intracellular chemical and biological reporter technologies developed by Roger Tsien together with the 3456-well plate approaches developed by Aurora Biosciences will have a lot of potential. As with all high-tech approaches, though, it remains to be seen how their technologies might be able to reduce screening costs or improve screening outputs.

Shifting the bottlenecks: HTS matures

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The tremendous volume of data generated by the Human Genome Project has resulted in an unprecedented level of opportunity for the pharmaceutical industry. As expectations turn to the HTS to fill the gaps in the drug discovery pipeline, the investment in this sector has increased. We are now approaching the post-genomic era, and with it a new set of threats and opportunities. The HTS benchmarks are moving steadily, as highlighted at the recent *Screentech 2001* conference in San Diego, CA, USA (13–16 March 2001). The meeting attracted a blend of industrial and academic technology developers along with end-users, to address the application of HTS to different targets classes. In addition, the neighbouring processes, such as assay development and miniaturization, compound logistics and data mining were also highlighted, indicating where the bottlenecks will shift as HTS principles change.

Although many themes were discussed, prominence was given to readout and screening technology development studies and examples of its application in case studies. These applications are increasingly shifting away from soluble systems and towards cellular assay systems. This was highlighted in the opening keynote presentation given by Roger Tsien (Howard Hughes Medical Institute, University of California, San Diego, CA, USA). Tsien set the scene for many of the presentations as he discussed the scope of applying labelling techniques and natural probe sources to investigation of biochemical interactions at the cellular level. Green fluorescent protein (GFP)-based fluorescent indicators are applicable to almost all organisms and can be targeted to specific tissues, cells, organelles or proteins. The comparison of fluorescence resonance energy transfer (FRET) between GFP mutants offers a general mechanism to build genetically encoded indicators and monitor dynamic protein–protein interactions. The potential of this method was demonstrated for the Fas/TNFR receptor superfamily as an example. Tsien also discussed the application of FlAsH (fluorescein-based arsenical hairpin binder) reagents for intracellular labelling of CCXXCC sequences for use in fluorescence anisotropy to monitor protein orientation. In the future, FlAsH analogs might be used to label proteins that are altered by GFP fusions (for protein expression and enhanced purification), which would be a key step in making reporter gene assays universally applicable.

Target validation – challenging assays

Potentially, efficiency within the gene-to-lead process is, in the most part, determined by target validation. Michael Kuranda (Millennium Pharmaceticals, Cambridge, MA, USA) reminded the audience that parallel target validation is thought to shorten the target validation process from 3–7 years to 2–3 years. To achieve this goal, Millennium has set up an Integrated Target Discovery Platform (involving 200 people) with competitive strengths in process optimization using SHERPA, a knowledge management tool, to assess expression and/or activity space, and predict assay configuration success. This platform enables screening to be performed in parallel to target validation, and enzyme targets have already been used to test this paradigm.

In two presentations, Jeremy S. Caldwell and Daniel Sipes (The Genomics Institute of the Novartis Research Foundation, San Diego, CA, USA) underlined the importance of an appropriate robotics infrastructure for cellular screening. Caldwell presented chemical and biological strategies for high-throughput gene functionalization using pathway-based screens. The company has built an impressive 1536-well-plate-compatible screening platform for cellular assays, enabling a production throughput of >125,000 compounds per day. The platform fluidics is composed of the Robbins Hydra (Robbins Scientific, Sunnyvale, CA, USA) for compound pipetting, and the synQUAD™ (Cartesian Technologies, Irvine, CA, USA) for cell dispensing.

In addition, they introduced a new concept to whole-genome functional analysis based on cDNA-HTS. Former methods have relied on the generation of a cDNA library from the cells of choice and subsequent cloning of each cDNA into a retroviral expression vector, which typically needs a very high signal-to-noise ratio (S/N) and limits the investigation to dominant effectors only. The new approach involves cDNA expression arrays and a 384-well-plate screening system that can be used in low S/N situations, providing access to both dominant and recessive effectors. Sipes outlined the principal screening concept at GNF, which enables a cell-based screening capacity of >60 screens per year. An interesting technical solution to compound

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logistics, are custom compound storage carousels that are kept under dried nitrogen gas, and an associated computer system that enables access to the entire library at any time.

