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Innovation in academic chemical screening: Filling the gaps in chemical biology

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Abstract

Academic screening centers across the world have endeavored to discover small molecules that can modulate biological systems. To increase the reach of functional-genomic and chemical screening programs, universities, research institutes, and governments have followed their industrial counterparts in adopting high-throughput paradigms. As academic screening efforts have steadily grown in scope and complexity, so have the ideas of what is possible with the union of technology and biology. This review addresses the recent conceptual and technological innovation that has been propelling academic screening into its own unique niche. In particular, high-content and whole-organism screening is changing how academics search for novel bioactive compounds. Importantly, we recognize examples of successful chemical probe development that have punctuated the changing technology landscape.

Introduction

Operating at the frontier of biology, drug discovery, a technologically-dependent and multidisciplinary activity, will be all the more successful with an *engaged* academic presence. Advancements in laboratory automation and the wide availability of libraries of RNAi reagents and small molecules have allowed academic institutions to enter the arena of high-throughput screening (HTS) to evaluate its potential and problems first hand. The ability to execute thousands and even hundreds of thousands of experiments in parallel with high reproducibility appeals to academic and industrial research alike. However, the practice of drug screening has become a ubiquitous endeavor in both, yielding examples that simultaneously reinforce the technique's power [1] and tribulations [2,3]. Whereas the goal with industrial drug screening is to identify a new therapeutic entity, the aims of academic researchers range from developing chemical tools and mechanistic explorations to translational research. Therefore, we seek to shed light on major developments arising from academic chemical screening that are enhancing the potential of chemical biology. Since the term *drug screening* is rather biased towards therapeutic development, *chemical screening* more appropriately addresses the breadth of programs conducted by academic centers.

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This review focuses on the recent conceptual and technological developments emerging from academic screening centers and the exciting results they have produced. Ultimately, the field is maturing and chemical screening is becoming less defined by the *industrial* or *academic* dichotomy in light of the increasing interconnection between biotech, pharma, and noncommercial entities (universities, governments, etc.). As the public screening initiatives sponsored by the NIH are transitioning to complement and support NIH's public health mission, the need has arisen for greater specialization and cooperation across the screening centers.

Recent highlights in academic HTS

Over the past several years, we have seen many successes in academic HTS and probe development efforts. Even with the myriad of skepticism waged against screening centers in the academic domain, we feel it is critical to recognize examples of the excellent progress being made. Interestingly, academic screening has embraced a wide range of assay types, each with its own strengths and weaknesses (Figure 1). One notable instance came from collaboration between researchers in the UK and Canada to inhibit pathogenic *Trypanosoma brucei* by chemo-copying RNAi phenotypes [4]. HTS with purified N-myristoyltransferase, a previously proposed drug target against the parasite-induced African sleeping sickness, identified promising *in vitro* hit compounds. Lead-optimization chemistry [5] was then used to develop a molecule with nanomolar potency and effective at eliminating the parasite in an animal model of the disease. Although their lead compound lacked species-specific selectivity, the authors demonstrated the druggability of a novel target and a therapeutic proof-of-concept *in vivo*. It is also important to note that the Dundee library was specifically developed to facilitate economical screening campaigns against neglected disease targets [6]. An important aspect of successful academic screening has been the coupling of high-quality assays with a diverse, thoughtfully-constructed library [7].

A second example of notable academic screening comes from the Scripps research institute where Schurer and colleagues used a cell-based reporter-gene assay to identify agonists of sphingosine lipid receptor subtypes S1P₁ and S1P₃ [8]. The Scripps study pursued modulators of these therapeutically-relevant receptors by integrated HTS hit profiling, structure-activity relationship (SAR) studies, and ligand modeling to provide new insights into sphingosine lipid receptor biology. Ultimately, the authors were able to develop selective and potent probes against both receptor subtypes. The S1P₃ agonists led to a subsequent study exploring therapeutic optimization of the probe [9]. We feel that the author's analysis of the hits from their large-scale screening is one the few to discuss the vastly underappreciated problem of reporter interference. The Schurer *et al.* study highlights the dire importance of orthogonal reporter assays to eliminate hits that speciously manipulate readouts in primary screening. Other studies have found that the vast majority of screening hits can be attributed to reporter interference [10,11], exposing the extent of this challenge to investigators pursuing HTS actives who are often limited in their follow-up resources.

