

SciTECH Briefs

Biopolymer Mercury Mop

Historically, a lack of knowledge regarding the environmental and biological impacts of heavy metals such as mercury meant that factory effluents were often flushed into surrounding areas, causing many sites to become potential hazards to wildlife and humans. To remediate this problem, researchers have searched for an ideal heavy metal sorbent that combines high affinity, selectivity, and ease of use. Biopolymers produced with genetic engineering



Mercury contamination in waterways is a widespread environmental issue.

techniques that allow precise control over their chemical and physical properties are promising candidates.

Recently, Wilfred Chen and colleagues at the University of California, Riverside (<http://engr.ucr.edu>), developed a fusion protein using *E. coli* broth media that combined the mercury-binding properties of MerR, a bacterial protein involved in mercury detoxification, with the solubilizing-precipitating behavior of elastin-like polypeptides (ELPs) (*Environ. Sci. Technol.* **2003**, *37*, 4457–4462). ELPs are biopolymers composed of pentapeptide repeats, which undergo reversible phase transitions in response to temperature changes, which

could allow for easy recovery of the sequestered metal.

The researchers found that each of the ELP-MerR fusion proteins tested could undergo the reversible phase transition and retain full mercury-binding capacity, as shown using cold-vapor atomic absorption spectroscopy. They also determined that the fusion protein was fully functional from pH 4 to 7, which is important because metal-contaminated wastewaters are often acidic. The researchers then tested the mercury-binding kinetics and found that most of the metal was bound almost immediately.

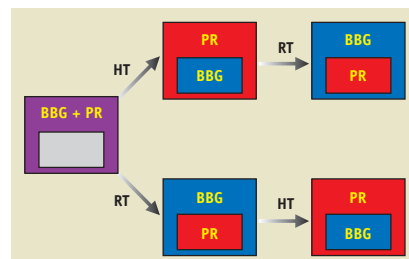
To verify specificity, the researchers repeated the mercury-binding experiments in ~100-fold molar excesses of cadmium, zinc, or nickel. They found that the mercury-binding capacity was unchanged and that binding of the other metals was minimal. This was also true in the presence of strong metal-chelating agents such as 2-mercaptoethanol. In fact, to clean the bound mercury from the fusion protein, the researchers had to extract the metal from the protein twice in high concentrations of 2-mercaptoethanol at pH 4. The extracted protein retained full mercury-binding activity for several reaction cycles.

In general, the researchers are pleased with their initial efforts. In their words, "As nature offers a wide selection of metalloregulatory proteins, a similar strategy could be used to generate ELP-based biopolymers specific for other pollutants."

—Randall C. Willis ♦

Heat Switch

Synthetic materials that can be controlled with biological precision are widely sought for the development of a new generation of industrial products. One vital biological stimulus is heat—thermal regulation is an intrinsic control mechanism in important processes such as ion transport and catalysis. Thus, constructing artificial systems with well-defined temperature-dependent behavior is an important component of this research. Researchers at Southern Illinois University (www.siu.edu) recently demonstrated promising thermoresponsive



Selective intake and release of dyes due to sequential changes in temperature. (Adapted with permission from Rao, M. S.; Dave, B. C. *J. Am. Chem. Soc.* **2003**, *125*, 11826–11827.)

properties in a porous glass sol-gel material (*J. Am. Chem. Soc.* **2003**, *125*, 11826–11827).

Specifically, the scientists looked at the effects that temperature changes had on their material's hydrophobicity. The silica-based material was prepared simply with sol-gel methods in which bis[3-(trimethoxysilyl)propyl]ethylenediamine was mixed with water to obtain gels. Hydrophobic and hydrophilic interactions were probed by measuring the intake and release of two dyes, phenol red (PR) and brilliant blue G (BBG)—the latter being the more hydrophobic of the two molecules.

