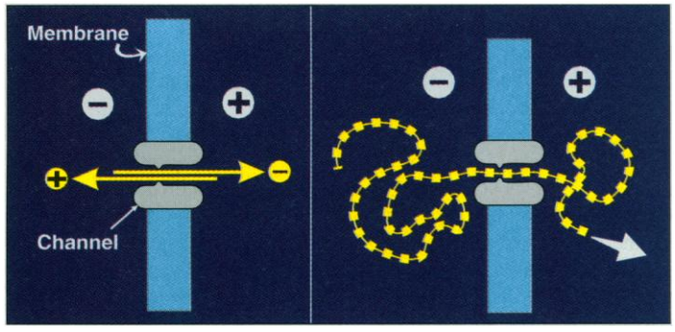
MEETING BIOTECHNOLOGY INDUSTRY ORGANIZATION

From the Bioweapons Trenches, New Tools for Battling Microbes

SEATTLE, WASHINGTON—Some 5700 biotech buffs gathered here from 16 to 20 May for the annual meeting of the Biotechnology Industry Organization. Most sessions focused on business, but one drew big interest from scientists: a showcase of projects funded by the U.S. Defense Advanced Research Projects Agency's antibioterrorism initiative.

Warp-Speed DNA Sequencing

Star Trek fans know that a tricorder can sniff out and identify molecules or pathogens in a matter of seconds. With such a device, officials could identify pathogens quickly enough to save lives during a bioterrorist attack, and physicians could rapidly type tuberculosis strains, for example, to determine which combination of drugs would work on which patient. Unfortunately, current techniques for rapid DNA decryption—using electrical separation of nucleotides or mass spectrometry (*Science*, 27 March 1998, p. 2044)—don't come close. "We need something that's off the wall, an approach to sequencing that's completely different from the way we think of doing this now," says chemist and gene sequencer Richard Mathies of the University of California, Berkeley.



Tomorrow's tricorder? A novel bacterial ion channel, inserted in a membrane, can quickly decipher DNA sequences.

Just such a wild idea was aired at the meeting by cell biologist Daniel Branton of Harvard University. He and biophysicist David Deamer of the University of California, Santa Cruz, have built a device that can read nucleotides from single strands of DNA as they pass through a well-studied bacterial ion channel called α-hemolysin.

The duo embedded the 1.5-nanometer-wide channel in an artificial membrane that splits a buffer-filled chamber. A voltage across the membrane keeps the pore open and pulls DNA—which carries electrical charge—through. Double-stranded DNA is too wide to fit, but the molecule's strands unzip and zip up at various spots all the time. Like a frazzled sneaker lace jammed into an eyelet, single-stranded DNA segments pass through the pore and the rest of the molecule unravels. As each nucleotide crosses the opening, it produces a characteristic dip in current. So far the researchers have tried discerning only long stretches of the same nucleotides, such as 30 adenines followed by 70 cytosines.

Mathies is impressed by the early results. "They are on to something very interesting," he says. Getting the machine to work for real DNA, however, will require impeding the strands so they pass through a pore more slowly. Right now they whip through at about a base per microsecond—so quickly that several nucleotide signatures blur together. Branton estimates that they will have to be slowed to about a millisecond per base for the signature of each nucleotide to register separately. One strategy is to add short pieces of single-stranded DNA, which bind temporarily to the DNA of interest, to the buffer on the input side. A string of deoxythymines, for example, slowed the transit of a 100-nucleotide stretch of deoxyadenines from 320 microseconds to 4400 microseconds.

As they work on DNA braking techniques, Branton and Deamer are also each developing ways to insert the α-hemolysin pores into materials more durable than membranes that could be easier to scale up for commercial use. To gouge narrow holes, Deamer and his Santa Cruz colleagues have turned to a 30-year-old technique called the nucleopore method, which relies on highly energetic fission products from uranium or californium to break bonds in a target material, in this case mica. A brief wash with hydrofluoric acid removes the defects, leaving 6-nanometer-wide holes in the mica. "While 6 nanometers is too big for sequencing, we may be able to use such a structure to provide a robust support for the α-hemolysin pore," said Deamer.

