New Method of DNA Testing Promises to Transform Medical Diagnostics

Single Approach Easily Adapted to Probe for All DNA-Substances Affords Significant Diagnostic Cost Savings

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Santa Barbara, Calif.-- Researchers at the University of California at Santa Barbara (UCSB) report in the *Proceedings of the National Academy of Sciences* a new method for detecting DNA, which could transform medical diagnostics. Currently, tests for the presence of DNA--to identify, for instance, the presence of a bacterium such as anthrax, or a virus, or a specific gene--require that the DNA be amplified or grown. The UCSB researchers combine the use of a light-emitting polymer with peptide nucleic acid (PNA) probes to make a test so sensitive that the costly DNA amplification can be reduced and perhaps eliminated.

The article "DNA Detection Using Water-Soluble Conjugated Polymers and Peptide Nucleic Acid Probes" appears in the Aug. 5 on-line edition of the *Proceedings* and in the Aug. 20 print version.

The authors are Brent Gaylord, a graduate student in the Materials Department; his adviser, chemist Guillermo Bazan; and physicist Alan Heeger. Both Bazan and Heeger also hold joint appointments in materials.


Prof. Guillermo Bazan

Heeger won the 2000 Nobel Prize in Chemistry "for the discovery and development of conductive polymers." Before the work of Heeger and his co-recipients, polymers -- a type of long skinny carbon-based molecule shaped with repeating units like beads on a string -- were thought of preeminently as insulators -- i.e., the plastic casing for the electron-carrying wires that run from lamp to wall socket. For a polymer to be conducting or light-absorbing and emitting, it has to alternate single and double bonds along the backbone of the polymer, which is called "conjugation," and the resultant polymer is described as "conjugated."

Bazan and his student Gaylord and other researchers have recently made light-emitting polymers soluble in water by attaching to the long polymer molecule via little molecular side chains a charged group, which behaves like a soap, thereby enabling the polymer to be dissolved in water.

Bazan and Gaylord made a water-soluble conjugated polymer that emits blue light when irradiated with ultraviolet light. Put the polymer in water, shine ultraviolet light on the solution, and it glows bright blue. A key property is the multiple positive charges of that polymer, whose scientific name is poly(9,9-bis(6'-N,N,N-trimethylammonium)-hexyl)-fluorene phenylene.

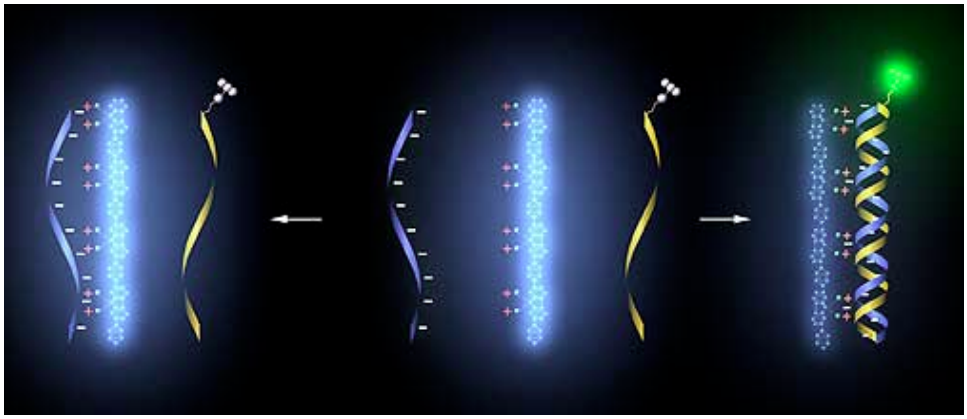
In addition to the light-emitting polymer, the other ingredient of the test is peptide nucleic acid (PNA). Understanding PNA's role in the testing method requires some understanding of what is being tested for--the DNA.

We are accustomed to think of the DNA molecule as a double helix with two strands of DNA that come

together via specific base pairing rules for DNA's constituent four types of nucleic acids whose sequential pattern determines the genetic code. Note that the DNA molecule is similar to a polymer molecule in that both are long chains of repeating units--repeated in the polymer exactly and patterned in DNA via the four-element code. DNA is negatively charged.

The testing method focuses on a small signatory piece of the DNA strand. The question the test asks is whether that small piece--characteristic of, say, anthrax and only anthrax--is present in the solution or not.

PNA is a synthetic analogue of DNA, which replaces the phosphate sugar backbone of DNA with a peptide backbone. "Peptides" are the molecules out of which proteins are made. PNA, which is soluble in water, is neutral--neither positively nor negatively charged. One strand of PNA comes together ("hybridizes") with one strand of DNA more readily than do two strands of DNA with each other because each of the DNA strands is negatively charged, thereby causing each to repel the other slightly.