John Hefti (Signature BioScience, Hayward, CA, USA) and Nancy Allbritton (University of California, Irvine, CA, USA) discussed two new technological approaches that have been developed to discover new targets. The multiple coupling spectroscopy technology (MCS) has been developed to examine charge distributions in molecules by considering the biological system as an electrical circuit. The resulting signals have a unique pattern 'signature' that can be applied to different assay types for the study of, for example, protein–protein interactions. Allbritton discussed the application of the Laser Micropipet System (LMS) to investigate signal transduction cascades in single cells. The LMS combines laser spectroscopy (shock waves) with microfluidics, capillary electrophoresis and cell biology. Cells are lysed using an Nd:YAG laser source (which terminates the reactions to be investigated), and then the cell content is loaded into a capillary and fluorescent signals measured by the analysis of peak area ratios in comparison to a fluorescent reporter. The value of this system lies in the possibility of measuring the activation of multiple enzymes in a cell within one experiment.

Multiplex and micro-volume assays – screening hardware news

Another focus of screening technology development is the implementation of new or even improved detection principles. This session was chaired by Dan Brown (Bristol-Myers Squibb, Wallingford, CT, USA), who set the scene for the following speakers by asking how important multiplex target assays are in the drug discovery process, and when conventional fluorescence would suffice. Several speakers discussed their experiences with cellular and bead-based assays. Flora Tang (Sugen, South San Franscisco, CA, USA) discussed the concept of using cell-motility analysis using the ArrayScan® System (Cellomics, Pittsburgh, PA, USA) for an Src kinase assay. The advantage of this method is that the assay is able to quantify a motility unit precisely thereby making the assay more suitable for HTS. Another high-resolution analysis system applicable to cell morphology assays was discussed by Pascale Roux (Aventis Pharma, Paris, France). The Acumen Assay Explorer (The Technology Partnership, Royston, UK) can be used to detect both cells with different shapes, and subpopulations of cells that have the same shape. However, this system is still under evaluation from a software point of view.

Several technology workshops took place throughout the week, including one joint-act from Carl Zeiss Jena GmbH (Jena, Germany) and Cellomics (Pittsburgh, PA, USA), highlighting the Zeiss uHTS system, which has a new plate-transfer concept and is capable of providing an automation platform for the Cellomics ArrayScan® Kinetics (ASK) workstation (Cellomics). The Amersham Pharmacia Biotech workshop demonstrated the progress that has been made using LEADseeker technology together with the Praelux confocal line-scanner principle, resulting in the LEADseeker Cell Analyser. In this unit, three charge-coupled device (CCD) detectors operate simultaneously to enable analysis in the broad wavelength range of 430–760 nm (simultaneous three-colour line illumination) in high-throughput cell analysis for membrane trafficking, intracellular signalling and nuclear activation. Although the audience raised the important issue of what the price would be for such a system, this information was not disclosed during the demonstration.

Success stories in miniaturized HTS

In a keynote lecture, John Houston (Bristol-Myers Squibb, Wallingford, CT, USA) reviewed the future demands on screening, which he outlined to be essentially improvement in quality and success rate by integration and parallelization of the R&D process. In this context, miniaturization must enable a throughput explosion while maintaining overall costs at a reasonable level. Wilma Keighley (Pfizer Global R&D, Sandwich, UK), Steve Ashman (GlaxoSmithKline, Harlow, UK) and Dirk Ullmann (Evotec OAI, Hamburg, Germany) presented an update on their applications of the EVOscreen™ Mark II uHTS platform in miniaturized 1 µl enzymatic target screening. Comparisons of conventional automation in 384- and 1536-well plate formats illustrated the benefits of confocal fluorescence detection by multi-parameter readout. The most interesting finding was that a high proportion of compounds appeared as hits in both formats, when the probability of such an overlap occurring by chance is very low. These results clearly demonstrate that miniaturized uHTS is maturing, as proved by data comparison sets on different screening formats and platforms.

Conclusions

In many respects, the conference was a timely reminder that the rate of change, particularly in HTS as a key step within the drug discovery process, has never been greater. Furthermore, integration and re-focussing of the surrounding disciplines, such as compound handling, detection technology, fluidics and data handling, to match the HTS to uHTS paradigms, will continue to challenge screeners in the coming years. A personal take-home message from the conference was that miniaturized screening is not routinely applied throughout the industry and quality is more important than pure throughput. There has as-yet been no decision as to whether integrated or modular screening platforms will be the favourable solution in the future. However, John Comley (PerkinElmer Life Sciences, Turku, Finland), while talking about challenges in liquid handling for miniaturized screening, suggested that the trend is towards rapid assay assembly by automated 'rapid plate filling devices', where miniaturization affects both the assay and the screening platform itself.