds

NASA/GSFC

Etna erupting, seen from space in July 2001. Sicily lies off the toe of Italy.

The geology of Etna

Etna has several layers of solidified lava, ash and pumice on its slopes and changes height periodically, following an eruption.

Etna is a type of volcano known as a stratovolcano. Other stratovolcanoes include Vesuvius and Krakatoa. Most volcanoes have iron-rich magma, but these three stratovolcanoes have a high silica (silicon dioxide) content which makes the magma more viscous. This means that the lava covers a shorter distance before it solidifies and gives stratovolcanoes a characteristic conical shape with steep sides.

The thicker magma has another, more catastrophic effect too – it makes it difficult for trapped gases to escape from the magma chamber. Pressure builds up inside the magma causing periodic explosive eruptions. As part of the volcanic mountain suddenly gives way there is an explosion of hot gases and a rain of extremely hot large boulders, known as volcanic bombs, and finer ash called pyroclastic flow. Two clues that this have happened in the past on Etna include 'graded beddings' where larger rocks are found at the bottom with smaller rocks, dust and ash on the top, and pumice stones.

Pumice stone is so light that a piece 15 cm long can balance on a rolled up banknote. You can see the holes in the stone made by expanding gases.

Pumice is such a light rock that some samples can float on water. It is formed when super-heated, highly pressurised rock explodes violently out of the volcano. The unusual foamy structure forms because of rapid cooling and rapid depressurisation which happen at the same time. The depressurisation creates bubbles by lowering the solubility of gases including water and carbon dioxide which are dissolved in the lava. This causes the gas to come rapidly out of solution, like bubbles of carbon dioxide when a carbonated drink is opened. It cools quickly leaving large air bubbles which give it a low density. You may have used pumice to rub dead skin off the soles of your feet.

A brief history of Etna

Etna started its life as an underwater volcano during the Quaternary era, about 600 000 years ago. The area lies above the convergent plate margin where the African plate and the Eurasian plate meet. This causes the presence of other volcanoes (nearby in the volcanic Aeolian Islands, for example) and devastating earthquakes.

Etna has experienced a variety of eruption styles and for some time has also had basaltic (iron-rich) lava. Since the 1970s more explosive eruptions known as 'paroxysms' have been observed, especially from the craters at the summit. These have included lava fountains and gas and ash columns.

Igneous rocks

Igneous rocks are rocks originating from magma or lava. Magma that cools down inside the Earth's crust will cool down more slowly than if it was on the Earth's surface. The rock thus formed is called intrusive and it typically contains large crystals because the crystals have had time to grow. If, instead, the magma comes out to the surface (where it is now called lava) it will cool down more quickly. Such extrusive rock will have small crystals as the crystals will not have had time to grow much before the rock solidified. If magma is rich in iron it will be less viscous than the silica-rich magma typical of Mount Etna. The table shows the types of igneous rock which are typical of these different conditions.

	Iron-rich	Silica-rich
Intrusive	Gabbro	Granite
Extrusive	Basalt	Rhyolite

Rock samples for sale in a tourist shop near Mt Etna

An erupting hornito on Etna seen from behind in November 2006. Hornitos (from the Spanish 'little oven') are small cones formed on the surface of a basaltic lava flow when the lava is forced up through an opening and accumulates around it.

Magma or lava? When molten rock is under the Earth's surface we call it magma; when it comes to the surface it is lava.

The summit consists of four craters but eruptions can also happen from the craters along the side of the volcano, all the way down to a few hundred metres above sea level. The top craters are generally active continuously for many years while the side craters have interruptions in activity which, in the last forty years, have lasted only 2 years on average.

In December 1991 the longest eruption of the 20th century started which lasted 473 days and prompted the inhabitants into quick action. Diggers worked round the clock to create an earth barrier 20 m high to protect the nearby town of Zafferana Etnea. The barrier kept the lava away from the town for two months. In 2001 this technique was not sufficient to keep the lava flow away from inhabited areas. On that occasion the Italian navy had to use 7 tonnes of plastic explosive inside the main canal.

Agriculture on Etna

So, who on earth would want to live near such a dangerous beast, above such an unstable spot on our planet? As a matter of fact 25% of the total population of the island of Sicily does! Catania, the 10th biggest town in Italy, is situated just a pumice stone's throw from Etna's craters and it has been periodically destroyed by it. You might think that the fatalistic attitude of Sicilians has made them particularly resigned to live under the shadow of an active volcano, but in fact it is the fertile soil that is the attraction.

When the volcanic rock breaks down it releases valuable minerals and a rich volcanic soil is created. Vineyards, apple orchards, and groves of olive, pistachio and hazel trees all thrive along the slopes of Etna.

Olive trees growing below Mt Etna

The importance of agriculture and the human influence on the landscape of the volcano, with all the richness of tradition and culture, has been acknowledged by the organisation which manages the nature park. While in Zone A no human settlements are allowed, Zone B has smallholdings with beautiful old farm houses, terracing works, storehouses, millstones, and wine-processing structures as well as the old landlords' villas. (Zones C and D are different still, mostly devoted to tourist facilities.)

Flora and fauna

As you can imagine, living on a volcano can be an unsettling experience for a plant: as well as the harshness of the weather and the lack of water (at higher altitudes) you also have to factor in periodic lava flows which could burn down entire forests. At lower altitudes there are oak forests as well as orchards. From 2000 m above sea level beeches (reaching here their southernmost distribution limit) and a very special tree: the Betula aetniensis. This is a rare relict species, endemic to Etna. This species of Betula (birch) has had to adapt its vascular system in order to survive extreme heat and cold. It is only found on the East and West slopes of Mount Etna, between 1300 and 2100 m, where other trees cannot survive.

Trekking on Mt Etna – note the limited vegetation and the ski lift used in the winter.

Higher still are astragalus, senecio, violets, cerastium and pioneer plants. Beyond the limit of the astragalus, between 2450 and 3000 m, the conditions are so harsh and the rock so new that no vascular plants are to be found.

Although wolves, wild boar and deer have long disappeared from Etna, there are still porcupines, foxes, wild cats, martens, rabbits and hares together with weasels, hedgehogs, dormice and several species of mice and bats. As well as many insects, snakes and birds of prey are common: the poisonous viper has increased in numbers in recent years due to the fall of the numbers of its predators.

Stefania Hartley is a science teacher who grew up in Sicily.

Look here!

Etna is one of the most accessible active volcanoes in the world:
www.parks.it/parco.etna/Eindex.php

Take a look at these live webcams on Etna:
http://tinyurl.com/b3x37wr

Try This

ICE - WATER - STEAM

Boil water in a cup made of ice

Using a microwave oven, water can be made to boil in a cup made of ice and all 3 states of water can be seen at once.

You will need

- A large mug or plastic tub, preferably with straight sides
- A small plastic tub such as a small yoghurt pot
- A few coins
- Sticky tape
- Access to a freezer and a microwave oven

Ask permission of the owner of the freezer and microwave before you start.

What you do

Nearly fill the large mug with water and float the small pot in it, weighing it down so that it just floats in the water. You want the small tub to be in the centre of the mug, not at the side (see Diagram 1). Sticky tape can help with this. Place the mug into the freezer until completely frozen (at least overnight).

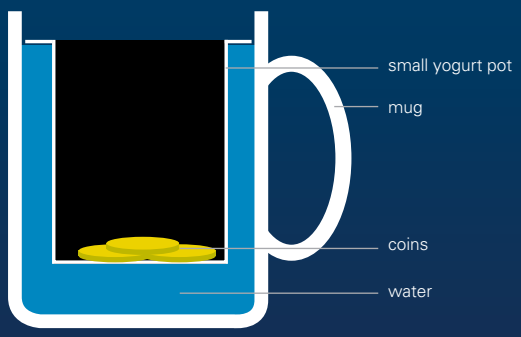


Diagram 1: Making an ice cup

Remove the coins and take the ice cup out of the mug. You may need to leave the mug out of the freezer for a few minutes to allow you to get the ice out of it. You can re-freeze the ice cup at this point until you are ready to use it.

