The *Discussion Forum* provides a medium for airing your views on any issues related to the pharmaceutical industry and obtaining feedback and discussion on these views from others in the field. You can discuss issues that get you hot under the collar, practical problems at the bench, recently published literature, or just something bizarre or humorous that you wish to share. Publication of letters in this section is subject to editorial discretion and company-promotional letters will be rejected immediately. Furthermore, the views provided are those of the authors and are not intended to represent the views of the companies they work for. Moreover, these views do not reflect those of Elsevier, *Drug Discovery Today* or its editorial team. Please submit all letters to Rebecca Lawrence, News & Features Editor, *Drug Discovery Today*, e-mail: Rebecca.Lawrence@drugdiscoverytoday.com

HTS: understanding the physiology of life ▼

The recent review by Wölcke and Ullmann in *Drug Discovery Today*[1] entitled 'Miniaturized HTS technologies – uHTS', provides a thorough summary of the current status of development in HTS. It will not only be interesting for everybody working in assay development for HTS to read, but can also be used as a short catalog of equipment that is currently available to succeed in this key step in the drug-discovery process. What the authors did not discuss however, is that there are still as many different views on how to set up the best hit-compound discovery platform as there are screeners and assay developers, and that their philosophy of miniaturization and automation is not shared completely by everyone.

One of the main reasons that many companies still act conservatively and continue to use the 384-well plate format is the cost of integrated fully automated workstations with highly sophisticated detection technologies. At the time of lead declaration, it is still the cost per test sample or per well and the pharmacological properties of the compound that count, rather than the high-tech method that was used to find it. The 1536-well plate format, however, will most probably become the standard plate format soon, and it will remain the standard format for years. Using volumes of ~5 μl, these high-density plates are applicable to nearly every currently used fluorescence detection method, producing stable signals with low enough background and minimum assay variability required for robust HTS. Comparably cheap standard fluorescence plate-readers are available that combine most of the common fluorescence-detection signals. The only method that is not yet feasible when using these readers is 'real' (in contrast to transient-state) single-photon-counting time-resolved fluorescence, for detecting lifetimes of between 1 and 20 nsec to run assays with standard dyes, such as fluoresceins, rhodamines or BODIPYs® (Molecular Probes, Eugene, OR, USA).

Approximately five years ago, Novartis was the first company to integrate single-molecule spectroscopy (SMS or SMDetection) into HTS in a collaboration with Evotec Biosystems (now EvotecOAI, Hamberg, Germany). This would not only enable miniaturization to <1 μl assay volumes, but would also allow us to: (1) explore new ways of miniaturized natural-product separation and screening, (2) study the storage and solution properties of large-compound collections in small wells, and (3) allow us to use the femtoliter-sized confocal illumination and detection volumes for completely new ways of screening on the solid surface. Furthermore, the aim of achieving the ultimate in 'high-content screening' was already in our minds. Although fluorescence correlation spectroscopy (FCS), which is used to distinguish particles based on their translational-diffusion time, was the only established SMD technology at that time, it could be foreseen that fluctuation analysis offered the possibility of distinguishing particles based on their translational and rotational speed, brightness, intra- and intermolecular distances, co-localization and fluorescence lifetimes. Despite sophisticated optics and electronics, a homogeneous solution single-molecule experiment is easy. The focus of the diffraction-limited optics is placed in the center of any type of well or drop, and the molecules of interest can freely fluctuate producing stochastic intensity signals at the detectors; the remainder of the technique is software analysis. Intensive programming efforts from excellent theoretical spectroscopists have led to the establishment of many techniques, such as fluorescence intensity distribution analysis (FIDA), two-dimensional (2D)-FIDA anisotropy, 2-color-2D-FIDA, fluorescence intensity multiple distribution analysis (FIMDA), photon counting histogram (PCH), burst intensity fluorescence lifetime (BIFL), fluorescence cross correlation spectroscopy (FCCS) and others, which now enable us to monitor virtually every molecular property change of a single molecule on binding or cleavage.

All of the investments in automation, dispensing, plate and data-management systems described in the review by Wölcke & Ullmann are surely worth the effort. The new dimension in fluorescence-based high-content screening, however, will come from the global and parallel analysis of the

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molecular parameters of interaction partners *in vitro* or on a cellular level. Then, the biochemists' dream of understanding the physiology of life on a molecular level might meet with the drug discoverers' dream of understanding the biochemical mode-of-action of a compound already after the primary screening phase.

Reference

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Phage display: increasing the rewards from genomic information ▼

These are interesting times in the ongoing war between humans and bacterial pathogens. Until recently, it appeared that the development of penicillin and other antibiotics had guaranteed victory but, unfortunately, it has become clear that the excessive use of these same antibiotics has led to the evolution of resistant bacterial strains that threaten to overwhelm human health. Thus, we again find ourselves in desperate need of new weapons against bacterial pathogens.

While bacteria have been busy evolving new forms of resistance, humans have been busy unraveling genomes. Whole-genome sequencing has provided access to the complete proteomes of dozens of bacterial species and, in doing so, has revealed numerous potential targets for antimicrobial action. In a recent review in *Drug Discovery Today*, Christensen *et al.*[1] point out that any essential protein could be a target for antibacterial drug discovery but, to effectively use this genomic information in drug development, two important goals must be achieved. First, newly discovered proteins must be validated as legitimate targets for therapeutic intervention and, second, high-throughput screens must be developed to enable rapid identification of compounds that inhibit protein function. Christensen and coauthors convincingly argue that phage-displayed combinatorial peptide libraries can aid both these goals.

Using current optimized methods, phage-displayed peptide libraries containing tens of billions of different sequences can be readily constructed and used to select specific ligands against essentially any protein of interest[2]. Thus, highly specific peptidic-ligands can be obtained rapidly without any previous knowledge of a protein's function. These ligands can then be used for target validation and HTS. For example, binding peptides can be expressed inside a bacterial cell and, in such a system, growth inhibition can be used as strong evidence that the peptide's binding partner is an essential protein, and thus, a potential drug target[3]. The same peptides can then be used to set up high-throughput assays to identify small molecules that bind at the same site and inhibit protein function[4]. In this way, new targets and inhibitors could quickly be obtained with only a minimal knowledge of a protein's structure and function.

The approaches described by Christensen and coauthors are indicative of a larger trend in the life sciences. The full benefit of genomic information can only be realized with combinatorial approaches that speed up the process of characterizing the tens of thousands of proteins that comprise a living proteome. Phage display and other combinatorial biology methods have a major role in modern biological research[5], and this role will continue to expand as we tackle the difficult, but highly rewarding, task of deriving novel therapeutics from genomic information.

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