



ELSEVIER

Chemical biology and bacteria: not simply a matter of life or death

Deborah T Hung^{1,3} and Eric J Rubin^{2,3}

Chemical biological approaches to understanding bacteria have largely been confined to screening for antibiotics. More complex phenotypes, such as virulence, have largely been studied using bacterial genetics. However, it has recently become clear that these two methods are complementary and that combining chemical biologic and genetic approaches to studying bacteria brings new power to old problems.

Addresses

¹ Department of Microbiology and Molecular Genetics, Harvard Medical School, USA

² Department of Immunology and Infectious Disease, Harvard School of Public Health, USA

³ Division of Infectious Disease, Brigham and Women's Hospital, USA

Corresponding author: Rubin, Eric J (erubin@hsph.harvard.edu)

Current Opinion in Chemical Biology 2006, **10**:321–326

This review comes from a themed issue on
Next-generation therapeutics
Edited by Clifton E Barry III and Alex Matter

Available online 30th June 2006

1367-5931/\$ – see front matter

© 2006 Elsevier Ltd. All rights reserved.

DOI [10.1016/j.cbpa.2006.06.016](https://doi.org/10.1016/j.cbpa.2006.06.016)

Introduction

The concept of chemical biology is old, dating back billions of years. Nature has long exploited the ability of small organic molecules to regulate cellular processes. From steroids acting as hormones in eukaryotic systems [1] to quorum sensing molecules in prokaryotic communities [2], these small-molecule effectors enter the cell and modulate gene and protein expression and function.

This same phenomenon has also been artificially exploited by modern medicine with the recognition that small molecules can be used to control cell phenotypes that impact human disease. With the first use of nitrogen mustards as anti-neoplastic, alkylating agents in 1942 and Alexander Fleming's discovery of penicillin as an antibiotic in 1928 came the recognition that the phenotype of cell death can be conditionally generated by small molecules with clinical utility.

As our understanding of cell biology in both eukaryotes and prokaryotes has grown more sophisticated, we have come to realize that a multitude of phenotypes exist that are far more subtle than simply alive or dead. The past decade has seen a marked acceleration of interest in using

small molecules as tools to systematically dissect the pathways involved in these complex phenotypes, culminating in the generation of a new name for an old phenomenon — chemical genetics [3].

The field of chemical genetics has predominantly fallen within the domain of eukaryotic biology, in part for historical reasons. Some of the earliest demonstrations of the power of chemical genetics resulted in important contributions to eukaryotic biology, using both specific natural products as well as small molecules identified in high-throughput chemical screens. Examples include one of the earliest applications of chemical biology, using colchicine to identify tubulin [4], as well as more recent examples. These include natural products FK506, cyclosporine and rapamycin, which have helped to elucidate immune signaling pathways [5], and monasterol [6] and blebbistatin [7], found in high-throughput screens, which have helped to elucidate steps in mitosis and cytokinesis [8]. By contrast, in prokaryotic systems, much of chemical biology has been relegated to the arena of antibiotic development, a life or death phenotype.

Chemical genetics has also been particularly applicable in eukaryotic systems because, until recently, targeted genetic methods have been largely lacking. In contrast, prokaryotic systems have a rich tradition of classical and molecular genetics with diverse phenotypes often easily generated through manipulation at the genetic level. Despite the power of classical bacterial genetics however, we would argue that chemical approaches can be extremely valuable in the study of diverse and complex bacterial processes. In fact, the potential for chemical genetic to complement classical genetics (and genomics) may well make prokaryotic biology an optimal domain for small-molecule approaches (Table 1).

Definition of chemical genetics

Chemical genetics generally refers to the use of small molecules to conditionally perturb gene function, resulting in the generation of phenotypic changes [3]. In contrast to classical genetics where manipulation occurs at the DNA level, small molecules more typically modulate protein function by inducing conformational changes or competing for naturally occurring protein–ligand or protein–protein interaction sites, resulting in altered activity (Figure 1). Although previously the repertoire of small molecules that were known to function in this manner was relatively small, the advent of high-throughput screening of large chemical libraries has provided a new opportunity to identify such compounds.