Fill the yoghurt pot in the centre with water and place it into a microwave. Microwave for about 30 seconds until the water boils. You have water in all three states at once – ice, water and steam.

Why does it work?

A water molecule has a slight charge on it as the hydrogen atoms are slightly positive and the oxygen slightly negative. It shape is shown in Diagram 2.

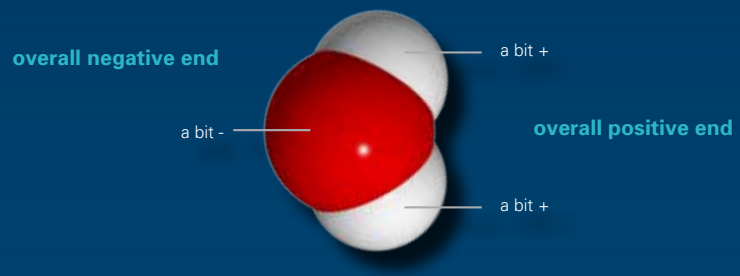


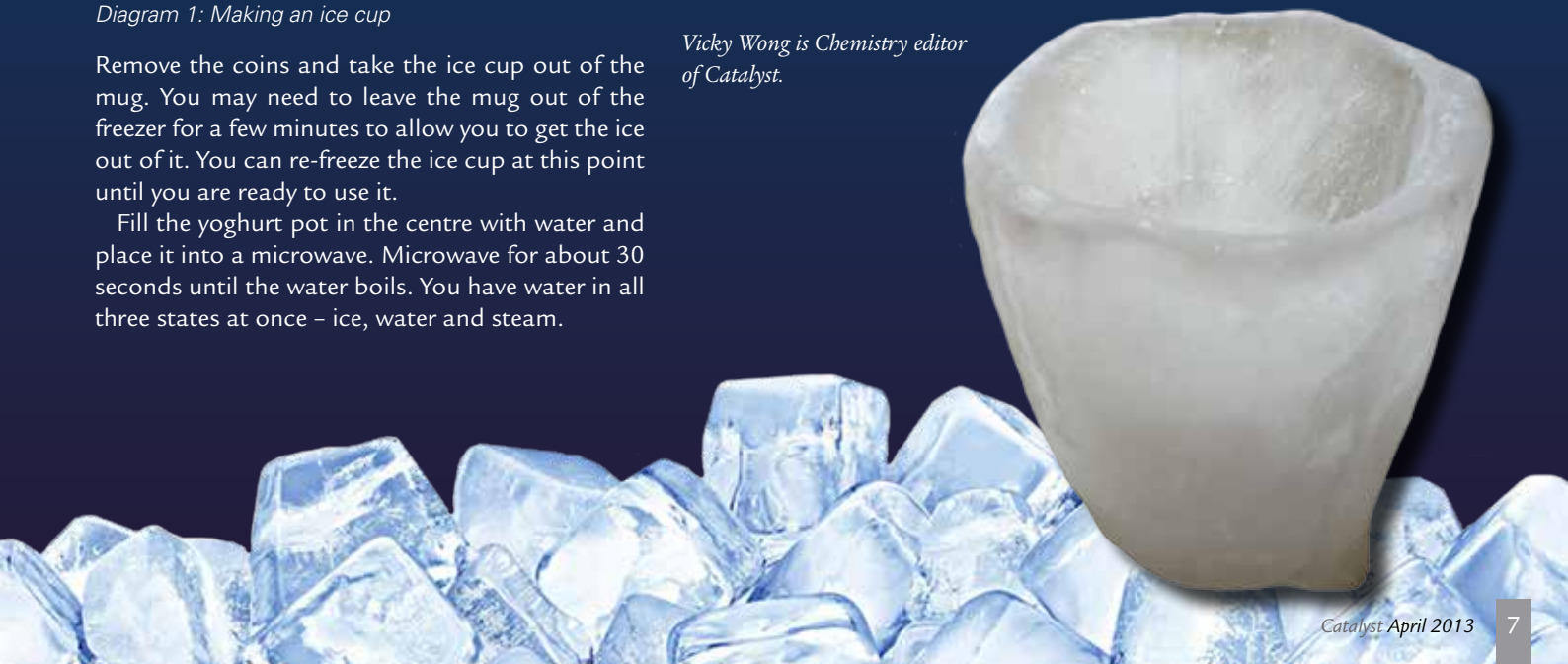
Diagram 2: The charge distribution on a water molecule

A microwave oven heats using microwave radiation which, like all other electromagnetic radiation, has an electric field which is constantly reversing its direction. In water, the molecules try to line up with the electric field but, as it keeps changing direction, so do they and they end up rotating. They have gained energy and eventually the water will boil.

In ice, the molecules are held in a lattice structure and are not free to rotate. So ice cannot absorb the energy of the microwaves and does not heat up as quickly as water.

There are some other factors to bear in mind: the ice and the water do not start at the same temperature; heat will be conducted by the water into the ice surrounding it; and the heat in a microwave oven is not evenly distributed – there are 'hot spots'.

Vicky Wong is Chemistry editor of Catalyst.



Louisa Preston

Lewis Dartnell

Extremophiles
How to find life on Mars

The surface of Mars – a picture taken by Mars Pathfinder in 1997.

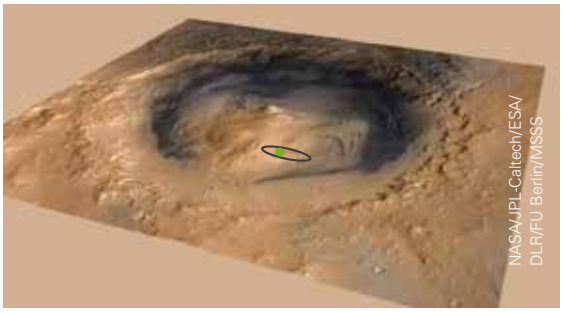
Key words
extremophile
astrobiology
bacteria
life on Mars

Where should we look for signs of life on Mars? Perhaps some of the strangest forms of life on Earth can give us ideas, suggest ***Louisa Preston*** *and* ***Lewis Dartnell****.*

When the Viking landers arrived at Mars in 1976 they sent back images to Earth of a rocky, dusty, red-brown landscape with no obvious signs of life. The world was watching, eagerly awaiting the results that would finally demonstrate that we are not alone in the Universe.

Since this time we have not given up hope that we might one day find life on Mars. Missions since Viking have shown us pictures of ancient rivers that used to flow on the surface and seas that may have covered large areas of the planet, and have provided evidence that water ice currently exists beneath the surface. Water has therefore been present on Mars in many forms throughout its history, providing a crucial ingredient for life.

In August 2012 our latest mission to the red planet, NASA's Mars Curiosity, finally reached and landed on the planet, searching for signs that Gale Crater – a 154 km wide impact crater just south of the equator - may have once been, or still is, a habitable environment i.e. a place with the right conditions for life to be able to survive.



NASA's Curiosity rover landed in the Martian crater known as Gale Crater, which is approximately the size of Yorkshire and Lancashire combined. A green dot shows where the rover landed, well within its targeted landing ellipse, outlined in blue. The picture of the rover is an artist's impression.

What are we looking for?

The search for life on Mars focuses on two main questions – what are we looking for and where should we be looking? The answers, however, lie right here on Earth. One of the developments in recent years that really opened up scientists' eyes to the possibility of life on Mars has been the realization of just how adaptable and versatile life here on Earth is. The **extremophiles** are a broad class of organisms found surviving in the most hostile and extreme environments on the planet. These range

Two extreme sites: an acidic boiling lake and a geyser, both in New Zealand

from volcanic lakes of boiling hot, acidic water, to freezing-cold, bone dry deserts, and high up in the atmosphere bombarded by harmful radiation from the Sun. The fact that earthly organisms can tolerate such extremes bolsters our optimism that life could thrive in similar habitats on other planets and moons, but it is the survival mechanisms that these endurance superheroes use that are truly fascinating. How are these extremophiles built differently to our own cells to ensure their persistence?