When the gel was kept in contact with equal amounts of the dyes in solution for 30 min and the solution monitored by absorption spectroscopy, distinctive temperature effects were observed. At 60 °C, there was almost a 2:1 selective absorption by the gel of BBG, leaving the PR absorption peak to dominate the outside solution. But at room temperature, BBG was largely released and the more hydrophilic PR was favored for absorption. This behavior was also found to be reversible. For example, the gel would take in large amounts of BBG at higher temperatures, and then upon subsequent cooling of the system the dyes would reverse directions. The same results were confirmed in the release experiments, in which the dye mixture was encapsulated during the construction of the gel.

In a previous study (*Adv. Mater.* **2001**, *13*, 274–276), the researchers observed a structural transition in this material from a hydrophilic to hydrophobic state with an

increased temperature. Thus, they propose that the distinctive thermal regulation is due to heat-induced adjustments in noncovalent interactions with the dye molecules. They also suggest that at high temperatures the gel shrinks and expels water, which favors release of the more hydrophilic PR and retention of BBG—and upon cooling the reverse occurs.

In any case, these results have raised the possibility of a range of potential applications in the design of novel devices such as temperature-regulated molecular flow pumps, molecular filters, separators, and molecular sorters.

—David Filmore ◆

Scaling Up

Since the first reports of using microwave heating to accelerate chemical transformation reactions in 1986, the progress of



Using a multichambered reaction vessel, researchers can scale up microwave-assisted organic synthesis. (Reproduced with permission from Stadler, A.; et al. *Org. Proc. Res. Dev.* 2003, 7, 707–716.)

R&D in microwave-assisted organic synthesis (MAOS) has been steady. In recent years, MAOS has extended beyond simple organic reactions and now includes applications in combinatorial chemistry, polymerase chain reaction, and electrochemistry.

But for all of its benefits, one problem associated with MAOS has been the challenge of scalability. Traditionally, the reactions have been small in scale (milliliter reaction volume) and yield. Part of the problem is the inability of the microwave energy to penetrate beyond the outer few centimeters of the reaction vessel. Several companies, such as CEM (www.cem.com) and Milestone, Inc. (www.milestonesci.com), have tackled this problem head-on, but

recently, Oliver Kappe and colleagues at Karl-Franzens-University Graz (www.maos.net) decided to pursue another angle to reaction scale-up: Rather than simply increase the volume of a single reaction, they aimed to increase the number of reaction vessels in a single apparatus (*Org. Proc. Res. Dev.* 2003, 7, 707–716).

The researchers developed a multimode system composed of eight reaction chambers (~60 mL each) in a 1400-W microwave system with magnetic stirring in each vessel and online temperature and pressure control. They tested their system with a variety of reaction chemistries—multi-component reactions, solid-phase reactions, metal-catalyzed couplings—and with different solvents, catalysts, reaction times, and temperatures, comparing the large-scale results with those from smaller-scale reactions under the same conditions.

In one example—a standard multi-component cyclocondensation reaction (Biginelli protocol), the researchers found that the experiment using four chambers with an 80-mmol reaction scale in each chamber provided an isolated percent yield similar to the more conventional single 4-mmol-scale reaction (73 vs 88%, respectively) and that each of the chambers in the multimode system produced a virtually identical yield.

In the other experiments, including Heck coupling reactions and Diels–Alder cycloadditions, the researchers determined that it was possible to achieve comparable yields in 5-mL and 500-mL processing volume reactions using conditions optimized in the small scale. In doing so, they have opened the door to future industrial MAOS applications.

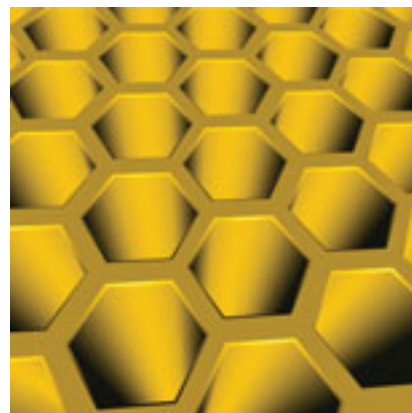
—Randall C. Willis ◆

Honey, I Shrunk the Sample Prep

Food purity is an ever-increasing concern for consumers and a growing area for regulation. Analytical techniques for the determination of contaminants must keep up with these dual demands. Determining phenol contamination of honey is one instance where improved methods are required—especially if large-scale analysis using automated techniques is to become practical. In the case of honey, naturally occurring phenol contamination (generally below regulatory proscriptions) can be exacerbated by errors in the harvesting process.