Another cell-based reporter-gene assay was used by researchers at UT Southwestern to meet the need for chemical probes that modulate Wnt pathway signaling [12]. Relevant to developmental patterning, tissue maintenance, and tumor growth, Wnt/ β -catenin inhibitors are desirable for both therapeutic and basic-science applications. Following HTS and important orthogonal counter-screens, the authors pursued promising chemical series with extensive chemical genetics and biochemistry to characterize mechanism-of-action (MOA). To explore the *in vivo* phenotypes and toxicological implications of Wnt pathway inhibitors as therapeutics, Chen *et al.* diverged from the conventional use of rodents and instead choose zebrafish. Since rodent models can be more time-consuming and cost-prohibitive for

academic labs, it is notable that Chen and colleagues demonstrated the effective use of zebrafish as a first-line *in vivo* system for post-HTS analysis. We anticipate that model organisms such as fish and worms will advance as a valuable method for evaluating the *in vivo* mechanism and potential of lead compounds. These emerging models are also well-suited for SAR and optimization stages where many compounds must be tested in standardized assays. As with any animal platform, chemical phenotypes in these model systems do not always translate into humans and the physiological differences should be considered. Although it is unclear if the UT Southwestern campaign was a contributing factor, it should be noted that Novartis has recently introduced a Wnt-pathway inhibitor to Phase I clinical trials for cancer.

These previous academic screening examples have a common theme: chemistry support is critical to the successful development of chemical probes. This is especially true with the increasingly high expectations being placed on academic centers in terms of a lead compound's specificity, selectivity, potency, and utility [13,14]. Overall, greater collaboration [15] needs to be planned into academic screening campaigns for follow-up studies that are crucial to the production of useful chemical probes (Figure 2). It is important to recognize that both academic (e.g. Chemical Methodology and Library Development initiative, <http://www.nigms.nih.gov/Research/FeaturedPrograms/CMLD/>, see PNAS volume 108 issue 17) and industrial entities (e.g. Structural Genomics Consortium, <http://www.thesgc.org>) [16] can be a source of partnerships in probe optimization, chemical library development, and MOA studies.

High-content and the pursuit of phenotypic screening

The rise of HTS outside the classical pharmaceutical environment, especially in cases of therapeutic discovery, has been characterized by some as lacking innovation and effectiveness [17]. However, this view is clearly misguided (many successes are highlighted here) and we regard phenotypic screening as playing to the innovative strengths of academic research. More than just an opportunity to move beyond the limitations of biochemical target-based HTS, cell-based phenotypic screening also allows access to the broad spectrum of targets that have been neglected in chemical biology. The infection of macrophages by *Leishmania donovani* is exemplary of a target that necessitates cell-based phenotypic screening [18]. While reporter-gene assays are a powerful and straightforward implementation of phenotypic screening, the technique is complemented by the simultaneous analysis of sub-cellular, cellular, and population-level phenotypic patterns obtained with well-designed high-content screens (HCS).

Incorporating microscopy readouts into microplate-based experiments [19], HCS offers greater physiological relevance and context to the resulting data. High-content microscopy has also been used as a tool to decipher MOA of bioactive compounds [20–22]. By monitoring a myriad of parameters from a cell exposed to a chemical agent (target expression, localization, cell morphology, organelle function, and cytotoxicity/cell health to name few), understanding that agent's MOA can become more straightforward [22,23]. Joseph and colleagues use of a high-content assay to profile the drug-induced mechanisms of apoptosis illustrates the multi-parametric paradigm [24]. Additionally, high-content has been a boon for exploring emerging targets such as mitochondrial dynamics [25], stem-cell differentiation [26,27], and epigenetics [28] where few existing tools are available.