There are various ways in which an environment can be deemed extreme (from our point of view). Different organisms tolerate extremes of pH (acidophiles and alkaliphiles), temperature (thermophiles and psychrophiles), salinity (halophiles), or high concentrations of toxic substances like hydrogen peroxides or heavy metals. Acidophiles, for example, thrive at very low pH by protecting the vital molecules inside their cells, like DNA and proteins, from the high concentration of protons in their environment. They're toiling constantly to pump protons back across their membrane to the outside, like a shipwrecked sailor desperately trying to bail out his leaking lifeboat. Alkaliphiles face the opposite challenge, and struggle to generate energy with too few protons in their environment.

Microorganisms can't regulate their own temperature (they can't cool themselves in the same way that we mammals can, for example, by sweating) and so instead must adapt all of their cellular machinery to different operating conditions. Thermophiles – heat-lovers – have evolved reinforced proteins to hold themselves together against the violent shaking of thermal motion at high temperatures (and so some have been co-opted for human technology). Psychrophiles, instead, must have enzymes and membranes that are loosened to remain dynamic and active even at very low temperatures. Some of the most extreme psychrophiles reside in tiny channels of salty water inside solid icebergs, growing at temperatures right down to −20°C, and are killed at human body temperature.

The halophiles grow in high salt solutions – it turns out that the Dead Sea isn't actually all that dead at all. The danger posed by very briny water outside of cells – more concentrated than their interior – is that it draws water out of the cells by osmosis and effectively desiccates them (that's the very reason we use salt to preserve meat and butter). There are two ways to deal with this. Some halophiles have modified their inner workings to cope with higher salt, keeping their inside balanced with the outside, and so safe from osmosis. Others have taken a different approach and stuff their cells with different solutes to produce an equally concentrated solution to guard against osmosis, but avoiding the issues of a briny interior. These cells protect their innards from getting too dry or salty by keeping them nice and sugary.

Article continued on page 12

The photograph on pages 10-11 shows the Mars Curiosity rover. This is a composite of 66 images taken by the rover's Hand Lens Imager and shows Curiosity on the flat outcrop of rock chosen for its first drilling operations.

For more about Curiosity's instruments, see Catalyst Vol 23 issue 1.

A self-portrait of the Mars Curiosity rover, at its first drilling site on the surface of Mars in February 2013.

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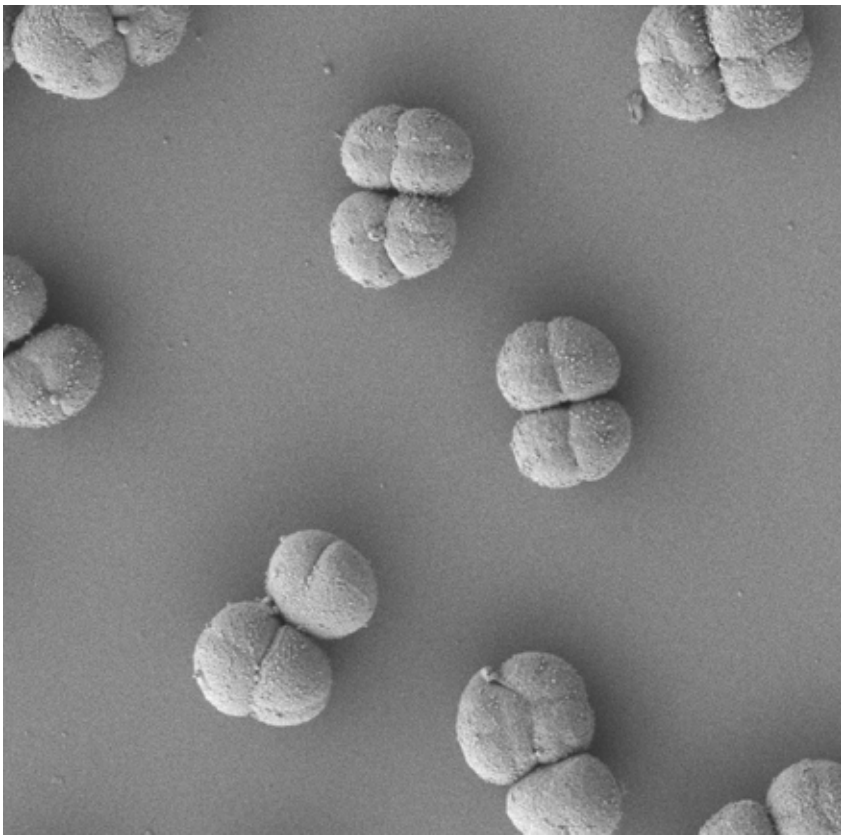
Surviving radiation

The sorts of extremophiles we focus on in our research are those able to endure very high levels of ionizing radiation. This is important because, unlike the Earth, Mars receives no shielding against cosmic radiation from its thin atmosphere and failed magnetic field. So if there is any life near the Martian surface it's going to need to tolerate this hazard alongside the cold temperatures and lack of liquid water. The most radiation-resistant organism known on Earth is a bacterium called *Deinococcus radiodurans* that can endure doses thousands of times higher than would kill human beings. In fact, this hardy little bug can prevail in such a range of hostile conditions that it's sometimes nicknamed *Conan the Bacterium*.

At first, it was a mystery as to why *Deinococcus* had ever developed such enormous tolerance of radiation - it can survive exposures far higher than those encountered in any natural environment on Earth. It is now thought that since being irradiated results in similar internal damage to the cell as being dried out, that *Deinococcus* actually evolved for desiccation tolerance, and the radioresistance is an adaptive spin-off. The organism is a master repairman, and can reassemble its radiation-damaged DNA after it is shattered into thousands of fragments, piecing it back together again like a life-or-death jigsaw. Exactly how *Deinococcus* protects itself is the subject of intense research at the moment; the hope being that we could learn how to better shield our own bodies against the harmful effects of radiation.



Radiation resistant Deinococcus*; the name means 'fearsome balls'. Each group of cells is about 3 μm across.*

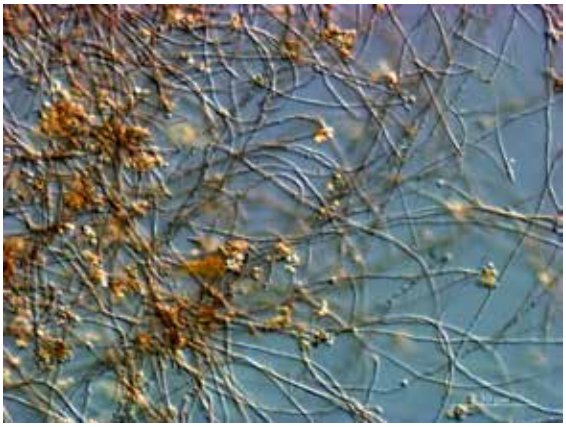
Analogue environments

All the above extremophiles can be found within hundreds of places on the Earth that mimic a physical, chemical and/or biological feature on Mars. We call these places 'analogue environments'. No analogue environment is ever a perfect replica of a site on Mars but it will have a number of specific features that can be compared and studied. We combine analogue research with laboratory simulations and data collected from Mars itself to create theories about where to look for life and what life to look for. In addition, analogue environments help us to practise methods for life detection; landing and driving rovers; and even test astronaut spacesuits and vehicles. Three places that are particularly like Mars and have been used to practise tools and techniques for searching for extra-terrestrial life are Rio Tinto in Spain, The Antarctic Dry Valleys and Iceland.

Rio Tinto, a highly acidic blood red river system in SW Spain, resembles ancient river channels on Mars. Here, the pH is so low (2.3) that only acid-loving extremophiles can survive in the waters, such as the bacteria *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans*. Banks of iron-rich rocks line the sides of the river building up for the last 2 million years.