Table 1

Comparison of classical and chemical genetics		
	Classical genetics	Chemical genetics
Modulate activity	Yes	Yes
Complete inhibition of function	Yes	Rarely
Rapidity of onset of inhibition	Slow (except for conditionally stable proteins)	Fast
Useful in pathogens during infection	Sometimes (with conditionally regulated promoters)	Sometimes (with bioavailable compounds)
Useful in non-replicating bacteria	Sometimes	Yes
Useful for studying proteins essential for <i>in vitro</i> survival	Sometimes (with conditional mutations)	Yes

By analogy to classical genetics, both forward and reverse genetic strategies may be employed in high-throughput chemical screens to find small molecules of interest (Figure 2). In the reverse approach, small-molecule libraries are screened against purified protein targets. Interesting small-molecule candidates can then be used to study mechanistic aspects of the protein or potentially to generate phenotypes related to that particular protein target if it is sufficiently cell-permeable and potent. In this approach, there is relatively little distinction between eukaryotic and prokaryotic systems [9].

In contrast, a forward approach screens for phenotypes of interest. The challenge, then, is to identify the protein targets of the small-molecule candidates as a means of identifying regulators of a given phenotype. It is in this type of approach that the opportunities in eukaryotic and prokaryotic biology diverge.

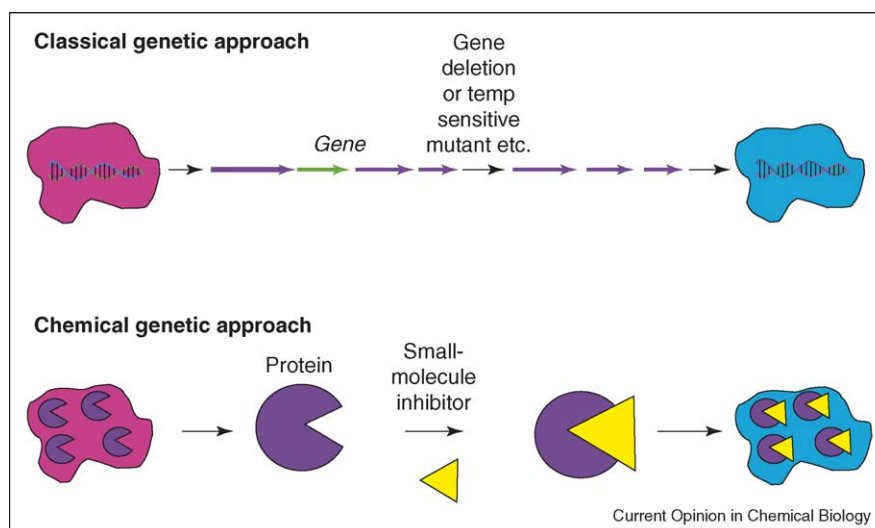
Applying chemical genetics to bacteria

There are several reasons why bacteria are well suited for chemical genetic approaches. The design of phenotypic screens in bacteria is typically easier and the assays less

expensive and more robust, having been performed in diverse organisms including *Yersinia*, *Vibrio cholerae*, *Staphylococcus aureus* and *Escherichia coli* [10,11^{••},12,13,14[•]].

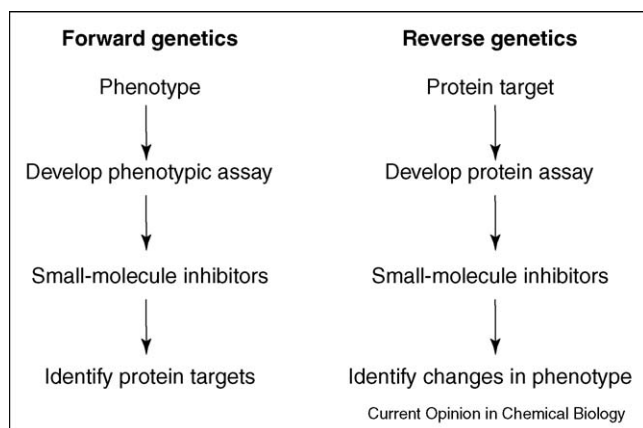
In addition, the marriage of chemical and classical genetics in many bacterial systems can facilitate understanding of the mechanism of action of the small molecule. An example of this is the use of classical genetics to understand the mechanisms of new vancomycin analogues by generating resistant mutants [15]. This example shows that generation of random or transposon mutants, or complementation with cosmid or expression libraries to generate resistance to a given small molecule are easily attainable goals with the help of conventional genetics. The inherently smaller size of bacterial genomes relative to eukaryotic ones (specifically, mammalian genomes) also allows straightforward systematic approaches to identifying and understanding cellular targets of small molecules (Table 2). For example, arrayed libraries of bacteria that knock-out [16,17], knock down [18] or overexpress every opening reading frame (ORF) can be used to assay compounds and quickly determine their effects. In eukaryotes, however, such systems are far more difficult to use

Figure 1



Genetic approach to obtaining conditional phenotypes.