Beekeepers use phenol layered onto an apparatus known as a phenol board,

which, upon solar heating, creates an effective vapor repellent to drive bees away from the hive to allow the honey to be collect-



ed. Although used less frequently in the United States, this technique is still common in other countries. If the beekeeper improperly handles the chemicals, significantly high residues of phenol can contaminate the honey.

Typical methods for detecting phenol contamination using HPLC-UV or GC-flame ionization require extensive sample preparation, including steam distillation, solvent extraction, and/or solid-phase extraction. To simplify the sample prep stage, researchers Marta Gyorik and colleagues at the Institute of Food Science, University of West Hungary (www.nyme.hu), developed a new technique (*J. Agric. Food Chem.* 2003, 51, 5222–5225). One gram of honey was dissolved in 5 mL of HPLC-grade water, homogenized, and then diluted to 10 mL, followed by remixing.

The researchers then analyzed 100- μ L aliquots of the filtered sample using a gradient reversed-phase HPLC system followed by high-sensitivity fluorescence detection. Using these techniques, the limit of quantitation, defined as the lowest concentration of phenol with an acceptable signal-to-noise ratio, was shown to be 5 μ g/kg. Of particular importance was the use of the high-sensitivity fluorescence detector with the emission slit width held to 10 nm or narrower because of the closeness of the phenol excitation and emission wavelengths.

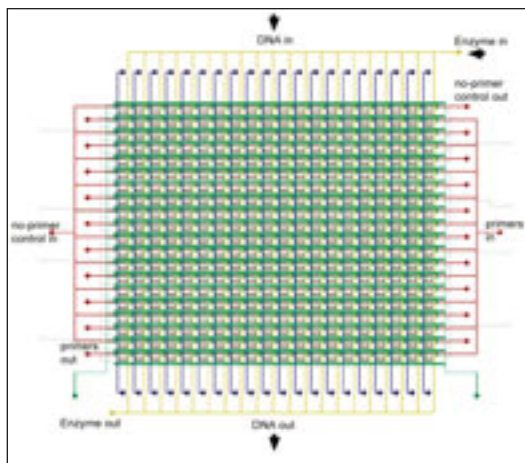
Although honey samples differed dramatically in their chromatographic patterns, the region of the phenol peak showed no sign of confounding compounds. The majority of commercial samples tested exhibited phenol levels well below the generally acceptable phenol contamination

limits (50 $\mu\text{g}/\text{kg}$). However, several samples showed phenol concentrations exceeding 500 $\mu\text{g}/\text{kg}$, considered by the researchers to be evidence of improper harvesting techniques.

—Mark S. Lesney ♦

Microfluidics in a Macro World

To take advantage of the great levels of efficiency offered by microfluidics, it goes without saying that somewhere along the way, samples have to actually be loaded



Schematic diagram of the 20×20 matrix chip layout, including valve (green) and pump (white) layers, and channels for DNA templates (blue), primers (red), and polymerase (yellow). (Adapted with permission from Liu, J.; Hansen, C; Quake, S. R. *Anal. Chem.* 2003, 75, 4718–4723.)

into the microfluidic device. It is at this so-called world-to-chip interface where the exceptional efficiency and economies of scale promised by microfluidics—in which nanoliter- to subnanoliter-scale experiments have been demonstrated—are threatened.

Because the minimum volume that can be practically introduced into a device is about 1 μL , it is not possible to limit the consumption of expensive reagents to nanoliters (or lower) per experiment in a conventional system. However, in a recent study, Stephen Quake and colleagues at the California Institute of Technology (www.caltech.edu) demonstrated a microfluidic matrix device that gets around this problem (*Anal. Chem.* 2003, 75, 4718–4723).