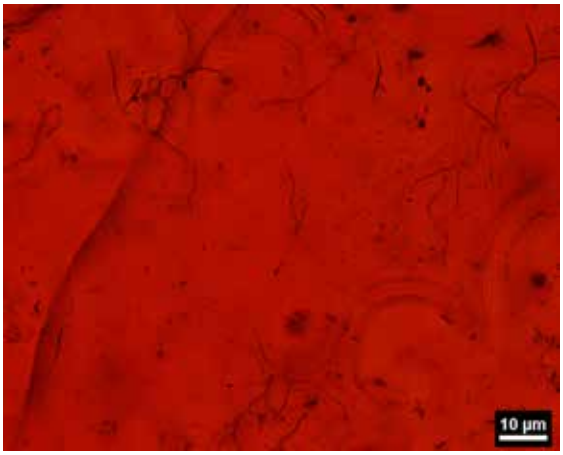
Lewis Dartnell by the Rio Tinto in Spain

The Rio Tinto in Spain resembles the rivers that might have flowed once on Mars.



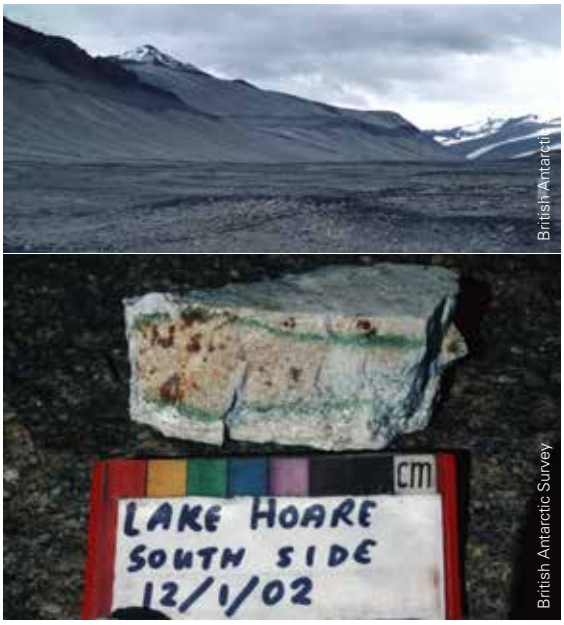
The acid-loving extremophile Acidithiobacillus ferrooxidans

Over this time, the bacteria and other microbes living in the river have been trapped inside the rocks forming black filament-like fossils, which we can study today. This site is therefore a perfect natural laboratory to study life living in the river and to compare it to that which lived millions of years ago. We can test instruments designed to identify the biomolecules that make up the bacteria, such as proteins, fatty acids and even DNA, experiment with cameras that can image the micro-organisms and rocks, and develop drills that penetrate the surface to access life buried deep within the rocks.



Fossils of bacteria from the rocks of the Rio Tinto

The Antarctic Dry Valleys are one of the most Mars-like environments found on Earth and the largest ice-free region in Antarctica. Conditions here are extremely dry and cold with temperatures recorded down to − 40 °C. The Sun's UV radiation is also very strong here which combined with the cold and lack of liquid water creates an environment that mimics the conditions on Mars today i.e. a world that is incredibly difficult for life to survive in. Life does exist here, however, as extremophilic communities of microorganisms, lichen and even tiny animals, living within the pores of sandstone rock to avoid drying out on the harsh Antarctic surface. Cryptoendolithic communities, typically a combination of cyanobacteria and lichen, are found living within the pores of sandstone rock to avoid drying out on the harsh Antarctic surface.



A dry valley in the Antarctic; a sample showing signs of the community of organisms living within the local rock

Finally Iceland has been used for decades as an analogue environment for Mars and also the Moon. Here, NASA have tested rovers and instruments, and trained their astronauts in planetary exploration techniques and geological protocols. Iceland has numerous volcanoes and lava flows, glaciers, ice sheets and hundreds of hot springs which have all been used as analogues to similar features that are currently observed, or would once have been found, on Mars, together with many forms of extremophilic life.

Louisa Preston at a hot spring in Iceland

The search for life outside of the Earth is the backbone of the fast growing and highly exciting field of astrobiology. This subject is an oddity within science as it has yet to prove its subject matter i.e. extra-terrestrial life, actually exists. But this doesn't matter. For us it is all about the hunt for life, and at the heart of this search is the study of Earth's most exceptional organisms and the Mars-like environments they live in.

Dr Louisa Preston is a TED Fellow and astrobiology Postdoctoral Research Associate at The Open University. Dr Lewis Dartnell is a UK Space Agency astrobiology research fellow at University of Leicester and author of 'Life in the Universe: A Beginner's Guide'

Charis Cook

The Other Genome Project

Key words
genome
Arabidopsis
database
Green Revolution

*In this article, **Charis Cook** explains the significance of a cress called Arabidopsis, the plant with the best-understood genome.*

Food security hit the headlines in the 1960s, when leading economists were predicting a catastrophic food crisis. That food-based apocalypse never arrived due to the unforeseen Green Revolution which was largely down to a long 20 years of work by Nobel Peace Prize winner Norman Borlaug. Today, global population growth and climate change means the world's food supply is again facing an uncertain future. Fortunately, plant research has changed beyond recognition since Borlaug started his wheat breeding programme in 1944, and scientific progress moves much faster today. The reason for the step change in plant science is extensive research into an insignificant member of the cabbage family, *Arabidopsis thaliana*, subject of The Other Genome Project.

Arabidopsis rows: Arabidopsis growing in a glasshouse at the University of Warwick. Image courtesy of Ruth Bastow, GARNet

Arabidopsis thaliana is a model species like the fruit fly *Drosophila*, yeast, and the bacterium *Escherichia coli*. Scientists work on these species in order to understand as much as possible about a particular kind of organism. Arabidopsis was first suggested as a plant model species for developmental and genetic research in 1943. A German scientist named Friedrich Laibach noted that *A. thaliana* was easy to grow inside in a small space, showed a lot of natural variation, was amenable to cross-breeding between varieties, and generated large numbers of seeds per plant.

Arabidopsis ecotypes: A selection of Arabidopsis ecotypes growing at the 1001 Genomes Project at the Gregor Mendel Institute, Austria.

Auxin mutant: An example of an extreme phenotype in Arabidopsis thaliana. *Wild type* Arabidopsis thaliana *is on the left. An auxin signal-transduction mutant,* axr2, *is on the right. Auxin is a plant hormone involved in many processes, including growth.*

Laibach's research group and a number of other German researchers worked on Arabidopsis well into the 1960s, but it was not until the 1980s that Arabidopsis became widely used worldwide. By this time, it was clear that artificially adding genes to Arabidopsis by gene transfer using *Agrobacterium tumefaciens* was easier than in other plant species, and genetically modified seed were shared between the growing number of Arabidopsis research groups.

Box 1 Transferring DNA into plants

Bacteria called *Agrobacterium tumefaciens* naturally insert their own DNA into plants. Scientists use *A. tumefaciens* to put specific pieces of DNA into plants. First, the DNA fragment that needs to be inserted into the plant genome is put into a circular piece of DNA called a plasmid. The plasmid also contains an antibiotic resistance gene which will act as a 'marker' for bacteria and plants containing the plasmid DNA.

