Packard and Aurora Biosciences to codevelop ultra-HTS technologies

In the July issue of *Drug Discovery Today*, Derek Hook eloquently described his vision for the Lilliputian future of high-throughput screening (HTS) technologies. A recent collaborative agreement between Packard Instrument Company (Meriden, CT, USA) and Aurora Biosciences Corporation (La Jolla, CA, USA) may be the first indication that Hook's futuristic view of a super-miniaturized, high-resolution robotic world of ultra-HTS, in which hundreds of thousands of assays will be conducted daily, may become a reality. The May agreement provides for codevelopment of three different technologies:

- high-density assay plates, NanoPlates™, which will be used to simultaneously conduct thousands of cell-based fluorescence assays,
- computer-controlled robotic arrays of miniature pipettes for aspirating and dispensing reagents in nanoliter to picoliter volumes, and
- optical imagers that can quantify and analyze thousands of fluorescence assays within a matter of minutes.

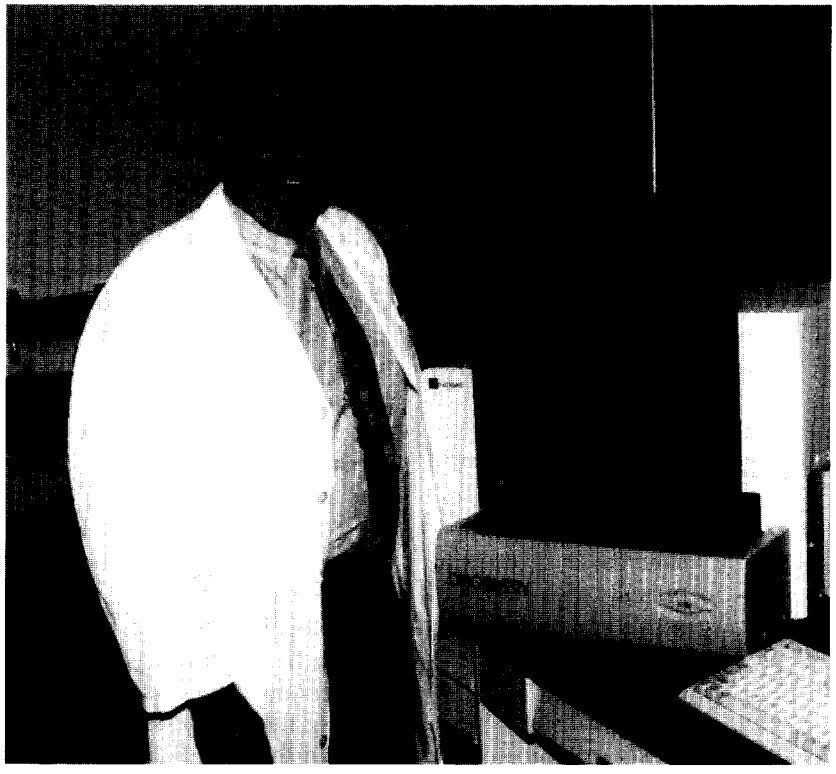
Packard, a privately held company with annual sales of about $110 million, brings to the collaboration a strong position in the instrumentation industry. Its current products for the screening laboratory include scintillation and luminescent microplate readers and robotic liquid-handling equipment for standard 96-well and the new 384-well plates. Packard's screening products are currently used by over 300 companies worldwide.

High-density plates

Based upon its extensive experience in instrument development and use of new ink jet technologies and piezoelectric methods, Packard is well on the way to having instrumentation that can automatically aspirate and dispense nanoliter volumes of reagents. They have also collaborated with Cambridge Imaging Ltd (Cambridge, UK) to integrate state-of-the-art camera technologies, originally developed for military applications, with appropriate software to generate image-analysis systems suitable for simultaneous measurement of the 2,000–5,000 wells that are likely to reside on the new NanoPlates™ in the same footprint as today's 96-well plate. Such image-analysis systems will be able to detect the emission of a single photon from a bioassay and provide a resolution of 20 microns. Packard is likely to first develop these technologies for the new 384-well format, followed by development of 864-well plate technology and the NanoPlates™.

New assay technologies

Packard also has a cooperative agreement with CIS bio international (Bagnols, France) to develop new assay technologies. Dr Al Kolb, Packard's HTS Business Development Manager, described some of these newly-emerging technologies at a short course on HTS held in Rheinfelden, Switzerland, in May. One of the most exciting advances is the development of homogenous time-resolved fluorescence assays (HTRF). In this assay, fluorescence resonance energy transfer between a lanthanide complex, such as europium cryptate, and an appropriate acceptor molecule is used to monitor the integrity of a chemical bond or molecular interaction. In one assay scenario, disruption of a chemical bond results in the separation of the fluorescence donor and acceptor, rapidly shutting down resonance energy transfer; the result is a drop in the fluorescence signal in proportion to the number of chemical bonds broken. For example, an assay for a proteolytic enzyme could be developed



Dr Al Kolb, Packard's HTS Business Development Manager.

