Messenger

A high-resolution visualisation of the Ion Channel Switch developed by the CRC for Molecular Engineering.

TODAY . . . You turn up at the hospital with chest pain. Is it indigestion, or something more serious?

An electrocardiogram yields an indefinite result. No chances are taken. Immediate therapy with substance 'X' begins, at $1500 a dose. Extreme care is taken, because this drug can be fatal, as well as lifesaving. Your life is in danger, perhaps from a heart attack, perhaps from a medicine you don't need. Nobody knows for sure yet, they're just playing percentages.

THE FUTURE . . . You turn up at the hospital with chest pain. The nurse takes a drop of blood and applies a small desktop instrument. The instrument is a biosensor. It evaluates the levels of several key proteins and enzymes in your blood. In five minutes, the results are available.

A heart attack has begun, but the prognosis is good. Therapy is begun in earnest, but the risk is low. Your body's metabolic response to the drug being administered is being measured in real time via more drops of blood being analysed by the same instrument.

This scenario describes just one of the countless potential uses for the biosensor technology being developed by the Cooperative Research Centre for Molecular Engineering and Technology. More information is available on the Internet at www.ambri.com.au/ institute/technology/index.html.

membranes

Graeme O'Neill introduces a microscopic membrane custom-built to detect molecules of almost any organic compound. Health care, DNA analysis and environmental and defence monitoring are among its potential uses.

As you read these words, tides of sodium, potassium, calcium and chloride ions are surging into and out of active neurons in your brain, through tiny molecular gateways called ion channels.

Without ion channels, there would be no life. When nature walled off the first prototype cells inside lipid membranes about four billion years ago, ion channels maintained essential lines of communication and supply with the external environment.

Ions (charged atoms) flowing through ion channels cause tiny but measurable voltage changes between the cell's interior and exterior. A decade ago, researchers at CSIRO decided to exploit this phenomenon to develop the ultimate biosensor – a device with moving parts that are only molecules in size – capable, in theory, of detecting individual molecules of almost any organic compound.

Since 1992, the project has operated from the Cooperative Research Centre for Molecular Engineering and Technology in Sydney. The centre was formed by CSIRO and the University of Sydney, and the Australian companies Pacific Dunlop and AWA.

In May this year, the centre announced it had developed a biosensor prototype that could detect the change in voltage associated with molecular interactions on the surface of a synthetic membrane. The interactions could be an antibody latching onto an antigen, or a DNA probe zipping itself to its alter-ego nucleotide sequence in a DNA sample.

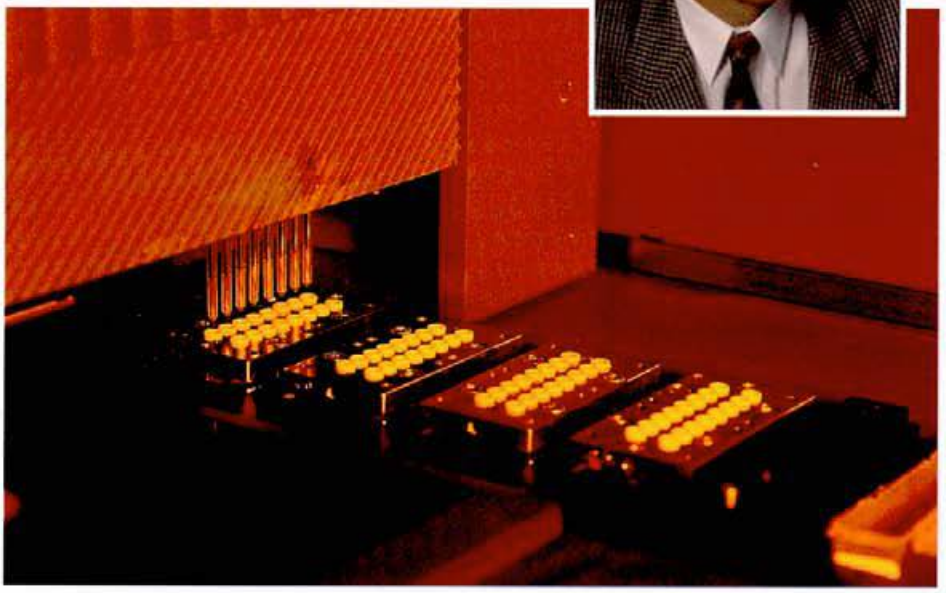
Director of the centre, Dr Bruce Cornell, says the biosensor can detect inter-molecular events at what he calls 'the last jumping off-point before you vanish into the quantum world' and report them to computers or other electronic devices.