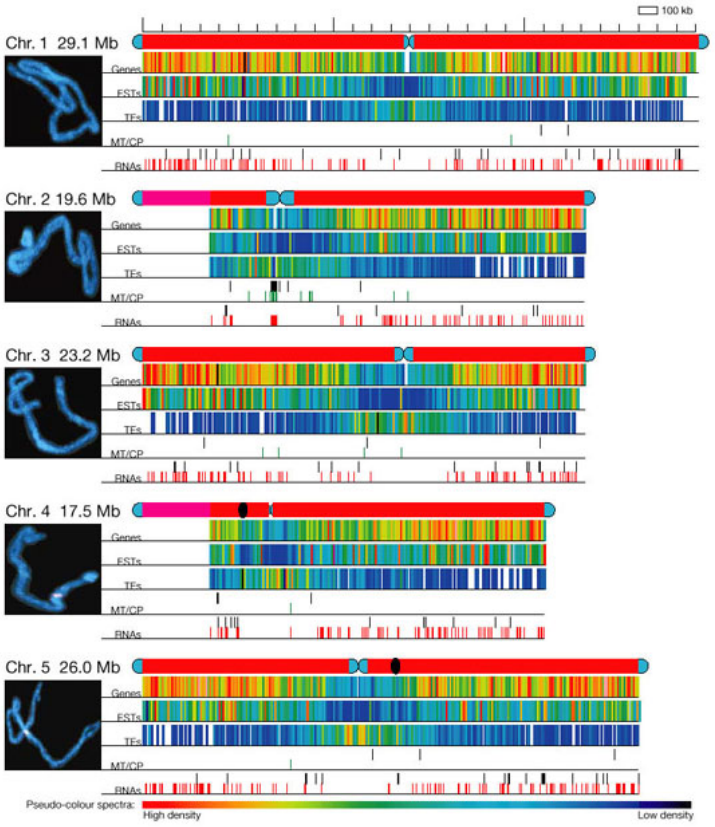
The plasmids containing the insert are put into *A. tumefaciens* cells which are grown on agar plates containing the antibiotic. The colonies that survive contain the plasmid, and are then grown in liquid culture.

Arabidopsis thaliana is dipped into the *A. tumefaciens* culture flower-first, so that the bacteria can transfer the plasmid to the pollen and ovules. This is called floral dipping. The seeds produced by these plants are grown on media containing the antibiotic, and the seedlings that grow contain the plasmid DNA. DNA analysis can confirm the presence of the required DNA fragment in the seedlings.

Participants at the First International Symposium on Arabidopsis Research in 1965, held in honour of the 80th birthday of Friedrich Laibach (standing at centre with glasses).

In 1989, James Watson, one of the team that discovered the structure of DNA and by then the Director of Cold Spring Harbour Research Laboratory in the USA, called a meeting to discuss the use of Arabidopsis as a model for genetic research. This was when the concept of an Arabidopsis genome project was first discussed. A year later, a group of leading plant scientists published a report outlining plans to sequence the whole Arabidopsis genome. At the time, that sounded overly ambitious and probably impossible – *A. thaliana* has a relatively small genome, but it is around 120 million base pairs long.

In 2000, after ten years of work from scientists in more than 50 research groups from all over the world, the genome was sequenced. The author of the research paper was 'The Arabidopsis Genome Initiative', recognition that this huge project was only possible due to the research community working together. The genome is published and publicly available, but its analysis is an on-going project.



A representation of the five genes of Arabidopsis, from the paper in the journal Nature where the details of the genome were first published (Nature 408:796-815, 14 December 2000).

The next Green Revolution

During the 1940s and 50s, scientists physically tested the wheat varieties that would spearhead the Green Revolution for pathogen resistance and higher yield. They grew them to maturity, observed the phenotype, interbred them, and repeated the cycle with the hybrid offspring. Thanks to the Arabidopsis genome project, today scientists get the information they need by analysing the genome of seedlings, a far faster process than breeding over many life cycles. This is because the genes conferring resistance to disease, or other beneficial characteristics, have been identified. Most were first found in Arabidopsis, and though crop genetics is advancing very fast and more genomes

have now been sequenced, many useful genes are still discovered by researchers doing fundamental plant molecular biology on *Arabidopsis thaliana*.

Box 2 Some definitions

Model species: An organism that is widely used in genetic studies because it is convenient to work on.

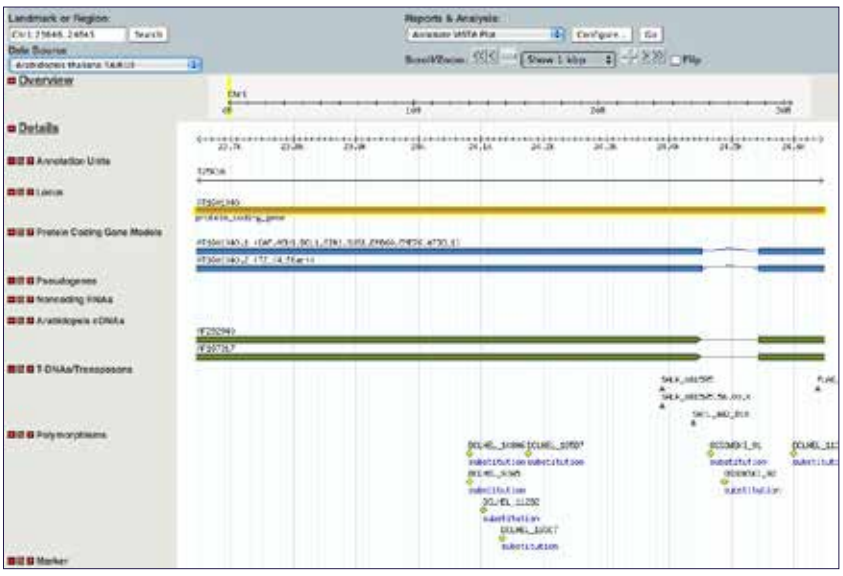
Phenotype: An organism's physical characteristics, defined by its genetic make-up and its interaction with the environment. An example is resistance to draught.

Homologue: Many genes are similar in multiple species. These genes are homologues of each other.

Genome-wide association studies: Analysing entire genomes to identify genetic features associated with a specific phenotype.

Ecotype: A genetically distinct variety of a species, which is usually adapted to a particular habitat.

With the sequence for 'Arabidopsis Protein A', a wheat researcher is likely to be able to find the homologue 'Wheat Protein A' quite easily – even if it turns out that there are actually two wheat proteins that do the job of one Arabidopsis protein. Searching through genomes and comparing gene sequences to find gene homologues in different species is done through free online databases. It is possible thanks to the open nature of modern science, which was born at the start of the Arabidopsis genome project. The world-wide effort to sequence the genome brought together a global research community in a way that had never been done before. Importantly, the resulting data were made public so the community continued to grow as new researchers used and built on the data.



A screenshot from TAIR, The Arabidopsis Information Resource, an online database of genetic information on Arabidopsis.

Working together

Community projects on the Arabidopsis genome are still on going and use new sequencing technologies. New sequencers are able to sequence multiple copies of the Arabidopsis genome in a single run. A notable recent large-scale research effort, the 1001 Genomes Project, was a change in direction for the plant science community. Genomics has traditionally been used to look within the genome to understand molecular processes, but the 1001 Genomes Project is a genome-wide association study that aims to link genetic variants to physical variation in *Arabidopsis thaliana* ecotypes from all over the world. As the name of the project suggests, it involves sequencing and analysing hundreds of genomes. Among the genes identified by the project so far are those responsible for flowering variation, and the single protein that is the primary determinant of levels of the metallic element cadmium in leaves. These could not have been found using traditional genetics experiments, which investigate one gene at a time.

The Arabidopsis Genome project changed plant science in the 1990s by giving researchers a common cause and community resources. Over the last 20 years, scientists working on other plant species have mimicked this, forming multi-group collaborations for large-scale projects. Arabidopsis researchers are still the pioneers of plant science. Although the public see the impact of plant science in crop plants, fundamental research is essential for progress in applied biotechnology. Now, as it was 30 years ago, *Arabidopsis thaliana* is the first choice for researching basic plant science and making new discoveries about how plants work.

Charis Cook is Liaison and Communication Officer for the UK Arabidopsis research network, GARNet (the Genomic Arabidopsis Resource Network).

Look here!

The Arabidopsis Information Resource (TAIR) is at *www.arabidopsis.org*. It provides tools for analysing the genome as well as pages of background information about Arabidopsis.

Nearly all gene sequences from any species (including Arabidopsis) are deposited at the National Centre for Biotechnology Information (*www.ncbi.nlm.nih.gov*). All the data and tools are free to access.