Branton's Harvard team, meanwhile, has taken a different tack. By modifying a standard lithographic technique, they build up cones of silicon nitride on a scaffold, then cover the cones nearly to their tips with a proprietary silicon-based material. Using a laser to remove the silicon nitride leaves tiny, round pores, which Branton's group has shrunk to about 4.5 nanometers wide. He believes that the 2 nanometers or so needed to sequence DNA is within reach.

The researchers predict that a single device with 500 pores—snipped from a bacterium or punched into silicon—would be enough to sequence a bacterial genome quickly. The plan, then, would be to use standard restriction enzymes to cut up a target genome (DNA or RNA) into 500 or so large pieces, with software available today quickly assembling the sequence data and identifying the microbe at hand. At an expected throughput of 1000 bases per second per pore, such a device could sequence a bacterial or viral genome in under a second—making the tricorder dream a reality.

Lobbing Nanobombs at Pathogens

"What do you do to an F-16 that's been contaminated by anthrax?" goes an old military joke. Answer: Crash land it on the enemy, because the severe measures for cleaning up the killer bacterium—and popular bioweapon—would turn the plane into an oversized paperweight. Today, the only practical methods to destroy anthrax spores are to incinerate them or to blast them with bleach and formaldehyde. Neither approach leaves an airplane's electronics—or contaminated personnel—in working order.

A more gentle solution may be a unique emulsion of oil, water, and two common lab detergents that form nanometer-sized droplets capable of fusing with and destroying not only tiny anthrax spores, but also other gram-positive bacteria, gram-negative bacteria, and most viruses. "We've basically created nanometer-sized bombs that attach themselves to and blow up most every pathogen known to man," said James R. Baker Jr., an immunologist and director of the Center for Biologic Nanotechnology at the University of Michigan. Influenza and Ebola—one of the most lethal known viruses—are dead within minutes after being doused with the mixture.

Experts say the emulsions could become a valuable addition to a growing arsenal of antimicrobial substances, including classes of peptides such as defensins and magainins,

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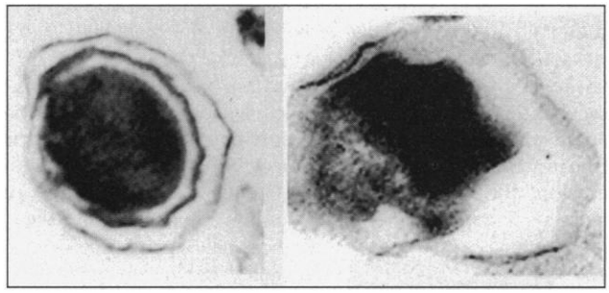
that kill by rupturing cell membranes. Trial and error led Baker's team to two formulations that demonstrate potent antimicrobial action. An emulsion including the detergents Triton X-100 and tributyl phosphate took out gram-positive bacteria and the vast majority of viruses sheathed in protein envelopes. (Naked RNA viruses are not susceptible, because they have no membrane for the emulsions to disrupt.) A second preparation killed a different spectrum of bugs: gram-negative bacteria and fungi. Combining the armaments yielded a potent killing machine. Exposing a variety of fungi, bacteria, and enveloped viruses to a 1000-fold dilution of the double-barrel emulsion for 15 minutes annihilated the life-forms, the researchers concluded from the absence of colonies in suitable growth media.



Decommissioned. *Bacillus cereus* spore, about 1 micrometer in diameter, before (left) and 4 hours after exposure to new emulsion.

To Baker's initial surprise, the emulsions proved effective against bacterial spores—a form of suspended animation in which the bacteria produce a hard protective coat—which tend to resist all but the harshest chemicals. "It appears that the oil acts as a nutrient that tricks the spores to start producing cell membrane, a process that the emulsions disrupt quite easily," said Baker. In one experiment, he and his colleagues infected skin wounds on mice with spores of *Bacillus cereus*, a cause of food poisoning and severe infections. One hour later, the wounds were rinsed with either a 10% solution of the two emulsions or with salt water. The wounds in the treated mice healed, while those in the control animals festered.