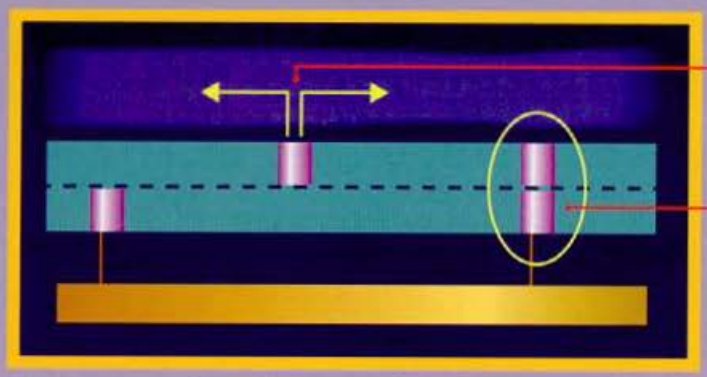
'This biosensor is a unique blend of the ability of biology to identify individual types of molecule in complex mixtures, with the efficiency and low cost of microelectonics,' Cornell says.

'It consists of a synthetic membrane that we make ourselves, chemically tethered to a thin metal film coated onto a piece of plastic. This membrane behaves like the outer skin of the cells of the human body in its ability to sense other molecules.

'When the membrane detects its target molecule, it turns these currents on or off by opening or closing molecular channels that pass through the otherwise insulating membrane.'

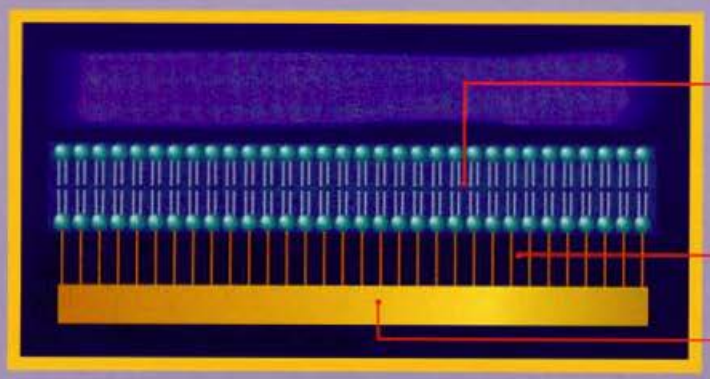
Right: Director of the Centre for Molecular Engineering and Technology, Dr Bruce Cornell: 'The biosensor can detect molecular events at the last jumping off point before you vanish into the quantum world.'
Below: Automated assembly of biosensors.



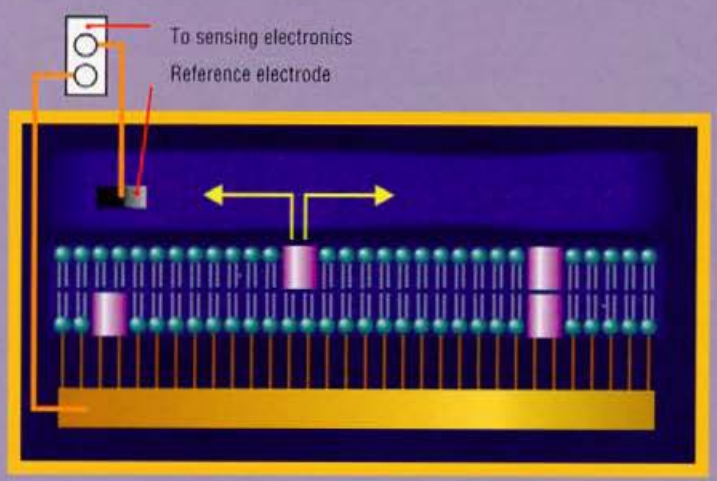


The biosensor can detect the presence of specific particles, and signal their presence by triggering an electrical current. It combines two, nanoscale building blocks: a sliding switch and an electrode.

The sliding switch (above) uses two halves of a molecule which create a channel through which charged particles can flow. When the two halves line up, the switch is 'on', and the ions flow. When the two halves are separated (by sliding sideways), the ion flow is cut, and the switch is 'off'. The two halves of the molecule move in the upper and lower layers of a membrane.



The electrode has a miniature reservoir formed between its surface and a biological membrane made from two layers of lipids. The 'bottom' layer of lipid is attached to the electrode by linking molecules. A slice through the membrane is illustrated below.



Super sensitive

When announced in the June 5 edition of *Nature*, news of the biosensor made international headlines. Cornell describes the sensitivity requirements in the state-of-the-art human diagnostics business as equivalent to detecting the increase in sugar content of Sydney Harbour after throwing a sugar cube from a ferry.

'The molecular sensor is vastly superior to the current generation of enzyme electrodes,' Cornell says. 'While our nearest competitor is operating at the millimolar level, we can detect compounds at sub-picomolar concentrations. That's a thousand million times more sensitive. In theory, it can detect a single molecule of a target compound.'