Seeing inside cells

Petra Kiviniemi

Key words
microscopy
plant cells
protein
imaging

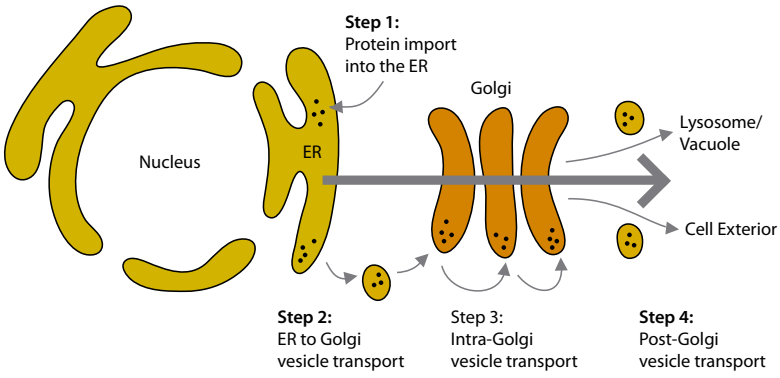
As you run across a grassy field, it would be difficult to imagine, without biological knowledge, the billions of intricately organized cellular processes which are occurring under your feet. Each individual blade of grass consists of various tissues, each made of cells in their thousands. In these cells an as yet unknown number of reactions and interactions are constantly taking place. The cells contain different parts known as organelles, which can be thought of as the cellular equivalent of the organs in animals and plants. Plant and animal cells differ in many ways (see the table below).

The secretory pathway

If you zoom into the cell cytoplasm you will see intricate organization of the different organelles which have processes occurring inside them.

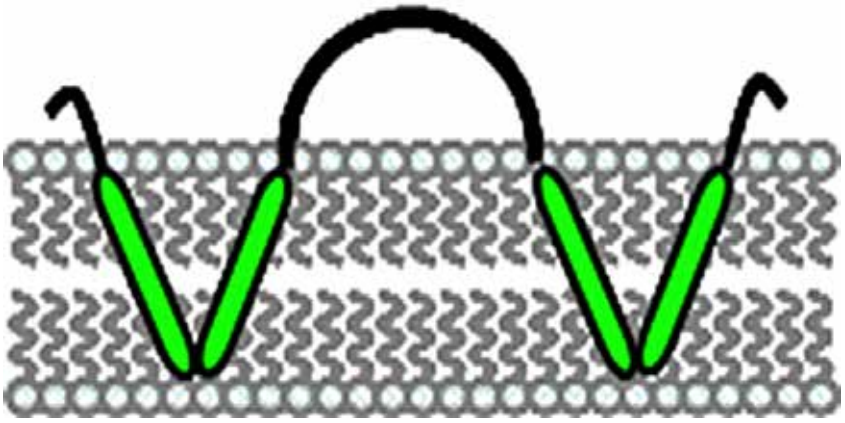
The genetic information that codes for all of the proteins of the cell is in the nucleus. Once a protein has been assembled in the cytoplasm, it enters the part of the endomembrane system known as the endoplasmic reticulum, or ER for short. The basic protein molecules, just a chain of amino acids, travel through this membrane system along a route we call the secretory pathway. Like an initially unmodified product sent down the production line of a factory, the proteins are folded and modified with additional components to produce the final product. Any faulty proteins are sent to be destroyed, like quality control on factory products.

Following this the proteins are moved to the next station in the secretory pathway, the Golgi apparatus. This is similar to the sorting centre of the factory where the proteins are packaged and sent for distribution based on the associated sorting signal, like an address. This occurs in little 'bubbles', or vesicles, made of lipids and proteins, which can merge with membranes, expelling their contents. This can be to the outer membrane so that the proteins are released into the surrounding area, to other organelles inside of the cell for a specific function, or to be destroyed.



The steps in the secretory pathway in a typical cell

Oxford Brookes University houses a whole group of plant cell biologists in the Department of Biological and Medical Sciences and we are very interested in finding out more about the structure and function of these organelles. I am researching a family of proteins called reticulons, known to shape the lipid membranes of the ER into tubules. To find out about the details of these structures which are invisible to the naked eye we use a range of different microscopes and techniques.



A generalized reticulon protein and how it is thought its topology may be in a lipid bilayer a membrane. The wedge like shape is what is thought to force the membrane to curve.

How can we find out more?

An optical (light) microscope is made up of multiple lenses, like magnifying glasses. Light passes through the specimen, is collected by the lenses, focussed and magnified into an image we can see.

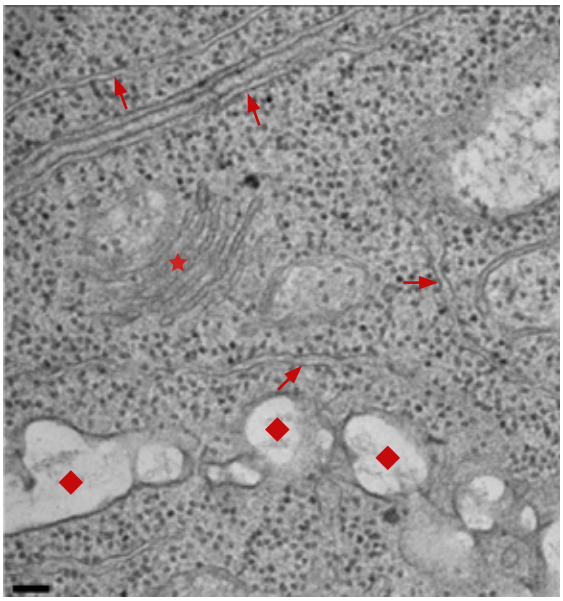
Since the beginning of microscopy it has moved far past the limitations of light microscopy and allowed us to see with great clarity the structural organization inside cells and even inside organelles. Powerful electron microscopy (EM) combined with other modern scientific techniques such as immunocytochemistry (the use of 'probes' to label specific molecular targets) allows us to see the precise place where specific proteins are located in a sample.

There are two types of electron microscope, transmission which can have a magnification of up to one million times, and scanning, which can magnify up to two hundred thousand times (TEM and SEM). Scanning electron microscopes look at larger 3D samples and scan an electron beam across the surface. The scattered electrons are used to create images of the surface detail of the sample.

I use transmission electron microscopy to look at the structure of the ER in my research. Firstly the samples need to be prepared by fixing the cells using chemicals. This keeps the structures preserved as they are at the time when they were 'fixed' in place, maintaining the shape of the organelles so they do not degrade. The cells are then embedded in resin that is hardened, so that thin sections can then be cut. The sections I usually cut of the cells are one one-thousandth of a millimetre thick and to do this I use a small diamond knife.

The sections are then placed on tiny grids to support them and placed inside the microscope for viewing and taking images to study. This entire process takes about a week to complete. The final information collected by the microscope depends on the density of the different parts of the sample and its interaction with the electrons. Therefore the images produced are black and white and not coloured.

A transmission electron microscope



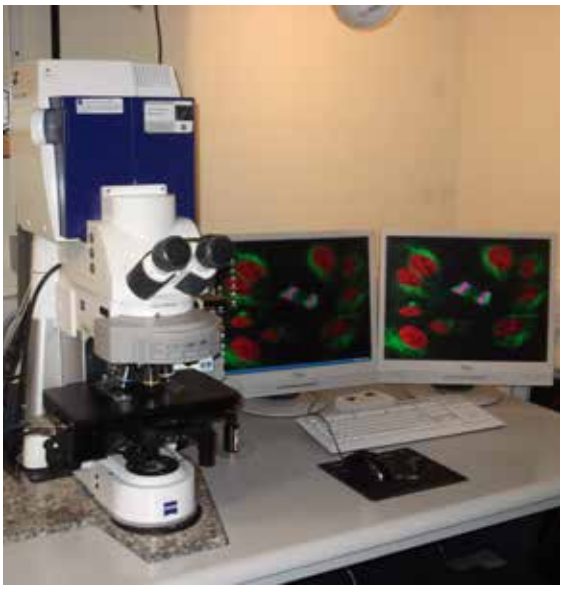
An electron micrograph of Nicotiana tabacum (tobacco) suspension culture cell showing a Golgi apparatus (star), ER tubules (arrows) and material forming the new cell wall in a dividing cell (diamonds). Scale bar = 100 nm

Electron microscopes, for all their power and the detail they reveal, have one major limitation due to the fact that they use electrons inside of a vacuum. This means that only dead cells can be studied and we cannot see how proteins move or interact over a period of time. I am interested in what happens to the proteins I am researching when the cells divide and how their distribution changes. I especially want to see what happens during the stage when a new cell wall forms between the two daughter cells.

