lurking unseen somewhere in the galactic disk, they should be observable through the "young isotope" gamma-ray light of Ti^{44} and Na^{22} (with lifetimes of 89 and 4 years, respectively), both created by the explosive process. If none are discovered, then the true galactic SN rate must be longer than previously assumed.

Barring the unexpected, SPI will be a long-distance runner: Its combined survey and in-flight calibration should yield their best results a few years into the mission. IBIS, on the other hand, will be a sprinter: Its wide field-of-view should yield a bounty of new, well-defined celestial sources, starting with a map of the center of our Galaxy. It will also provide important data on objects outside our Galaxy, especially active galactic nuclei (AGNs).

AGNs were first seen as 100-MeV gamma-ray sources by ESA's COS-B satellite in the 1970s (*8*) and then by GRO in the 1990s (*9*). The available evidence indicates that they are powered by central, massive black holes, with a peak energy output in the MeV region. Together with INTEGRAL's x-ray detector and optical monitor, IBIS will further explore AGNs at multiple wavelengths, continuing on from GRO and, more recently, the BeppoSAX satellite.

BeppoSAX also blazed the trail to identifying gamma-ray bursts with massive cosmological explosions (*10*). INTEGRAL will observe tens of bursts per year, performing germanium-resolution spectroscopy on them for the first time. NASA's SWIFT, dedicated to "catching GRBs on the fly," will be in orbit in 2003 (*11*). In 2004, the Italian mission AGILE (*12*) will provide the high-energy (>100 MeV) complement to INTEGRAL. The even more powerful GLAST should be launched by NASA a few years later.

Right now, two major x-ray observatories, NASA's Chandra and ESA's Newton, are in orbit, ready to start formation flight with INTEGRAL, providing simultaneous data at different wavelengths. Astronomers eagerly await the first data from this exciting mission. But even before take-off, INTEGRAL had bagged a result: technological innovation. In the best tradition of high-energy astrophysics, germanium spectrometers and CsI imagers, like Geiger counters or spark chambers, bring into space ideas developed for laboratory physics. In turn, space hones ground-based technology to a new sharpness, a cross-fertilization that makes both fields grow.

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PERSPECTIVES: ANALYTICAL CHEMISTRY

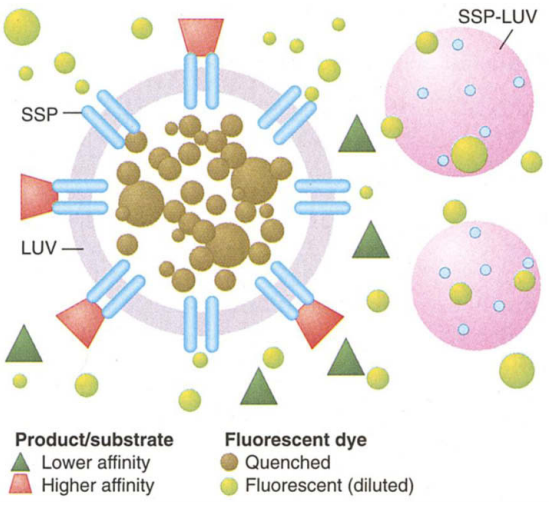
Analytes Ante Portas

Norbert Hampp

From genomics to proteomics and drug discovery, the demand for analytical tools enabling easy, reliable, and rapid screening of large numbers of samples is increasing constantly. On page 1600 of this issue, Das *et al.* (*1*) present a surprisingly simple but nonetheless effective and flexible method with potential applications in high-throughput screening.

In genomics, the main analytical task is to check for the presence or absence of specific DNA or RNA sequences. State-of-the-art assays make use of hybridization of complementary strains labeled with enzymes, fluorescent dyes, or nanoparticles. In proteomics, the analytical tasks are much more diverse. A specific marker is required to detect and quantify the activity of an enzyme. The selectivity of antibodies remains unchallenged, but it takes several weeks to get an antibody for a new target. Even minor changes in the chemical structure of the substrate require the same lengthy procedure. Modified enzyme substrates carrying a marker may be used, but they must also be synthesized in advance, limiting the flexibility of this technique.

The system introduced by Das *et al.* (*1*) should be particularly applicable to proteomics screening. The authors use large unilamellar vesicles (LUVs) containing a fluorescent dye. Self-assembling supramolecular pores (SSPs) span the LUV membrane (see the figure), defining the only spots where low molecular weight compounds like the fluorescent dye can diffuse from within the LUV to the outer medium. Binding of a compound inside the pore suppresses the diffusion of the fluorescent dye.



Pore control. SSP-LUVs containing fluorescent dye at self-quenching concentrations are added to an analyte solution. The dye can only diffuse through the SSP channels. When a SSP is blocked either by the substrate or by the product of an enzymatic reaction, the release rate decreases. The enzyme activity is measured as the ratio between blocking and non-blocking substrate/product.

The chemical building blocks of the SSPs can be easily modified. Octaphenyl groups determine the length of the pore spanning the LUV membrane. The eight phenyls in each octaphenyl chain do not form a planar structure; rather, every second one is oriented perpendicular to the plane of the others. Short peptides are attached to each phenyl group. According to the orientation of the phenyl rings, every second peptide chain is oriented perpendicular to the other side chains. Because the peptide side chains of one octaphenyl group interdigitate with those of the next, β-sheet structures are formed and a barrel-shaped pore is obtained.

