Biosensors Promise New Era in Marine Research

Each time you get a flu shot or other vaccination, you benefit from your immune system's exquisite ability to create antibodies that can recognize and bind with foreign agents-whether they're viruses, bacteria, or toxins.

VIMS researchers Erin Bromage, Steve Kaattari, and Mike Unger are now tapping the immune system's power-and the latest advances in electronic instruments-to address pressing issues in marine science.

The trio combines specially designed antibodies with digital instruments to create so-called "biosensors" that hold promise for detecting and tracking oil spills, monitoring harmful algal blooms, identifying toxins, and other, perhaps yet unrecognized, applications-all in real time.

Bromage, the project's lead researcher, says that as far as he knows, "no one else is doing this. They might have biosensors in the lab, or a sensor that someone is operating in the field, but no one else has something that can be deployed autonomously over a period of time to look for a specific target."

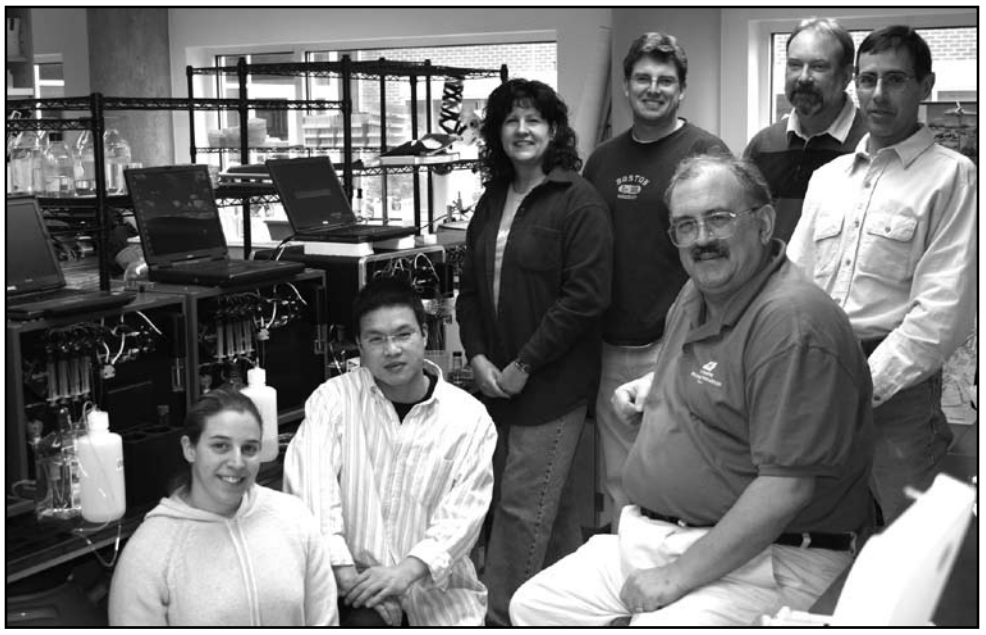
Kaattari, an immunologist, says, "We're developing antibody-based sensors with the idea of placing them on different platforms to sense the introduction and dispersal of aquatic contaminants." Those platforms include marine buoys, robotic submarines (see p. 8), and in the near future, a Buck-Rogers-like device small enough to clip on a researcher's belt.

Unger is an environmental chemist who analyzes samples using today's most high-tech instruments-mass spectrometers and gas chromatographs. He jokes that by working on the new sensors " I threaten to put myself out of business."

Using today's technology, says Unger, is both time-consuming and expensive. "For every hour I spend in the field collecting samples, I have to spend another 100 hours in the lab. There are multiple steps; it can literally take weeks to get one data point-at up to $1,000 per sample."

With biosensors, Unger says "We're looking at a new era, where there's a possibility of achieving fast, cheap environmental data."

Biosensors promise a new era by combining what Kaattari calls "an antibody's almost infinite power to recognize the 3-D shape of any molecule" with detectors that can immediately translate the antibody's recognition into an electronic signal. Bromage says the team's current device can process a sample in less than three minutes.



The VIMS biosensor team. Clockwise from L: Graduate students Candace Spier and Jianmin Ye, marine scientist Mary Ann Vogelbein, Dr. Erin Bromage, Dr. Mike Unger, marine scientist George Vadas, and Dr. Steve Kaattari.