Confocal microscopy

Fortunately we have another type of microscope with which we can also view living cells at a high resolution, although not to the extent that we can with electron microscopes. Fluorescence microscopes allow us to see fluorescent molecules such as different stains which bind to different parts of the cell, enabling us to see them clearly. A more advance system is known as a confocal laser scanning fluorescence microscope and is in essence a much further developed light microscope.

These microscopes enable us to look at different types of proteins in live cells at a magnification of up to 5000 times. We can not only see where in the cells the proteins are located but also how they move and interact with organelles in the cell. Using lasers we can produce light of different wavelengths and excite specific molecules in the specimen to produce fluorescence. The fluorescent molecules are excited by the wavelengths of light specific to them and then emit another wavelength of light which we can detect. The results are imaged on a computer which is linked to a detector in the microscope.

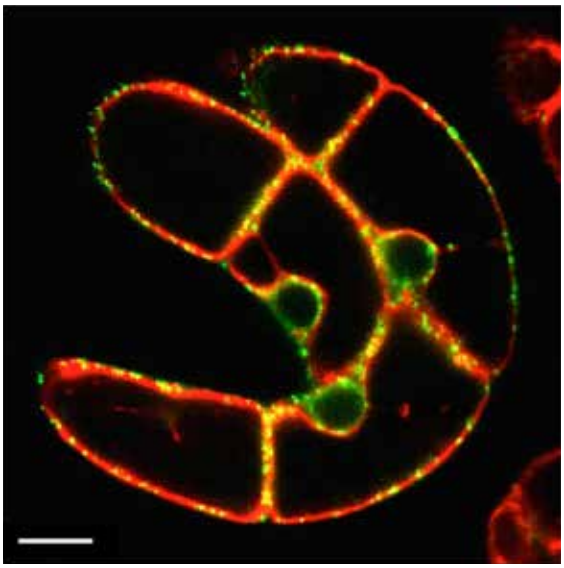


A confocal microscope in use to look at plant cells

I use confocal microscopy in my work to image cells which contain a piece of modified DNA with a portion containing the code for the protein I am interested in attached to the code for a fluorescent protein. Then, when I look at the cells under the microscope, I select a wavelength that excites the proteins produced from this DNA code and wherever the fluorescent proteins are they are excited and fluoresce.

As the fluorescent proteins are attached to the proteins I am studying, I know that the fluorescence indicates where those proteins are. Also, I know that the brighter the fluorescence in a certain area of the cell, the higher the number of my proteins in that location. From this I can look at how the distribution of protein changes as the cells undergo division and try to figure out how this is important to the functioning of the cell.

Plant Cells	Animal Cells
Have cell wall so stronger but less flexible than animal cells	No cell wall
Have vacuoles, membrane bound sacs which store various substances. They also contain water in variable amounts which can alter the rigidity of the cell	No permanent large vacuole
Have chloroplasts which trap light energy which is used to make food such as sugars. The chloroplasts also give off all the oxygen which is in the atmosphere.	No chloroplasts



A confocal micrograph of Nicotiana tabacum (tobacco) suspension culture cells expressing the Golgi marker ST-GFP (a green fluorescent protein fused to sialyltransferase). The plasma and vacuolar membranes are stained red with the dye FM4-64. Scale bar = 20 µm

3D and video

Prior to photographic technology and computer imaging, cells could only be viewed under the microscope by eye and diagrams drawn to record the data. With modern technology we can not only record individual photographs but also reconstruct 3D images and record live video of the movements of the fluorescently-labelled proteins inside the cells. We can also use stains to label larger structures such as entire organelles or their components, instead of individual proteins.

So next time you wander across a field try, to imagine the complexity of the billions of cells making up all of the living things that you see around you.

Petra Kiviniemi is a cell biologist currently working towards a PhD as a postgraduate researcher at Oxford Brookes University. She is investigating a family of proteins called reticulons in plant cells using a variety of cell imaging and molecular techniques.

Look here!

Some fascinating images made using a variety of microscopes: *http://www.nikonsmallworld.com/*

David Sang

Newly-built passive houses in Lockerbie, Scotland

Passive House
Low-energy buildings

Key words
energy consumption
heating
insulation
buildings

Heating accounts for over half of the energy used in homes in the UK. In this article, we look at buildings designed to cut heating demand almost to zero.

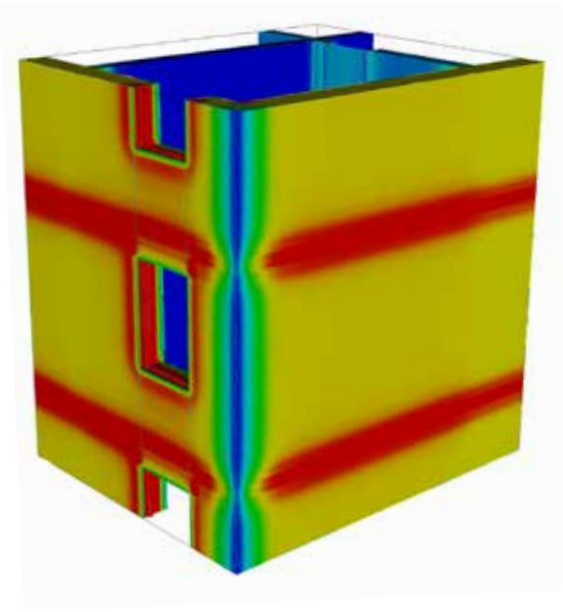
There are over 70 000 **passive houses** in Europe – just a few of them in the UK. To be described as 'passive', a house must meet strict criteria for energy use. In particular, its demand for heating must be less than 15 kWh/m^2 per year. Let's look at what this means.

1 kWh is 1 kilowatt-hour, a unit of energy. These are the units clocked up by an electricity meter. Big houses need more heating, so the floor area must be taken into account. A passive house with a floor area of 100 m^2 would need 100 ´ 15 = 1500 kWh of heating in one year. Compare this with the average energy consumption of 18 000 kWh and you will see what a demanding target this is.

Insulation

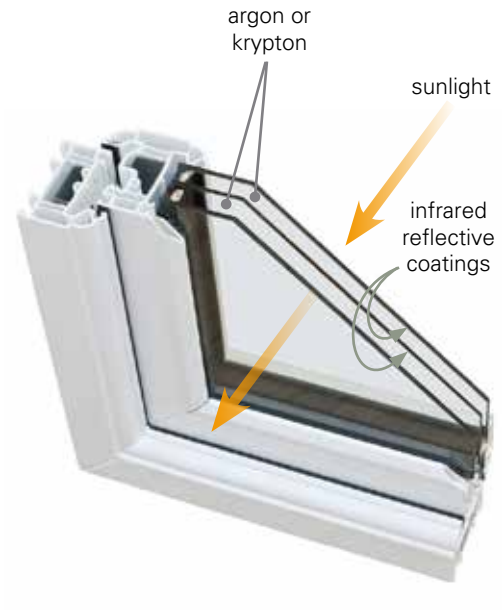
Of course, good insulation is essential in a passive house. However, there is more to this than thick walls and triple glazing. Passive house designers look for thermal bridges – any points where heat can conduct out of the building. These include window and door frames which must be insulated over, and points where walls meet floors and ceilings.

A typical newly-built house in the UK has a heating demand of 120 kWh/m^2, 8 times that of a passive house.