Recently published manuscripts highlight the capabilities of high-content in academic chemical screening [29]. The technology is poised to enable three-dimensional (3D) cell-based screening assays [30–33] with increased physiological relevance in tumorigenesis and angiogenesis (reviewed in [34]). Given the high degree of complexity in 3D culture screens,

HCS extends the capabilities of population averaging measurements (i.e. from conventional plate readers) and quantifies morphological features that are important for interpreting responses (e.g. tumor invasion, neuronal structure). As 3D assays are technically challenging applications of HCS, scientists must still innovate to simplify assay protocols, workflows, and image analysis routines.

A notable success from academic HCS highlights the integration of the technology into both primary screening and MOA determination [35]. In a campaign conducted at the Broad institute, a library of small molecules was screened to find those that shifted leukemia cells from a pathogenic proliferative mode to a benign differentiated state. Interestingly, their post-HTS investigations yielded insight into the role of Aurora kinase B in megakaryocyte polyploidization and demonstrate a new therapeutic proof-of-concept for leukemia. Infectious diseases are another area where HCS has played an important role in advancing academic chemical screening. For example, HCS was the backbone of a very recent study attempting to elucidate novel drug combinations that could extend the therapeutic landscape for HIV [36]. Having proven useful for determining host genes important for viral entry, assembly, and release [37], the HCS assay was uniquely suited for gathering mechanistic data about drug action (incorporating cytotoxicity monitoring and activity classification). Another interesting aspect of the Tan *et al.* chemical screen was the use of a well-annotated chemical library to make meaningful therapeutic conclusions from their data.

Furthermore, advances in HCS data-processing are streamlining the complex analysis [38] bottlenecks inherent to the technology. The creation of the Open Microscopy Environment (OME) tailored for HCS [39] and CellProfiler [40] for image analysis have helped to uncouple the instrumentation from closed, vendor-supplied solutions that are often incompatible with existing infrastructure. The creation of the OME data management framework is an important step to allow HCS data to be exchanged between multiple platforms and analysis tools, a major frustration shared among the users of proprietary systems. The recent increase in open-source HCS analysis tools such as PhenoRipper [41] emerging from academic laboratories represents a growing ecosystem of ideas surrounding the technology. Additionally, the dissemination of high-content data analysis and visualization tools from industry is aiding academic centers manage the *big data* challenges of HCS [42]. In terms of hardware, the current generation of automated microscopes have substantially increased image acquisition speeds, implemented LED light sources (reducing operating costs), and have achieved more user-friendly interfaces. Furthermore, competition between multiple hardware vendors has driven costs down while increasing the range of options available to meet the specific needs of each academic facility.

As more academic centers seriously engage high-content imaging as a flagship assay paradigm, they inevitably face logistical challenges: scanner speed, data storage, data analysis, and management. With multiple parameters in a given assay readout, best practices for even the most basic questions, such as how to evaluate assay robustness [43], are still being developed. Additionally, without a set of scanners running in parallel, scientists can still expect high-content screens to tie up automation for much longer periods. Clearly, further enhancement of the speed, efficiency, and logistics surrounding high-content screening is needed before the practice fully matures in academic environments. However, these are primarily engineering hurdles and the true challenge of HCS lies in the assay development. Even as the technological aspects of HCS have substantially advanced, the design of assays that make use of the platform's full potential remain formative. Looking forward, we expect this to improve as high-content assays are extrapolated from MOA experiments to discovery assays. Finally, a standard for data sharing, curation, and collaboration is an important unmet need of the academic HCS field to ensure the usefulness of the technology in chemical screening. This latter point is foreseen to be a topic of

discussion and action for the recently formed Society for Biomolecular Imaging and Informatics.