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in which the opposite ends of a peptide would be labelled with the europium cryptate and an appropriate fluorescence acceptor molecule. The peptide would hold the europium cryptate and acceptor in close proximity, maintaining energy transfer between the pair. Introduction of a protease capable of breaking one of the peptide bonds would disrupt the energy transfer and result in a decrease in fluorescence signal. Such assays are elegant and particularly suited to the ultra-HTS format because there is no need to separate the products of the reaction from the starting materials; the assay involves simply combining the reagents and reading a signal. Moreover, because the reagents are all in solution, there need be no concerns regarding the degree of dispersal of the reagents, as can sometimes be a problem with bead-based homogenous assays.

Fluorescence acceptor

Packard is marketing a proprietary fluorescence acceptor for HTRF termed XL665 that emits light at 665 nm in response to energy transfer from the europium cryptate complex. A major advantage of this system is the long lifetime of the 665-nm signal. By waiting just a fraction of a millisecond after termination of an excitation flash before measuring the fluorescence response from XL665, most of the endogenous fluorescence arising from the assay and test components is dissipated, resulting in a time-resolved signal primarily from the XL665. Spurious fluorescence signals from test substances, one of the most vexing problems in using fluorescence assays for HTS, can thus be avoided. By coupling the europium cryptate donor and XL665 acceptor to specific antibodies or to either biotin or avidin, strategies have been developed to use the HTRF technology for a number of different assay types. Such assays include immunoassays, receptor binding, protein–protein binding, kinase and phosphatase assays and nucleic acid hybridization.

Fluorescent probes

Aurora Biosciences is a small company, currently with only 15 employees, which was founded in 1995 on the basis of the research of Dr Roger Y. Tsien (University of California, San Diego, CA, USA). Dr Tsien's laboratory has led the development of numerous fluorescent probes and techniques for use in studies of cell activation and intracellular signaling. These probes provide a window into the intracellular environment of intact, living cells and enable a real-time picture to be developed of the changes in the ionic environment, metabolite concentrations or the biosynthesis of specific macromolecules upon cell activation or perturbation. Fluorescent probes have become widely used in the study of basic mechanisms in cell activation but have so far been used only sparingly in HTS.

One of the original probes developed by Tsien is used to monitor changes in the concentration of intracellular calcium ions. Such changes frequently occur upon binding of a ligand to a receptor on the cell surface, but because the change in calcium ion concentration occurs rapidly and is transient it is difficult to capture the real-time fluorescent signal under screening conditions. Fluorescent probes that act as calcium ion sensors have now been developed that 'memorize' increases in the intracellular calcium ion concentration; such reagents should prove valuable for screening applications. The company also has proprietary fluorescent probes for measuring membrane depolarization and ultra-bright mutants of green fluorescent protein, which can be used for real-time assays of intracellular enzymes. They also have fluorescent proteins that emit yellow light and two different shades of blue light, and these can be simultaneously transfected into cells to monitor multiple proteins or to conduct intracellular fluorescence resonance energy transfer experiments. Aurora expects to license from the California Institute of Technology (Pasadena, CA, USA) additional fluorescent probes, which will be used in monitoring G proteins.

Another fluorescent probe in Aurora's tool kit is a β-lactamase reporter system for monitoring gene transcription. A proprietary fluorescent substrate has been developed that will detect the presence of β-lactamase in the cell. According to Dr Harry Stylli, Aurora's Senior Director of Screen Technology, the substrate is loaded into the cells as an ester, which has no fluorescence properties and easily penetrates plasma membranes. Once inside the cell, and following attack by esterases, the substrate becomes unable to penetrate the cell membrane and produces an intense green fluorescence. Then, β-lactamase, if it has been produced, converts the substrate into a compound with blue fluorescence. The intensity of the different colors is easily monitored using current imaging techniques; the level of green fluorescence is a measure of cell viability, and the blue fluorescence indicates the level of β-lactamase activity. Stylli claims 1–10 molecules of β-lactamase in a single cell can be detected, which is 100-fold more sensitive than the luciferase system.

Because the procedure relies on the measurement of the ratio of the blue and green fluorescence levels, the assay is independent of the number of cells in a given well. As a result the 'edge-effects' caused by a reduced cell population in the outer wells, which are a common problem in cell-based HTS assays, can be avoided. "The β-lactamase system will be extremely useful in bioassay miniaturization, rapid screen design and, most importantly, in finding functions for genes of unknown function in mammalian cells", says Stylli.

Next century

Using its proprietary fluorescent technologies and its alliances with strategic partners such as Packard, Aurora will focus primarily on the development of ultra-HTS assays for intact mammalian cells. However, it will also develop assays applicable for biochemical targets, such as enzymes and binding assays. In addition to developing its own novel screens, the company will establish screening systems in the customer's facilities and license its technologies. The major goal of the co-development program between Aurora and Packard is to bring to reality the ability to test in excess of 100,000 discrete samples per day in a given assay, a goal which Aurora claims is essential for any organization hoping to be competitive in the drug discovery business in the twenty-first century.

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