To realize the desired economies of scale in microfluidic devices, the Caltech team has dual goals of minimizing the number of loading (i.e., pipeting) steps and maximizing the efficiency of each step by distributing the individually pipeted volume over a large number of independent assays.

The three-layer matrix device (see figure), which they fabricated with soft lithography techniques, accomplishes both of these objectives.

The middle layer of the device consists of three sets of microfluidic flow channels that together form a 20×20 matrix with 3-nL reactors at each of the 400 intersects. One set has a single input/output port that can transport a sample to each of the 400 reactors. The other two sets are perpendicular to one another, each with 20 separate input/output ports that bring samples through a single row or column, respectively. The outside layers are composed of thousands of valves and pumps for precise, automated control of sample loading and mixing at each of the reactors.

Thus, 400 three-component reactions can be carried out with only 41 pipeting steps; the same reactions would require 1200 steps with standard liquid handling. In addition, microliter-volume loading can be used for 3-nL-volume reactions without wasting material. The researchers demonstrated the practicality of this approach with an assay essential for genetic analysis—the polymerase chain reaction (PCR).

DNA fragments (or controls) were loaded into each of the vertical inputs, primers (or controls) were pipeted into the horizontal inputs, and DNA polymerase was introduced into the single input channel. Fluorescent dyes were used to detect the occurrence of PCR amplification. Of the 3200 reactions that were performed, 98% produced the expected results (a positive signal when all reagents were present and a negative signal when something was missing). PCR products isolated from the matrix reactors were also shown to compare well by gel electrophoresis with products produced under equivalent PCR conditions using a conventional apparatus.

In addition to the diagnostic and genetic testing applications that this PCR example immediately suggests, Quake and his team propose that their microfluidic matrix chip provides an effective platform for a broad array of high-throughput biological and chemical assays, particularly those that require expensive reagents.

—David Filmore ♦

Science Bits

Plug It In. A researcher at the University of Missouri–Columbia (www.missouri.edu) found that the development of a plug-in fuel cell hybrid, with as little as 20 miles of range from rechargeable hydrogen, could cut the amount of gasoline consumed in the United States by more than 50% (*Int. J. Hydrog. Energy* 2003, in press).

Ozone Depletion Gene. University of California scientists—at Berkeley (www.berkeley.edu), Irvine (www.uci.edu), and San Diego (www.ucsd.edu)—identified a gene that controls the production by terrestrial plants of methyl halides, which contribute to the destruction of the ozone in the stratosphere (*Curr. Biol.* 2003, 13, 1809–1813).

MIP Monitor. Chemists at West Virginia University (www.wvu.edu) reported selective organic vapor detection with a molecular imprinted polymer-based piezoelectric crystal sensor (*Anal. Chem.* 2003, 75, 5387–5393).

Plant Impersonation. Scientists at Rutgers University (www.newark.rutgers.edu) have designed and characterized an artificial reaction center protein that can participate in multiple reduction/oxidation cycles with acceptors and donors following photoexcitation, thus mimicking a natural photosynthetic reaction center. They say it is the first example of a protein-based artificial reaction center (*J. Am. Chem. Soc.* 2003, 125, 11814–11815).

Bendable Electronics. Researchers at Nanomix, Inc. (www.nano.com), reported a process for transferring nanotube network transistors onto flexible polymer supports, producing devices with charge mobilities of $12 \text{ cm}^2/\text{V}\cdot\text{s}$ —what the researchers say is the highest reported values to date for flexible organic transistors (*Nano Lett.* 2003, 3, 1353–1355).

PCB Prototype. Scientists from Mitsubishi Heavy Industries (www.mhi.co.jp/index.html) demonstrated real-time PCB monitoring using time-of-flight MS with picosecond laser ionization. A prototype apparatus was constructed and field-tested for PCB monitoring in MHI's treatment plant (*Environ. Sci. Tech.* 2003, 37, 4737–4742).