The test for a specific type of DNA (whether anthrax, tuberculosis, or breast cancer gene) is either positive (far right) or negative (far left). In the center are the components of the new DNA testing method: the light harvesting and positively charged conjugated polymer is shown in blue; to its immediate right is a specific neutral PNA sequence (gold ribbon) topped with a small fluorescent dye molecule (gray); and to the polymer's immediate left is the DNA (single blue strand) in the sample being tested. At far right, if the negatively charged DNA complements the PNA, they hybridize (gold and blue intertwined ribbon); the negatively charged DNA/ PNA associates with the positively charged polymer; and the little fluorescent molecule atop the intertwined blue-gold ribbon glows green; the test is positive. At far left the test is negative because the PNA (yellow ribbon) does not complement the DNA being tested for. Although the negatively charged DNA is attracted to the positively charged polymer, the PNA does not match the DNA, so the little molecule atop the PNA does not turn on.

For the purposes of the testing method the PNA is tailor-made to come together with whatever DNA is being sought. Attached to the PNA is a small fluorescent molecule. If the DNA has the complementary code of the PNA, the two strands come together. The resultant pairing will be negatively charged overall due to the negative charge of the DNA fragment.

The negative PNA-DNA pairing will be attracted to the positively charged conjugated polymer. When that happens, the little light attached to the PNA tail goes on, and the test is positive for whatever DNA is being sought. If the light doesn't go on, the DNA is not present, meaning anthrax is not present, tuberculosis is not present, a breast cancer gene is not present.

The little fluorescent molecule attached to the PNA tail is designed to absorb the blue light from the conjugated polymer and re-emit lower frequency green light. If the substance being tested for is not present, then when ultraviolet light is shown on the solution, the tester will see blue light. Green light means the substance being tested for is there; blue light means it's not there. "This is," said Gaylord, "a very on or off diagnostic," whose results are readily evident to the technician conducting the test. Gaylord said that he had successfully used the procedure to detect the presence of a DNA sequence

characteristic of anthrax in a solution.

"The real advantage over standard protocols," said Bazan, "pertains to the use of the conjugated polymer as a light harvesting molecule. The polymer is what makes the test sensitive. It is capable of collecting a large number of photons. The ability of the polymer to absorb and emit a photon relative to the ability of that small fluorescent molecule attached to the PNA tail is huge, and that is what we call 'optical amplification.'

"The light harvesting properties of the polymer mean that we can sense the presence of what we are trying to detect at much lower concentrations," said Bazan. "The goal will be to use this invention and not have the need to grow the DNA taken from the patient, but test directly the sample taken from the patient. In the developed world, we have the ability to grow DNA and thereby to identify an illness. However, in poorer countries, it is the cost of diagnosis, not the therapeutic drugs, which often prevents treatment. This method will significantly decrease the cost of diagnostics."

Gaylord points to the advantages this method affords for detection of infectious agents over today's standard methods. "Most medical diagnostics now," he said, "operate not by DNA detection, but protein or antibody detection. For each possible infecting agent, a whole system has to be devised. A person is infected; that person's immune system makes antibodies. Somebody has had to figure out what those antibodies look like and what antigens they bind to, and then a test for detection has to be devised. Today's diagnostics detect the reaction to the invader rather than the invader itself. We have one testing method that enables direct detection of the invader, and can be readily adapted to direct detection of other invaders merely by altering the PNA. We already have huge DNA data banks; the tester just has to get the complementary PNA."

"You can buy the PNA," said Bazan. "A supply chain for these components is in place. You can't buy the conjugated polymer; we made it."

"And," said Gaylord, "multiples of little fluorescent molecules can be used to test for more than one type of DNA at the same time--each giving off a different color--to distinguish, for instance, between infectious agents with indistinguishable symptoms. And that means one test in place of two or more, and that translates into cost savings."


Prof. Alan Heeger

The University of California will own the patent on the invention by Gaylord and Bazan.

"We use the electrostatic interaction as a means of mediating energy transfer," said Bazan. "The light harvesting polymer gives our testing method the advantage; the electrostatics make it work. The beauty of this bio-recognition test, compared to what else is being done, is really in the simplicity."

"I am pleased to see new applications for conducting polymers in biology," said Heeger. "This PNA/DNA bio-sensor concept, which uses a water soluble semiconducting polymer for light harvesting, is simple, elegant, bio-specific, and sensitive. More generally, I view this progress in DNA sequence detection as another important example of successful interdisciplinary science."

The research was conducted under the auspices of UCSB's Institute for Polymers and Organic Solids. Both Bazan and Heeger are participants in the California NanoSystems Institute (CNSI), initiated by Gov. Gray Davis to foster research breakthroughs that will seed the future of the State's high tech economy.

[Note: Professor Bazan is available to discuss the research and invention by e-mail at bazan@chem.ucsb.edu and by phone at 805-886-1076.]

High-resolution versions of graphic images related to this article can be obtained by contacting Kathy Kramer