Evolving whole-organism technologies leads academic screening on its own path

Model systems such as *Caenorhabditis elegans* (nematodes) and *Danio rerio* (zebrafish) have gained a broad representation in basic-science research as excellent models of development in multicellular organisms. Unlike the case of HCS however, model organism screening in academia has suffered from a lack of mature technologies. Now, whole-organism chemical screens with zebrafish and nematodes are growing in popularity due to key technological advancements such as improved imaging techniques, genetic manipulation, and screening equipment. In terms of screening, an advantage of both nematodes and zebrafish over other model organisms such as *Drosophila* (fly) is that they are transparent and can be grown in a fully aqueous environment. Compounds are dispensed directly into the culture medium and are accessible to the entire organism. Additionally, nematodes and zebrafish possess features such as a nervous system and innate immune system making them even more attractive for drug and probe discovery. In the confines of a 96 or 384-well microplate, zebrafish are typically screened in the embryonic or larval stage [44] while the size of *C. elegans* makes it amenable for screening of both juvenile and adult forms [45].

Of the recent advancements that are bolstering the capabilities of whole organism screening, TAL-effector nuclease (TALEN) [46] mediated genome editing has been adapted to zebrafish [47–50] and nematodes [51] allowing for rapid, low-cost transgenic engineering. We anticipate that the straightforward nature of TALEN-mediated genome editing relative to zinc-finger nucleases (ZFNs) will open a multitude of screening assays to academia. A second major development in zebrafish and nematode-based HTS has been in liquid handling instrumentation for fully automated screening. Traditionally, zebrafish embryos would be manually dispensed into 96 or 384-well microplates for HTS. Optical sorting systems with microplate dispensing have now been developed to integrate zebrafish embryos and nematodes into automated platforms. Specifically, the COPAS [52–55] and ZebraFactor [56] (modification of the XenoFactor [57]) systems offer commercial solutions to academic screening centers. The vision-based sorting capabilities of these instruments add a layer of quality control to whole-organism dispensing. Large-scale screening is also being facilitated by mass embryo production systems from Aquatic Habitats. Overall, we are greatly encouraged by the creation of end-to-end automated solutions for zebrafish in academic facilities [58].

In addition to drug and probe discovery campaigns, both *C. elegans* and *D. rerio* have shown much promise for next-generation toxicological screening assays as well [55,59–61]. In particular, nano-toxicity profiling assays are well suited for microplate-based zebrafish screening [62,63] and allow for the detailed mechanistic analysis of nano-particles [64] on organ systems and development. Although it is too early to predict if zebrafish can supplant toxicological screening in rodents, it is likely to become an integral part of academic endeavors due to their flexibility, higher throughput, and lower overhead costs.

Whole-organism screens can use a variety of different readouts amenable to HTS plate readers [65] or automated microscopes [45]. For example, using a transgenic luciferase readout, Gut and colleagues executed a high-throughput screen [66] for small-molecules that modulate the expression of *pck1*, a gene that regulates gluconeogenesis. Their discovery that mitochondrial Translocator protein ligands lower blood glucose is also another example of how well-annotated bioactive compound libraries can be leveraged to gain novel biological and therapeutic insight. Just as with cell-based assays, convergence of high-content

microscopy with screens in worms and zebrafish has been a promising development within academic centers. Detailed analysis of phenotypic modulation has been achieved by coupling multi-parametric, high-content microscopy readouts (bright-field, fluorescence [63], and *in situ* hybridization [67]) to whole organism screening [68,69]. To achieve cellular-level analysis of zebrafish larvae, a fully-automated confocal imaging system has also been demonstrated [70,71]. High-content has been used in conjunction with the *C. elegans* screening as well [45].

Given the difficulty of screening for compounds that modulate disease-relevant CNS targets, developing zebrafish larvae offer an exciting opportunity for academic screening. Moving away from traditional target and cell-based assays, Kokel and colleagues demonstrated a screening platform for psychotropic agents with zebrafish behavioral readouts [72]. Using light and touch-response assays, the team was able to record compound-induced signatures that resulted from CNS and neuromuscular modulation. It is our opinion that whole organism screening gives academic centers a chance to break out of standard HTS models and add higher-order physiology to their assay development and target identification toolbox.