This computer model shows thermal bridges – points where a house will lose energy most quickly.

Triple glazed windows are made of three sheets of glass. The spaces between are filled with argon or krypton, noble gases which are poor conductors of heat. The glass lets in light from outside and this contributes to the energy supply of the house. The inner surfaces of the glass are coated with a layer that reflects infra-red radiation back into the house.



The uPVC frame of a triple-glazed window is designed to minimise heat loss.

Ventilation

Most people enjoy a sense of fresh air in their homes. But when warm air leaves a building, it takes energy with it. There are two ways this can be reduced in passive house design.

Firstly, draughts and leaks must be minimised. With windows and doors closed, the building is pressurised to 50 Pa above the outside air pressure and the rate of air flow outwards is measured. For a passive house, less than 0.6 times the air volume of the house must escape each hour.

Secondly, a mechanical ventilation heat recovery (MVHR) system must be used. Outside air is gently blown into bedrooms and living rooms. Air is drawn out of bathrooms, toilets and kitchens (where it is more likely to be damp and smelly).

To avoid heat loss, the MVHR system includes a heat exchanger. Warm air leaving the house passes through tubes; its energy conducts through the walls of the tubes to warm the cooler incoming air. In this way 75% of the energy in the warm air can be recovered.

What the residents say

A passive house costs 5-10% more than a conventional low-energy house but the savings in fuel bills and the protection from future price rises makes the expense worthwhile in the long run.

Some people fear that the ventilation system will give a claustrophobic feel or produce a constant draught, but this is not so. And you can always open the windows.

Each person in a house contributes about 100 W to the heating, so invite your friends round and save on your fuel bills!

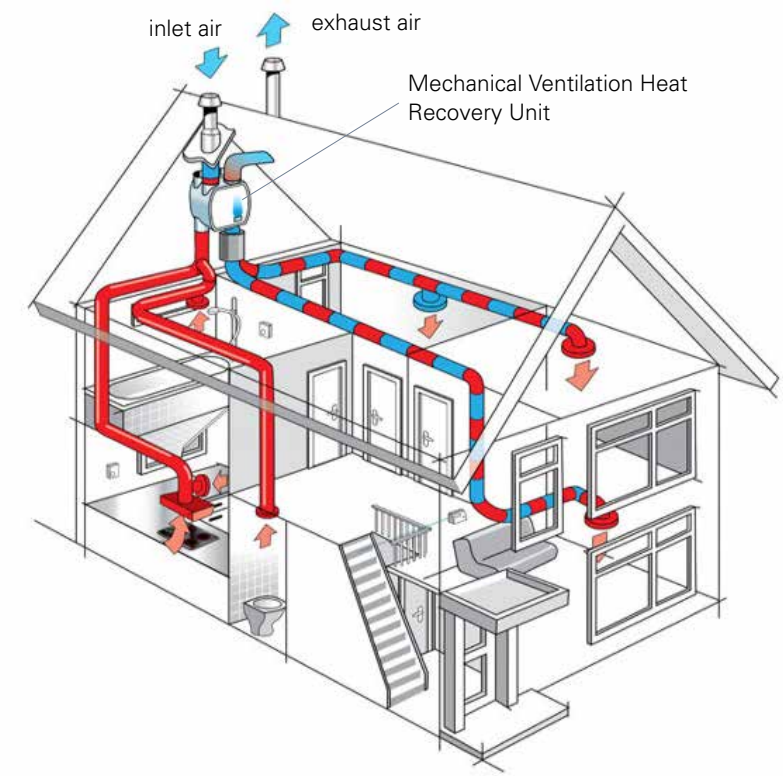
David Sang is Physics editor of Catalyst.

Units of energy and power

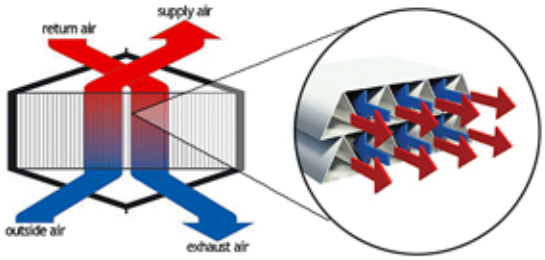
The watt (W) is the unit of power, the rate at which energy is being supplied. One watt is one joule per second.

The kilowatt-hour is a convenient unit of energy. A 1 kW appliance running for 1 hour uses 1 kWh of energy. (1 kWh = 3.6 million joules)

Building materials (including windows and doors) are rated according to their U-value. The walls, roof and floor of a passive house would have a U-value of less than 0.15 W/m^2 °C (watts per square metre per degree C). This means that, for a temperature difference of 1°C between inside and outside, energy will flow out at the rate of 0.15 W through each square metre.



Living spaces have fresh air pumped in (red/blue), while warmer, damper air is pumped out (red).



In the heat exchanger, energy from the warmer exhaust air is transferred to the incoming supply air.

Look here!

The Scottish Passive House Centre: www.sphc.co.uk

The Channel4 TV series Grand Designs has shown several low-energy and passive house projects. Check their archive.

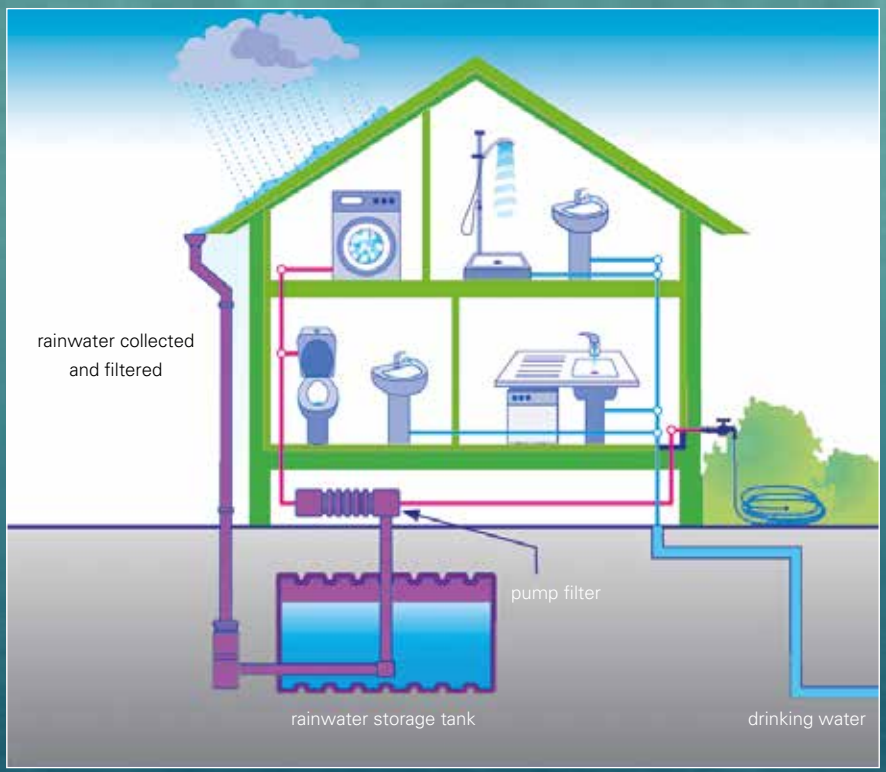
The back page of this issue of Catalyst shows a passive housing scheme in southern Germany.

PASSIVHAUS

In the German city of Freiburg, a series of apartment blocks has been built to meet passive house standards. Cars are excluded, making it possible for children to play safely outdoors. Some of the other environmentally friendly features are shown below.

The south-facing side of the blocks has big windows to allow sunshine to enter. The north side has smaller windows.

The energy of sunlight is used to heat water and to generate electricity.



Why use drinking water to flush your toilet and wash your clothes? It takes energy to purify water to a drinkable state, so rainwater is a better choice. Sewage is sent to a biogas generator.

Each block has a co-generator which burns fuel to produce electricity; waste heat is used to heat water, stored in the red tank.