Ultra high-throughput screening – a journey into Nanoland with Gulliver and Alice

A look at the promising but also challenging future that HTS holds for drug discovery

When Gulliver awoke in Lilliput he must have wondered whether he had grown or the world around him had shrunk. Alice spent her time shrinking and growing. Those who practice high-throughput screening (HTS) must begin to wonder whether recent trends towards miniaturization and ultra-high throughputs will take us into some of these worlds. Dr Harry Stylli (Aurora Biosciences, La Jolla, CA, USA) at the *First Annual Meeting of the Society for Biomolecular Screening*, held in Philadelphia in November 1995, must have stunned his audience when he envisioned a world where it is possible to screen up to and beyond 500,000 samples per day. Automated high-speed combinatorial chemistry efforts are now creating such vast compound sets, and such numbers will soon become the norm rather than the products of speculation. Just as the revolution in molecular biology and biotechnology revitalized the search for new targets, so will it be necessary to visualize revolutionary approaches to screening in order to increase throughput, cut assay costs and shorten discovery time to maintain competitive advantage.

'Most major pharmaceutical companies .... cannot continue to screen using conventional 96-well microplate technology for much longer'

Most major pharmaceutical companies with synthetic compound decks in the range of 10^5–10^6 compounds cannot continue to screen using conventional 96-well microplate technology for much longer. To screen 100,000 compounds in 1,400 microplates requires 30 days' screening at a rate of 50 plates per day per assay. Using present technologies it is not now practical to take more than 300 days to screen a deck of 10^6 compounds and remain competitive. As deck sizes get much bigger, approaching 10^6 or more compounds, and as screen numbers rise, microplate throughput rates will have to increase beyond the capacity of existing automation systems. Moreover, cost and supply of reagents escalate to uneconomic levels.

We cannot, however, neglect conventional automated HTS operations. It would not be practical to replace the existing infrastructure overnight. Existing technology will still stretch in the near future to quite impressive throughput rates using homogeneous reporter systems (such as fluorescence polarization, time-resolved fluorescence, scintillation proximity assays or real-time spectrophotometric systems with whole-plate imagers), parallel gantry-type robotics and sample-dense 384-well plates. Eventually, however, continuing competitive pressures will push towards integrated, miniaturized screening systems. First- and second-generation combinatorial chemistry systems on microchips are being built with the capability to synthesize up to 10,000 compounds per chip. To develop analogous screening systems, it will be necessary to integrate real-time, sensitive-detection assay and submicroliter fluid-handling methods onto microchips. Such HTS systems should have a throughput of 8×10^5 per day. With reagent demands in the low microliter range, this would require only milliliter volumes of reagent for a whole 10^6 compound HTS deck.

'Drug discovery management must anticipate and lead change in HTS'

So, is this technology the answer? Experience from the electronics industry would seem to answer 'yes'. The transistor replaced the vacuum tube to achieve cost reduction through ever-increasing levels of miniaturization, integration and economies of scale. In the HTS world, cost reduction will come not from economies of scale of the micro-miniaturized systems, but from reductions in the costs of reagents and manpower.

Derek Hook, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, PO Box 5100, Wallingford, CT 06492, USA. tel: +1 860 284 6366, fax: +1 860 284 6417, e-mail: d.hook@ccmail.bms.com

  1359-6446/96/$15.00. PII: S1359-6446(96)20006-6

Advances in micro-miniaturized sensor systems, such as Cytosensor® (Molecular Devices, Sunnyvale, CA, USA) pH monitoring using light addressable arrays, capillary electrophoresis on etched glass chips, GCs on a silicon chip and miniaturized microcalorimeter arrays, will yield a whole new array of miniaturized, integrated sensor and fluidic handling systems that will be developed into integrated miniature ultra HTS systems.