Academic screening as a complementary avenue to industrial pursuits

The implementation of HTS techniques by academic and government entities has on occasion been described as a futile attempt to compete with industrial programs; though as many academics anticipated, such a conflict has not materialized. Indeed, something altogether different has emerged: non-industrial chemical screening has defined a complementary path to interesting discoveries. Since academic researchers operate outside of the industrial therapeutic development paradigm, they are able to focus more on understanding the mechanistic aspects of pharmacological phenomena and the principles surrounding them. Several examples of these studies are highlighted in Table 1. In particular, Wells and colleagues used a unique assay to identify compounds that promoted auto-proteolytic activation of procaspase-3 in chemical HTS [73]. The subsequent characterization of compound 1541 and analogs suggested that they act as classical inhibitors that bind procaspase-3 and stabilize a self-cleavage-inducing conformation. However, upon deeper study, the team uncovered an unprecedented mechanism of action [74] where compound 1541 promotes auto-activation of procaspase-3 by forming ordered nanofibrils. These small-molecule nanofibrils have uncertain therapeutic potential but represent a completely unexpected scaffold-like mechanism that modulates enzyme function.

Overall, we would like to emphasize that even though academic chemical biology and screening may pursue similar targets as industrial counterparts, such endeavors are not redundant. While industry possesses the resources and expertise to develop a therapeutic agent, academic researchers have greater exploratory freedom as a consequence of being less encumbered by the pace of industrial drug discovery. Together, private sector and academic efforts can coexist as two paths of exploration, both reinforcing each other.

Conclusion and Future Directions

With over 60% of academic drug/probe discovery centers utilizing HTS [75], it is clear that the tools are now broadly available and considered generally useful. Looking forward, we anticipate academic chemical screening to continue as a center of innovation in high-throughput biology. Recently developed technologies will also have substantial impacts on the practice of chemical screening in academic environments. For instance, the ability to perform genomic manipulations on endogenous loci with the rapidly expanding genome-editing technologies (ZFN, TALEN, CRISPR, etc.) is an exciting opportunity to improve

assay design. Combined with disease-associated genetic backgrounds from patient-derived cells [76], HTS assays are edging toward an inflection point in physiological sophistication. These new cell-based HTS assays coupled with the considerable academic infrastructure and expertise in functional genomics (genome-wide siRNA, shRNA, and cDNA) are poised to accelerate target identification; for example, by the characterization of leads from chemical screens in functional-genomic modifier screens.

Finally, we foresee an interesting collateral consequence of the so-called *translational* activity in academia. As opposed to the ‘sky is falling’ attitude of some regarding the evolving nature of science and policy, we see this as an opportunity for a more rigorous assessment of basic research findings and publications [77]. An increase in the reproducibility of scientific advancements in biology may grow from the collaborative efforts of academic and industrial labs to translate initial findings into tangible tools. A key aspect to this is the field placing more value on informative negative data in addition to positive findings. Additionally, by encouraging collaborative ventures in screening and probe development, policy and funding agencies also have an important role to play in the development of rigorously validated compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Notable achievements in probe development highlight the potential of academic HTS
- Academic use of high-throughput chemical screening complements industrial programs
- The emergence of high-content and model organism screening extend phenotypic assays
- Drug discovery and development paradigm shift to cooperative initiatives with industry

Figure 1.

Spectrum of assays encompassed by academic chemical screening initiatives. Examples of each assay type (from left-to-right): protease assay with a fluorescent donor-quencher reporter, receptor agonist/antagonist assay coupled to a luciferase gene reporting downstream promoter regulation, high-content translocation assay of a fluorescent-labeled protein from the cytosol to the nucleus, whole-organism microplate-based assay looking at motor neuron development in zebrafish larvae, and a non-microplate whole-organism screen of adult mammals performed by the direct administration of compounds (or pools of compounds) into the animal. The adult mouse assay involves monitoring behavioral metrics or sacrificing the animal and examining tissue/cellular-level changes. (Middle) Each assay type has advantages and disadvantages relevant to chemical screening. PK; pharmacokinetics, PD; Pharmacodynamics. *High-content assays may offer more tools that simplify the mechanism-of-action (MOA) determination process. (Bottom) Example molecules from the above assay categories developed from recent academic programs making use of chemical screening.