To produce antibodies that can recognize the shape of a particular molecule-say one of the hundreds of types of hydrocarbons that might be found in an oil spill-the researchers essentially follow the recipe for making a vaccine using laboratory mice.

"Just like doctors vaccinate humans against the flu," says Bromage, "we vaccinate mice against contaminants. We chemically bind contaminant molecules with proteins that the mouse's immune system can recognize. The animal responds by producing antibodies, as if the contaminant were part of the vaccine."

Once the researchers have "trained" the mouse's immune system to produce antibodies that can recognize and bind to a specific contaminant, they ramp up production by fusing the mouse's antibody-producing "B" cells with tumor cells that can grow indefinitely.

"When you immortalize that cell it produces only that very specific antibody, forever," says Kaattari. "You grow it in culture, and it can produce a large amount of antibodies within a couple of weeks. It's not like having to go to a new animal every time we might need new antibodies." A cell line that was created in 1983 is still in use at VIMS today.

The researchers place the antibodies they've produced into newly developed devices that can measure the proportion of antibodies that bind to the targeted contaminant within a sample, thus measuring the contaminant's concentration.

The researchers stress that the ability to quantify the concentration of a contaminant is a key benefit of new-generation sensors. "We used to just get an on-off signal," says Unger. "The detector could only tell us whether or

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Bronk Lab Studies Nitrogen's Role in Red Tides

VIMS Associate Professor Deborah Bronk has received a $500,000 grant from the National Oceanic and Atmospheric Administration (NOAA) to study the role that nitrogen plays in generating red tides along Florida's Gulf Coast.

Red tides, known by scientists as harmful algal blooms or HABs, cause major economic and environmental harm around the U.S. and the world. In Florida, HABs are estimated to cost more than $20 million per year in tourism losses alone, and have been implicated in many fish-kill events. HABs also occur in Chesapeake Bay, most notably the widely publicized *Pfiesteria* bloom of 1997.

Bronk's funds are part of a five-year, $4.73 million grant from the ECOHAB program (Ecology and Oceanography of Harmful Algal Blooms) to the Florida Fish and Wildlife Conservation Commission. ECOHAB is a partnership among NOAA, the Environmental Protection Agency, and the National Science Foundation.

The overall grant will support nine scientists from seven research organizations as they explore the nutrient sources that support and regulate red tides along Florida's Gulf Coast.

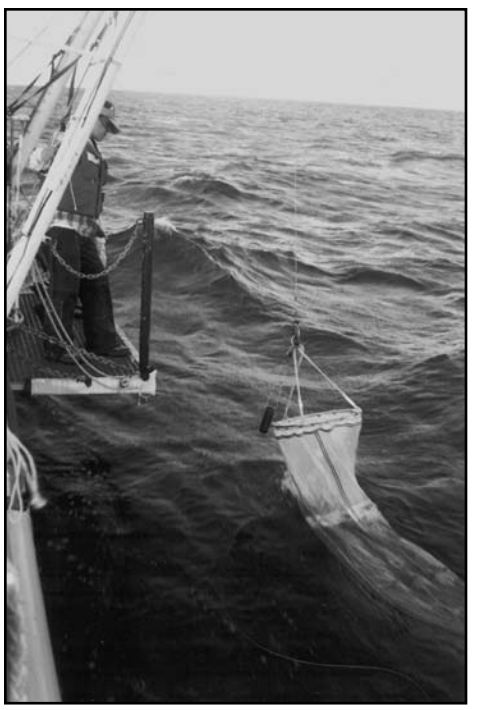
Florida's red tides are most commonly caused by blooms of the microscopic alga *Karenia brevis*. This organism intrigues scientists due to its ability to thrive in areas with both high and low nutrient concentrations.

Bronk's specific role in the project is to determine the types of nitrogen that *K. brevis* favors, and to calculate how quickly the alga can use the nitrogen to grow and reproduce. Her laboratory is one of the few in the U.S. with the expertise and equipment needed to make these types of measurements.

The basic question, says Bronk, is "What kind of nitrogen do they use, and how are they using it?"