'If we can screen million-size compound decks within a few days, rather than months or years, the supply of meaningful, validated and robust screens will become a bottleneck'

The next few years will be a time of unparalleled challenges within HTS. Not only must companies continue to invest in conventional automation and robotics in order to achieve higher rates of screening, but they must also plan and manage the forthcoming ultra-HTS microchip revolution. To avoid the analogous fate of giant computer companies who lacked the vision to see the development of desktop computing, drug discovery management must anticipate and lead change in HTS. Just as it took ten years from the first primitive microcomputers to become today's desktop workstations, the transition to new micromachined, miniaturized screening technology will take some time, but is inevitable. The current human and technological challenges of automation, robotics and data handling are simple compared to those in this next revolution. Flexibility and willingness to learn new skills will become important, just as Gulliver had to adapt to the land of Lilliput, and use the skills of the engineers of Lilliput to work in the new world of the small, and Alice had to adapt to the new thought process and scale of the world beyond the looking glass. We cannot become so blinded by the promise of technology that we neglect the vitally important need to gain human acceptance of the changes that these technologies will bring in the day to day work environment.

There are many other challenges. The effort in devising limited combinatorially diverse synthetic libraries is as much a response to limited screening capacity as for the widest effective novelty. Released from artificial shackles, rates become high enough and costs low enough that vast decks of compounds can be screened rapidly. This ability to screen vast numbers of compounds rapidly and at low cost will result in another cycle of combinatorial effort to develop chemical diversity space properly and to its full potential.

The relevance of natural product screening will be confirmed when the need arises to screen compounds with built-in 3-D conformational novelty. Indeed, there is some reason to believe that nature has selected for compounds with built-in biological activity, and that this activity is often realized through the positioning of the appropriate chemical functionality in 3-D space using elaborate chemical scaffolds. This ability of nature to amplify a number of weak interactions into significant biological activity is especially well illustrated by the cyclosporin/FK506/rapamycin set of compounds. These compounds have a remarkable ability to interfere with protein–protein binding, using a coordinated set of weak interactions. Developing such molecules from small molecular synthetic libraries is difficult to imagine.

There will also be a challenge to move from Gulliver's land of the miniature high-throughput assay system to the real *in vivo* biological world. Miniaturized high-throughput assay systems will need to relate to the phenomenological aspect of the therapeutic target as much as possible, so that translation from the micro to the macro world is meaningful and rapid. They will include whole cell-based assays or at least mimic them as closely as possible. An example of this trend is the use of whole-plate fluorescence imagers to measure ion fluxes within whole cells on treatment with test substances in real time. This methodology has already led to the development of lead chemotypes that show parallel responses in electrophysiological experiments.

The final challenge is the development of a continuing supply of novel, practical robust screens to the HTS juggernaut. If we can screen million-size compound decks within a few days, rather than months or years, the supply of meaningful, validated and robust screens will become a bottleneck in the drug discovery process. We need to break away from conventional targeted screening and leverage the power of ultra-HTS and the Human Genome Project to go fishing for disease targets, develop the drug and then find the target. This unconventional approach would allow a company to leapfrog the competition, and to develop truly innovative and completely proprietary therapies. The ability of ultra-HTS systems to work with the microscale reagent requirements really makes the approach practically and economically feasible. The small quantities of specialized proteins, nucleic acids or ligands required for these miniaturized systems will be produced *in situ* by genetically engineered cellular systems, during the assay, on the microchip. These systems would be part of the assay protocol, or part of a miniaturized bioreactor coupled to the sensor and test array.

'The winners emerging from this exciting chaos will be the patients'

Since I first entered the world of HTS in 1983, I am constantly reminded of that ancient Chinese curse 'may you live in interesting times'. This curse will still be with us, as we travel from the world of Fleming's laboratory, to the science fiction world of robotics and automation, and to the Lilliputian world of Gulliver's travels and beyond.

Whatever the outcome we must remember the goals of the pharmaceutical industry as healers and preventers of disease. The winners emerging from this exciting chaos will be the patients who suffer from disease and who will receive novel therapies from companies that discover them using ultra-HTS.

Derek Hook