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Editorial overview: Analytical biotechnology: New technologies for quantitative analysis of biological specimens and natural products

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Frank L Jaksch, Jr, co-founded ChromaDex®, Inc. in 1999 and serves as Chief Executive Officer. Under his leadership, ChromaDex has focused on developing a comprehensive natural products chemistry business, expanded into international markets and built an impressive roster of Fortune 500 customers. ChromaDex is now the leading supplier of botanical reference standards and phytochemical products, analytical services and novel ingredients to the dietary supplement, sports nutrition, food & beverage, cosmetic and pharmaceutical markets.

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Savaş Tay is an assistant professor of Bioengineering at ETH Zürich. He develops high-throughput microfluidic and optofluidic technologies for single-cell analysis, and uses them to decipher information processing mechanisms cells use when responding to external signals. His interests include space and time dependent analysis of immune pathways such as NF-κB, stem cell differentiation, and cell migration, all with single-cell resolution.

High-throughput screens to identify bioactive compounds that can benefit human health have become commonplace. While these screens have not been limited by imagination, they have been limited by the simple truth that analytical characterization of complex biological systems remains challenging. Traditional *in vitro* methods of cell culture, enzyme activity, DNA expression *etc.* used in large screens are well controlled but usually devoid of the normal biological microenvironment of the subject organism. Exciting recent advances in microfluidics technology empower investigators to control the microenvironment. This biotechnology can be applied to single cells and whole tissues.

Equally challenging can be the analytical characterization of the material being screened especially if that material is extracted from a natural source. Knowing precisely the complex makeup of the sample is essential for dereplication as well as identification of the bioactive compound (and any potential inhibitors). Recent advances in NMR, fermentation, DNA barcoding, chromatography and bioassays are discussed.

Although this issue is presented with two main story arcs, microfluidics and natural product development, the reader is urged to consider that integration of these biotechnologies during all aspects of development may produce the largest impact on human health.

Microfluidics for quantitative analyses of biological systems

Biological systems are comprised of hierarchical structures such as organs, tissues, cells and biochemical pathways. Quantitative and high-throughput analysis of such systems is a prerequisite in developing organism level computational models that will allow rapid, *in silico* testing of drug perturbations. Traditional pipette-and-dish methods are limited in terms of precision and throughput, and the complexity, heterogeneity and time-dependence of biological interactions require new analytical methods. Microfluidics addresses these limitations and has already impacted quantitative biological analysis. In this issue, we review recent developments in microfluidic analysis of biological systems, from single molecules to single cells and to intact tissue.

The review by Tabeling describes recent insight from physics of microfluidic systems. Standard theoretical analysis of low Reynold's Number conditions applied to most microfluidic systems assumes inertia free, continuous flow of fluids. New theory now has been developed to solve the droplet breakup in T-junctions in two-phase (oil and water) flow. Inertial microfluidics has been used to sculpt flow patterns, useful for separating,

sorting or combining fluids and cells. Ren et al. review how new materials improve the functionality of microfluidic devices and expand their applications. Elastomers such as PDMS have attractive properties such as easy fabrication by molding, inertness, gas permeability, elasticity and tight sealing, and have been to material of choice for constructing microfluidic chips. Biologists prefer plastics, however, and recent developments in fabrication techniques allowed plastic-based microfluidic chips. Hydrogels possess properties that are similar to extracellular matrix, making them useful for 3D cell culture applications. Fabricating hydrogel microstructures or infiltrating microfluidic chips with hydrogels now allow microfluidic systems that mimic *in vivo* tissue structures. On the other hand, PDMS based chips continue to improve. Araci and Brisk describe how they pushed the envelope in PDMS membrane-valve based systems by carefully optimizing multi-layer soft lithography. The size of the PDMS membrane valve has now been reduced from 100 μm × 100 μm to 6 μm × 6 μm, allowing the integration of 1 million control elements in a single, postage stamp sized device. They describe fabrication and automation of such valves in whole, and show applications of integrated chips in high-throughput biology.

The improvement of microfluidic systems in terms of precision, functionality and throughput paved the way for new applications in quantitative and systems biology. Streets and Huang describe how precision and efficient sample handling by provided by microfluidics made possible to analyze single-molecules in high-throughput. *In vitro* measurements of protein–protein and protein–DNA interactions have greatly benefited from microfluidics. Single copy measurements using digital-PCR have been applied to quantification of DNA and mRNA to detect fetal aneuploidy and point mutations in leukemia, and gene expression variations in various cancers.

Recent insight from quantitative cell studies revealed that each cell has its own composition of mRNA and proteins, creating a great degree of heterogeneity even among monoclonal populations. Single-cell analysis has become an important tool in biology and medicine. Weaver et al. review recent applications of microfluidics to single-cell analysis, with particular attention to understanding of tissue-scale phenomena in cancer biology, stem cells, regenerative medicine, and neuroscience. The study of pathogenesis and immune response have also benefited from microfluidic single cell analysis. Culturing cells in PDMS microfluidic chips has advantages in sample handling and in performing precision measurements on individual cells, but requires careful attention to culture conditions. Mehling and Tay discuss the pros and cons of microfluidics over traditional technique for mammalian cell culture, and describe potential issues and their trouble shooting. Optimized microfluidic protocols now allow automated long-term imaging of live-cells cultured under complex environments, mimicking *in vivo* signaling niches. Micro-organisms too can be efficiently cultured and analyzed in microfludic systems, and the review by Okumus et al. show how such methods can be used to decipher biological complexity. Controlled perturbation of cells in adaptation experiments address genotype and phenotype links, such as emergence of bacterial resistance to antibiotics.