The outer surface of the SSPs is designed to incorporate them into the lipid bilayer of the LUVs. Das *et al.* chose leucine residues for this purpose. The inner surface of the pores can be functionalized by introducing different amino acid residues. Depending on their size and charge, molecules may bind to the inside of the pore and block the pore cavity. If two different molecules with different affinities to the SSPs—such as

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the substrate and the product of an enzymatic reaction—are present, then the ratio between the concentrations of those species will determine how many pores are blocked.

A test procedure using the described SSP-LUVs comprises the following steps. First, the substrate and the enzyme are incubated and a fraction of the substrate proportional to the enzyme activity in the sample is converted into product. Second, SSP-LUVs filled with a fluorescent dye are added. Because of the high concentration of the fluorescent dye, emitted light is reabsorbed and not emitted from the sample volume. The dye can only get from the inside of the LUVs (with its quenching conditions) to the outer medium (with its nonquenching conditions) via diffusion through the SSPs. The number of SSPs per LUV determines the maximal diffusion rate, which is diminished proportional to the concentration and affinity of pore-blocking molecules in the analyte.

Das *et al.* show in several examples that the substrate and product of enzymatic reactions often differ substantially in their pore affinity—the only property needed for their detection by the new system. The change in pore-blocking efficiency causes a related change in the dye's flux rate from the inside of the LUV to the outer medium, where it becomes detectable as a result of dilution. No laborious separation steps of bound and unbound fluorescent dye, as required in other techniques, are needed. The released fluorescent dye can be detected with simple, readily available equipment.

The system is attractive because of its simplicity and easy adaptability to new tasks. Das *et al.* show that the pores can be engineered to produce a variety of different affinities. In addition, several fluorescent dyes could be used if different LUVs are used in a single test. Because the principle behind this system—the differential measurement of the pore-blocking affinities of the substrate and product of an enzymatic reaction—is less dependent on the analyte structure itself than are antibodies, the SSP-LUV test system might be developed into a platform technology for high-throughput screening. It should enable sensitive fluorescence detection without any separation steps to remove bound from unbound dye.

Natural and synthetic pores form the basis of several exciting applications, demonstrating the enormous potential of such pores as nanometer-scale sensing elements. For example, the sequences of single-nucleotide chains have been determined during passage through an engineered nanopore (*2, 3*). Pores have also been used for ultrasensitive chemical detection (*4, 5*). Das *et al.* have added a new, promising application for engineered self-assembling pores.

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PERSPECTIVES: STRUCTURAL BIOLOGY

Force and Voltage Sensors in One Structure

Francisco Bezanilla and Eduardo Perozo

It is difficult being a prokaryote. Throughout their life cycle, bacteria and archaea may be exposed to a wide range of environmental challenges, including large changes in the osmotic activity of their environment. Prokaryotes have thus evolved several medium- to long-term membrane mechanisms that help maintain osmotic balance (*1*). In the face of sudden osmolality decreases, an emergency response is needed to prevent the cell membrane from bursting. In most prokaryotes this response comes from stretch-activated or mechanosensitive channels that act as the molecular equivalent of an escape valve to quickly balance any hydrostatic pressure buildup (*2, 3*).

There are two major families of nonselective mechanosensitive channels in bacteria: the large-conductance channel (MscL) and the small-conductance channel (MscS). On page 1582 of this issue, Bass *et al.* report the structure of MscS that not only gives new insights into tension dependent activation, but also provides a first look at a voltage-dependent channel (*4*).

MscL opens a pore with a conductance of about 3 nS, while MscS has a conductance of about 1 nS. In addition, MscS mechanosensitivity is significantly modulated by voltage (*5, 6*) so that its open probability (P_o) is increased by a factor of e per 15 mV of membrane potential change. Mechanosensitive channel activity can be reproduced in both MscL and MscS by reconstitution in lipid bilayers (*7*), suggesting that in these systems, the process of mechanotransduction is governed by lipid-protein interactions. Both channels were identified in 1987 with patch-clamp technology (*5*), but it took several years to determine their molecular structures (*8, 9*). MscS and MscL have no apparent sequence homology, but they act as part of a two-step mechanism of osmotic emergency response. MscS opens first, having an activation midpoint near 4 dyne/cm. MscL acts as a last-resort mechanism; its activation midpoint occurs at ~12 dyne/cm, close to the breakdown point of the lipid bilayer.

The first breakthrough in our molecular understanding of tension-dependent gating in mechanosensitive channels came with the determination of the MscL crystal structure (*10*). MscL (see the figure) is a highly α-helical homopentamer, formed by the association of short individual subunits (~130 amino acids in length) containing two transmembrane segments each. Now for an encore, the Rees group has done it again, reporting the crystal structure of MscS at 3.9 Å resolution (*4*). The channel forms a symmetric homoheptamer with a well-defined membrane region and a large, mostly β-containing extramembrane region that protrudes toward the cytoplasm. The membrane region has three helical segments, with the loop between transmembrane helix 2 (TM2) and TM3 and half of TM3 lining a central pore. The other half of TM3 is abruptly bent away from the pore and lies almost parallel to the plane of the membrane in its intracellular face. The pore is about 10 Å wide, consistent with the expected dimensions of an electrolyte-filled cavity having a conductance of about 1 nS, indicating that the structure corresponds to the open conformation of the channel. This is in contrast to MscL, whose crystal structure shows that it corresponds to the closed state. Because the tension needed to open MscS is about one-third of that needed to open MscL, it is possible that the crystallization conditions favored the open conformation of MscS. The structure is of a remarkably high quality even at 3.9 Å, as a conse-

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