Answers to these basic questions have direct implications for management. That's because the form of nitrogen present in seawater reflects the nitrogen's source. Says Bronk "If we know the kinds of nitrogen that *K. brevis* uses during different stages of a bloom, and we know the source of that nitrogen, then managers will have a better idea of which nitrogen sources to control in order to better predict and perhaps limit future blooms and their effects."

Bronk's work with *K. brevis* in Florida should also help managers control other HAB organisms, both in the Gulf and elsewhere around the world, including Chesapeake Bay.



A plankton net collects Karenia brevis *for Bronk's kinetics experiments.*

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not a contaminant was present, not its concentration."

Kaattari adds "With an exact concentration, we can monitor the dispersal of a substance over real time. If we can see the concentration increasing or decreasing in a certain pattern, we can predict the path of oil spills or other toxic releases."

A number of commercial firms produce the new-generation sensors, using optical diffraction, fluorescence, and other detection techniques.

To date, Bromage, Kaattari, and Unger have collaborated most closely in sensor evaluation and development with Sapidyne, Inc., a commercial firm located in Boise, Idaho.

"Sapidyne has the most robust technology on the market," says Kaattari. That's important for plans to deploy biosensors in the field, where they will be exposed to environmental extremes.

The team is currently testing a Sapidyne-designed sensor that can simultaneously process 12 different samples and quantify in real time the concentration of any particular contaminant. The company loaned VIMS the prototype, which is worth more than $70,000.

"Working with Sapidyne has been wonderful," says Bromage. "It's the true meaning of collaboration between academia and industry."

Kaattari adds "They were excited to discover new applications for this technology, and we've purchased three more of their sensors, so we can start deploying them."

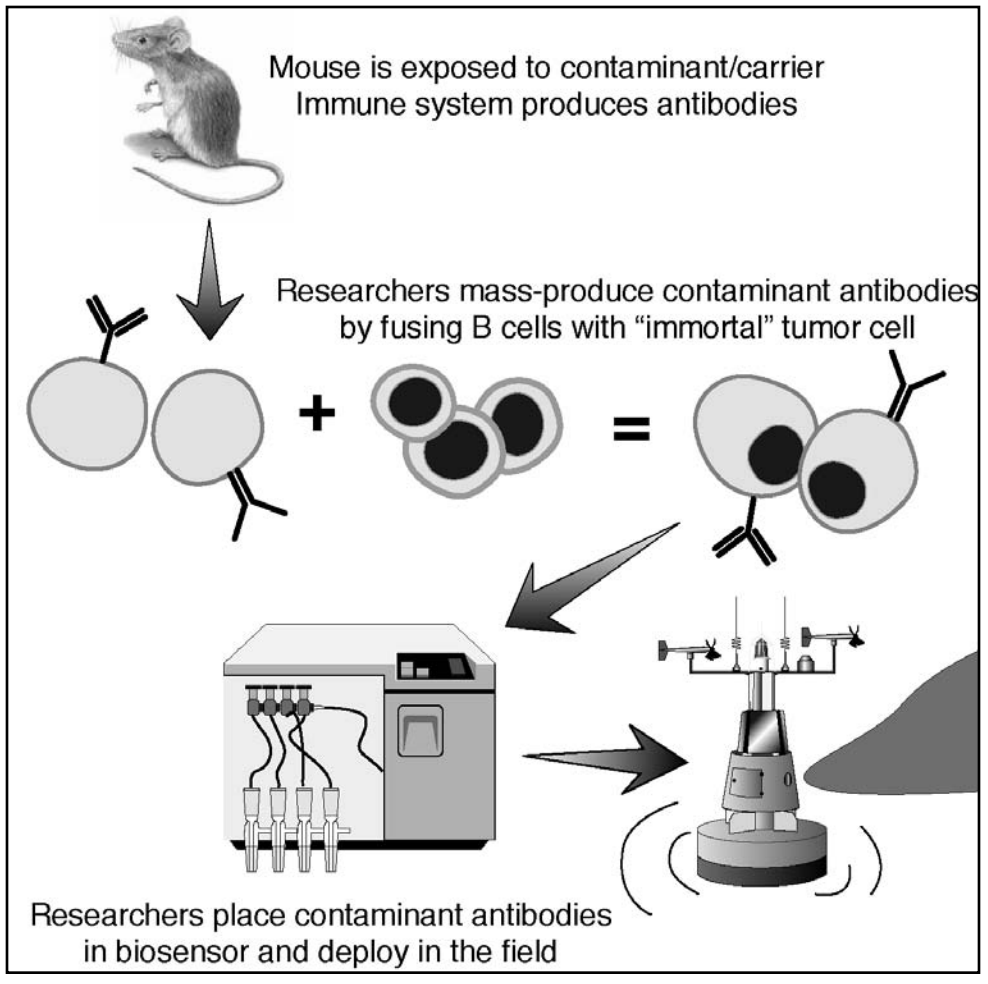
Kaattari notes, however, that the antibodies produced at VIMS can be used in many other sensing devices as well. "We have the ability to create antibodies that can go onto all the different kinds of platforms. A lot of people might want to use a standard clinical assay that they already have, and just need the antibodies. We can provide those too."