Generating whole tissues and organs *in vitro* may alleviate the increasing regulatory burdens in drug testing, and microfluidic technologies show great promise in this direction. Luni et al. describe how new 'organ-on-chip' devices can be realized by culturing *ex vivo* tissues such as biopsies, or by growing intact tissues from stem cells cultured in chips. Such 'microfluidic organs' can then be combined to create multi-organ *in vitro* models for testing drug compounds. Culture of model animals such as nematodes or fruit-fly embryos in microfluidic chips offers unique advantages in manipulation, throughput and control. Stirman et al. describe recent advances in animal microsurgery in microfluidic chips, especially using optical and mechanical micromanipulation. Various drugs or genetic material can be precisely delivered into small animals, and neuronal surgery can be performed on immobilized animals. A combination of flexible PDMS membranes, local cooling or CO_2 exposure has been used to immobilize animals in chip.

The promise of microfluidic total analysis systems for global health applications can be realized if such systems are combined with advanced sensing devices used in consumer electronics, such as cell phone cameras. Coskun and Ozcan review the emerging computational imaging and sensing platforms for global health, and describe how they developed general field-portable and cost-effective systems using these devices.

Advances in biotechnology aimed at unlocking the true potential of natural products

The demand for natural products continues to increase. More natural, effective, environmentally friendly, sustainable and healthy alternatives are continuously sought in a wide array of consumer products including supplements, energy drinks, health foods, pharmaceuticals and cosmetics to name a few. However, the molecular characterization of natural products is inherently complex. Consequently, development of natural products for novel indications is fraught with pitfalls from extraction to commercialization. Initially, the process of obtaining extracts from their natural sources results in mixtures of unknown number of compounds. Identification and quantification of these compounds presents a significant challenge. This is vital for both the initial characterization of the natural product and for the quality control of

subsequent extractions. Next, choosing a bioassay that is robust enough mimic the human situation presents its own set of limitations. These limitations often lead to 'false positive' or 'false negative' results and delays the development of natural products. Finally, commercial production presents yet more obstacles as scaling up of a natural product and/or complying with existing regulations in a responsible manner may be difficult.

Several of the innovative technologies described in this issue will aid in improving all aspects of natural product development. Improvements in NMR technology, preparative chromatography, fermentation, DNA barcoding and bioassays will all help streamline the development of natural products to the benefit of human health.

The unbiased view of sample composition provided by recent advances in quantitative NMR (qNMR) techniques described by Simmler and colleagues will alleviate concerns regarding compound identification and purity (quality control) of a natural product since qNMR can simultaneously quantify multiple compounds. Further advances in NMR technology are reviewed in Halabalaki et al. Miniaturized NMR probes allowing for smaller samples to be analyzed as well as cryogenic NMR probes which significantly increase the signal to noise ratio will accelerate discovery.

Complementing qNMR in the accurate identification of extracts from natural sources is the advancement of DNA barcoding technology reviewed by Techen et al. This emerging biotechnology applies various molecular biology techniques to ascertain the DNA sequences of well characterized regions of the target natural product and compares that to know databases for authentication.

Bioassay selection to screen for active compounds is at the heart of any discovery process. The review by Agarwal et al. discusses how *in vitro* bioassays can be effectively employed during all phases of natural product development. Unbiased design of *in vitro* bioassays to the targeted disease and advice on overcoming common pitfalls are discussed. Uncommon uses for *in vitro* bioassays such as assessing bioavailability and biological quality control are also presented.

Enhancement of preparative chromatography technology to allow more economical and efficient isolation of the desired natural product and increase ability to scale up production is reviewed by McChesney and Rodenburg. These advances require relatively simple modifications to current chromatography procedures that yield large benefits in terms of lifespan of each column and consistency throughout utilization.

One of the most critical issues in developing natural products for large scale commercialization is availability of the source material. Zhou et al. discuss novel fermentation processes for the manufacturing of natural products. This technology involves the metabolic engineering of microorganisms to produce the desired natural product. This technique has the innate advantage of being more environmentally friendly as well as the high probability of being more efficient and economical. It is self-evident that if a natural product can be produced in a lab responsibly and sustainably that is desirous over extracting it from the natural world which can have adverse effects on ecosystems. Advances in this field are allowing a more diverse array of natural products to be produced and strategies for future improvements are presented. Mora-Pale et al. also address advances and challenges presented by molecular engineering of recombinant microorganisms and plant cells. They review strategies for optimizing heterologous expression when complete biosynthetic pathways are transferred to the subject organism in order to maximize yields of natural products. Selection of subject organism is also highlighted as bacteria, yeast and plant cell systems are reviewed.

Technological advances enabling the more effective development of natural products coupled with the ever increasing consumer demand for natural products necessitates a standard method for validation of natural products in the interest of human health. Brown and Lister review current initiatives for the validation of analytical methods for natural product identification. Misidentification of natural products or inadequate quality control ensuring consistent composition are challenges essential to translating optimal efficacy of natural products to the consumer.

In summary, integration of the recent innovations in biotechnology discussed here will advance the entire field of natural product development. With each new tool in the arsenal aimed at the accurate characterization and scalable commercial manufacture of natural products during all stages of development, investigators can more effectively unlock the true potential of natural products.