Such feats are possible because 1000 sensors can be fabricated along a line just two millimetres long on a silicon chip. That means a million sensors can be packed into a 2 mm array, forming an ultra-fine net to trap single molecules from solution. In addition, a range of different sensors could be arrayed on a single chip, so that measurements of a number of compounds could be performed in parallel.

'You get a very quick answer, because you don't have to wait for a molecule to diffuse through a solution and find an isolated electrode. You can set a lot of hooks to catch a few fish,' Cornell says.

The extensive laboratories at The University of Sydney have been used in scaling up the chemistries of the biosensor.

The biosensor's exquisite sensitivity derives from its inherent ability to amplify the vanishingly small signals created by detection events. When the detection event disrupts a channel, a million ions suddenly stop flowing, creating a signal that is readily detected in an external circuit.

In addition, Cornell says the three-dimensional shape-matching reactions that occur between antibodies and antigens, or between complementary DNA molecules, are much more avid than reactions between enzymes and their substrates, making them inherently more sensitive, and thus detectable at lower concentrations.

From defence to disease detection

What use is the biosensor? Cornell says the list of potential applications is enormous. If one participant in a highly specific biochemical reaction has been identified, you can plug it into the biosensor, and it will seek out its partner.

Many potential applications exist in the human health care and diagnostics industries.

'You can take a drop of blood, place it on a biosensor and it will measure the compound you are interested in: a protein, an enzyme, a hormone, an antibody, therapeutic drugs, toxins, or infectious agents such as bacteria and viruses,' Cornell says.

'Selected DNA sequences can be plugged into the biosensor to detect mutations associated with cancers or genetic disease.

'Hybridisation will become a dominant component of the diagnostics market over the next 10 years, and we are tracking the Human Genome Project because our device would be very useful for detecting polymorphisms (natural variations) in genes.'

Cornell says the biosensor also could aid the search for new pharmaceuticals. It could be used to screen protein libraries from plants or animals for compounds with potential therapeutic activity.

'We also see it being used in a wide range of veterinary applications,' he says. 'And it could be used to monitor water and air pollution, in drug-detection of athletes, or the military could use it as an early warning device for biological or chemical warfare agents.'

Borrowing nature's design

TO CONSTRUCT the biosensor, the researchers copied several of nature's inventions. They began by using sulfur atoms to bond an artificial lipid bilayer – a synthetic membrane – to a substrate coated with an ultra-thin layer of gold to form an electrode (see diagrams opposite).

The artificial membrane is studded with synthetic ion channels, based on the design of a natural ion-channel made of a polypeptide called gramicidin A, found in the common soil-dwelling microbe *Bacterium brevis*.

Each synthetic ion channel is tethered by its tail to the gold electrode; its head can be attached to a range of receptors such as antibodies, small DNA sequences or cell-surface receptors, which project from the outer surface of the synthetic membrane.

If the antibody finds the antigen it is seeking – or the DNA probe detects a matching nucleotide sequence – the binding reaction tugs at the synthetic gramicidin molecule, preventing contact between two segments of the molecule (one in the inner layer of the membrane, the other in the outer layer).

The channel normally serves as a conduit for ions in a saline solution that bathes both sides of the membrane. While the channel remains open, ions flow, maintaining an external electrical circuit between the gold electrode and another electrode in the saline solution above the membrane. But when the ion channel is disrupted, halting the flow of ions, the event registers as a change in the external current.

If the gold electrode is integrated into a micro-electronic device on a silicon wafer, the circuit-breaking event can be relayed directly to a computer or other electronic instrument, which registers and records a detection event or 'match'.

A secret well kept

The biosensor research was conducted amid extraordinary security measures. Everyone involved in the project was sworn to secrecy and researchers forsook the right to publish their results in the scientific literature.

Even outsiders brought in to review the research were required to sign secrecy agreements. Only now are the researchers involved beginning to publish details of their work in the scientific and technical literature.

Cornell says the partners have invested $35 million in the project in the past decade, and the centre now has a large portfolio of patents in Australia, the United States and Europe that costs up to $250 000 million annually to maintain.

'It has been an interesting social experiment,' he says. 'Australian science traditionally has had a policy of open publication, but it's difficult to see how we could have gone forward as we have, gaining the interest of our commercial partners, had we released everything and had no proprietary knowledge to patent.'

The centre's biosensor has been operating successfully for several months in the laboratory and commercial products are expected to be launched in a couple of years.

abstract

The Cooperative Research Centre for Molecular Engineering and Technology has developed a biosensor prototype that can detect the change in voltage associated with molecular interactions on the surface of a synthetic membrane. Potential applications for the device exist in health care and diagnostics industries, environmental monitoring and in defence. The CRC has a portfolio of patents in the Australia, the US and Europe.

Keywords: Biosensors; Molecules; Synthetic membranes; Research