To date, VIMS' biosensor team, which also includes students Candace Spier and Jianmin Ye and research assistant Mary Ann Vogelbein, has created antibodies that recognize TNT, TBT, and various hydrocarbons.

TNT is, of course, an explosive that is widely used in weaponry. Because TNT residues contaminate many military bases and nearby groundwater plumes, the Department of Defense has expressed avid interest in developing biosensors that can detect TNT quickly and cheaply. Such a device would prove particularly helpful given the remediation efforts needed to allow full use of former military installations like Fort Monroe that have been decommissioned as part of the nationwide Base Realignment and Closure process (BRAC).

The TNT antibodies produced at VIMS are so sensitive, says Bromage, that they can readily distinguish between TNT and another closely related molecule that differs only in a single side branch.

"Our TNT antibodies are a hundred times more sensitive than those that are commercially available," says Kaattari. "We've already surpassed current spectroscopic technology, and we're way beyond EPA requirements for looking at TNT concentrations in water samples."



The VIMS biosensor team produces antibodies that can recognize specific contaminants in the field.

VIMS Green Team member Maggie Fagan led a group of more than 15 VIMS students, staff, visiting scholars, and family members during the Virginia Waterways Clean-up on September 16. The annual event is part of the International Coastal Cleanup. The team collected 371 pounds of trash and 49 pounds of recyclable materials from Gloucester Point Beach and Yorktown Beach. Here, graduate student Amanda Lawless and her husband Doug do their part in the clean-up.

Bromage has led the effort to produce antibodies for tributyltin, or TBT, which is used in boat paint to prevent fouling by marine organisms. TBT enters seawater through leaching or when a boat is scraped for re-painting, and remains toxic to marine organisms even at extremely low concentrations.

"The TBT antibody is actually how this project got initiated," says Unger. "Because the release of TBT from ships is so detrimental to the environment, it's good if you can detect it in real time." Newport News Shipbuilding has expressed particular interest in VIMS' work in this area.

VIMS' hydrocarbon antibodies hold great promise for detecting and tracking oil spills. Says Unger, "We can delineate the sources of oil now, but with very time-consuming techniques. The real advantage of hydrocarbon antibodies is their speed and low cost."

Unger has talked to Canadian researchers about using hydrocarbon antibodies to detect oil under ice. Currently, the researchers detect oil leaks the old-fashioned way: they dig a hole and look. Unger proposed using an under-ice biosensor with a protruding antenna.

"That autonomous, rapid answer, that's where this stuff really starts to shine'" says Unger. "There's no question that industry is interested in that. Industry, academia, and government are all potential end-users of the technology."

As technology providers, Unger notes that VIMS offers a unique mix of skills. "A key thing about our work is its interdisciplinary nature. One of the reasons we're having success is that Steve and Erin are immunologists, and I'm an environmental chemist—two completely different fields. We realize that working together gives us the best approach for developing these kinds of sensors. We have experience with toxins, identification, protein synthesis, and chemical conjugation-you need expertise in all these areas to develop these sensors."

Jim Golden, Associate Vice President for Economic Development at the College of William and Mary, concurs with Unger's assessment.

"There are very few places that have the immunology, the chemical capability, the biological capability, to pull this together," says Golden. "There's a lot of expertise involved in each of those steps."