Sperm and red blood cells are the only animal cells that Baker's team has found to be susceptible to the emulsions. Other cells are studded with carbohydrates that appear to somehow prevent the emulsion droplets from fusing to the cell membrane. This gentleness is a nice surprise considering that membrane-disrupting antimicrobial peptides have shown unexpected toxicity in animal tissues, says microbiologist Jill Adler Moore, director of the Institute of Cellular and Molecular Biology at California State University in Pomona. The bottom line, says Baker, is that the emulsion mixture is a drug candidate mainly for external uses, such as for treating skin ulcers.

This expectation will soon be put to the test. The National Institute of Child Health and Human Development in Bethesda, Maryland, is planning a clinical trial to see if the emulsions will work as a vaginal contraceptive cream that wards off sexually transmitted diseases. And the U.S. military intends to try to detoxify contaminated equipment by hosing it down with the emulsions, a procedure that could save the equipment from becoming expensive scrap—or paperweights. **–JOSEPH ALPER**

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NEUROBIOLOGY

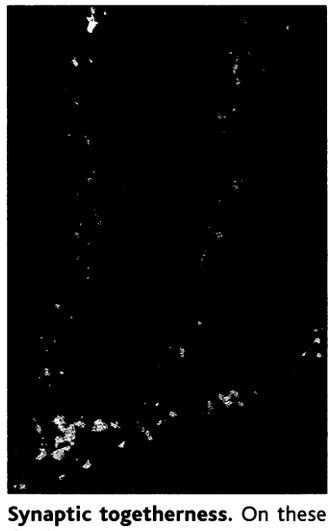
New Clues to How Neurons Strengthen Their Connections

New results point to the AMPA receptor for glutamate as playing a key role in the changes underlying long-term potentiation in brain neurons

Neurobiologists who study how the brain adapts and learns have long known that synapses—the specialized regions where one neuron receives chemical signals from another—are where the action is. For example, learning seems to be associated with an increase in the strength of those synaptic connections. Now, three teams—two of which report their results in this issue of *Science*, while the third published in the May issue of *Nature Neuroscience*—implicate a new player in the biochemical changes underlying a type of synapse strengthening known as long-term potentiation (LTP).

The neurons that undergo LTP respond to the neurotransmitter glutamate. Their synapses contain two kinds of glutamate receptors, but researchers studying LTP have largely focused on the one known as the NMDA receptor. That's because glutamate binding to this receptor is the first step in LTP. Exactly what happens after that is unknown—and the subject of fervent study and debate. The new work fingers the other, less famous glutamate receptor, the AMPA receptor, as a player in those synapse-strengthening events.

Previously, neurobiologists had thought that AMPA receptors are present at relatively unchanging levels in the vast majority of synapses on glutamate-sensitive neurons. But that no longer appears to be the case. Two of the teams, led by Roberto Malinow of Cold Spring Harbor Laboratory on New York's Long Island and Robert Malenka at the University of California, San Francisco, show that AMPA receptors move into and out of synapses as synaptic connections strengthen and weaken. The third team, led by Peter Seeburg and Bert Sakmann of the Max Planck Institute for Medical Research in Heidelberg, Germany, provides indirect evidence that the movements are needed for LTP to occur. Taken together, says Richard Huganir, who studies receptors at Johns Hopkins University School of Medicine in Baltimore, the results give "incontrovertible evidence" that "the regulation of AMPA receptors in general is going to be very key" to modulating synapse strength.



Synaptic togetherness. On these dendritic spines, AMPA receptors are stained green, NMDA receptors are red, and synapses where both are present are yellow.

The current findings are also likely to influence a long-standing debate over whether the changes that occur in LTP take place postsynaptically, that is, in the cell that receives the signal, or presynaptically, in the cell that dispenses it. They imply that at least part of the changes are postsynaptic. Ironically, however, the new findings trace back to an experiment done 9 years ago that was long viewed as strong evidence for presynaptic change.

At that time, the NMDA receptor had already been implicated in LTP, a task for which it is remarkably well suited. Brain neurons usually have thousands of synapses for

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