Figure 2.

A current view of academic screening with an emphasis on collaboration. Blue flow-paths represent inputs of collaborative expertise utilized by a screening center or lab to develop new small-molecule modulators. Red flow-paths represent the interconnection between key steps in the process (grey boxes). Note that instead of a purely linear model, data and outcomes generated at each step can feed back into the workflow to support an evolving non-linear discovery environment.

Table 1

Examples of how academic/academic-industrial partnerships can effectively pursue mechanistic and non-standard observations in chemical biology.

Target	Relevant molecule	Discovery
Activators of procaspase-3 in apoptosis (cancer)	<p>Compound 1541</p>	Compound 1541 was discovered in an <i>in vitro</i> target-based screen for activators of procaspase-3 [73]. After demonstrating that the compound was active in cell-based apoptosis assays, a subsequent study [74] concluded that its novel mechanism of action was the self-assembly of nanofibrils that may mimic cellular scaffolds.
Abl kinase in cell proliferation (cancer)	<p>GNF-2</p> <p>GNF-5</p>	GNF and Scripps Research Institute researchers screened for compounds that displayed differential cytotoxicity towards Bcr-Abl-transformed cell lines [78], searching for new inhibitory mechanisms for the resistance-prone Abl kinase. Optimization of lead compounds yielded GNF-2 and 5 as a new class of allosteric inhibitors. Further study at the Barnett and Dana-Farber Cancer Institutes revealed that that inhibition by GNF-5 binding to the Abl myristate pocket was likely due to allosteric modulation of the distant ATP binding site [79]. More importantly, the secondary study provided a set of biophysical principles to pursue further therapeutic development against Abl.
Inhibitors of caspase-6 in apoptosis (neurodegeneration)	<p>Compound 3</p>	High-throughput chemical screening was performed as a collaboration between UCSF and Genentech to identify inhibitors of caspase-6 to promote neuronal survival [80]. The chemical optimization of hits led to the development of compound 3, a selective inhibitor of caspase-6 with low nM potency. Interestingly, investigation of compound 3 revealed a new type of non-competitive binding and inhibitory mechanism. Overall, the analysis of compound 3 provided a useful proof-of-concept for future caspase- targeted drug development.
Epigenetic bromodomain-containing proteins in cell proliferation (cancer) and spermatogenesis (male contraception)	<p>(+)-JQ-1</p>	Building from results of chemical screening campaigns to modulate bromodomains (relevant to epigenetic regulation), the (+)-JQ-1 compound was developed as an anti-tumor agent by inhibition of BRD4 (BET family member) [81]. Interestingly, the (+)-JQ-1 mechanism of action demonstrated of how small-molecules can disrupt protein-protein interactions. Furthermore, the BET family also contains a member with testis-specific expression (BRDT). After selectivity profiling revealed that (+)-JQ-1 also had activity towards BRDT, a second study demonstrated that (+)-JQ-1 was also useful for reversibly inhibiting spermatogenesis <i>in vivo</i> and thus validated BRDT as a novel drug target for male contraception [82].

Target	Relevant molecule	Discovery
Gene reporter-dependent off-target activity/mechanism aided by chemical library profiling (HTS innovation/efficiency)	<p>NCGC00058026</p> <p>PTC124 (Ataluren)</p>	Our own experience of non-traditional observations of off-target activity has focused on their implications for HTS lead identification. Using HTS to profile luciferase enzyme inhibitors, many chemotypes were discovered that could act as confounding artifacts in reporter gene assays. The NCGC00058026 compound had a strong resemblance to the clinical candidate PTC124, a molecule derived from HTS using a luciferase-dependent reporter gene assay [83]. This finding initiated a study to explore the relationship between <i>in vitro</i> luciferase inhibition and increased luciferase activity elicited by PTC124 in the cell-based HTS assay. It was determined that the likely mechanism of PTC124's potent HTS activity was tied to ligand-dependent stabilization of the luciferase enzyme [84].