Figure 2



Forward and reverse approaches to using chemical genetics.

and, thus far, are limited to a small number of species [19,20].

What does chemical genetics add to traditional bacterial genetics?

Traditional bacterial genetics provides two distinct sets of tools: mutations that alter a gene product's function, and mutations that affect the amount of a gene product. Mutations that alter function (i.e., point mutations) can result in loss or gain of function or, in the case of enzymes, changes in K_d , k_{cat} , or substrate specificity.

Mutations that affect the amount of a gene product fall into two classes: those that affect RNA abundance and translation, and those that affect protein stability. In many bacterial systems, genetic techniques exist to make deletions in genes, either in a directed fashion or randomly through transposon mutagenesis. In what is essentially a chemical genetics approach, regulated promoters can be used to inducibly express genes at different levels [21,22]. It is far more difficult to reliably construct proteins that are conditionally stable. Most such mutations result in

proteins whose function is lost at high temperature. Such mutants may be problematic as they grow only under restricted conditions that can result in numerous unrelated changes to the cell, and the mutant proteins often lack full activity under permissive conditions. More importantly, temperature-sensitive mutants can only be isolated by a relatively laborious process that is only successful for about a third of known essential genes [23].

The limitation of these types of traditional mutants is that they are not truly conditional. One cannot turn the gene products on and off at will on a short time scale, in a tunable manner that is also reversible. This limitation has several implications. Essential genes are difficult to study because it is difficult to isolate mutations that result in lethality. Generation of mutations on the DNA level often results in compensatory changes such as up-regulation of other related genes that confound or distort the phenotype related to the single gene.

Enter chemical genetics, a method that targets proteins on a rapid time scale with the addition of the small molecule, and in a reversible manner with washout of the small molecule, and can be fine-tuned to inhibit one but not another domain of a given protein. This approach is particularly useful in the study of bacteria for several reasons. It allows the study of wild-type bacteria that have no deleterious mutations. Small molecules can be used to cause immediate changes in function, an attractive feature in circumstances where protein or RNA half-lives are prolonged (for example, in non-dividing bacteria such as *Mycobacterium tuberculosis*). Lastly, small molecules can be used to 'conditionally' probe the functions of pathogens during infection *within the context of the host*, or of bacteria as they interact in complex communities *in their natural ecological niche*. Examples show how small molecules can be used to study the *in vivo* requirements for quorum sensing in an *S. aureus* abscess model [24[•]] and for virulence regulators in a *V. cholerae* intestinal colonization model [11^{••}]. With the increasing recognition of the limitations of studying bacteria *in vitro* outside of the context of their natural environment, the development of tools to study bacteria in their natural environment will be vital to the next phase of microbiological discovery.

Current challenges

While the marriage between chemical genetics and prokaryotic biology holds great potential, several challenges must be met to make these approaches widely applicable. These challenges include systematic methods for identifying protein targets, specificity and potency of small molecule inhibitors, and development of appropriate chemical libraries suited for prokaryotic systems.

Target identification is a major challenge in forward genetic, phenotypic screens. While target identification

Table 2

Comparison of size of selected genomes

Organism	Genome size ^a	Genes
<i>Haemophilus influenza</i>	1.8	1738
<i>Staphylococcus aureus</i>	2.8	2929
<i>Mycobacterium tuberculosis</i>	4.4	3959
<i>Escherichia coli</i>	4.6	4377
<i>Pseudomonas aeruginosa</i>	6.3	5570
<i>Saccharomyces cerevisiae</i>	12.5	5770
<i>Plasmodium falciparum</i>	22.9	5268
<i>Caenorhabditis elegans</i>	100	19 427
<i>Drosophila melanogaster</i>	122	13 379
<i>Homo sapiens</i>	3300	20–25 000

^a Megabases.

is not always critical, it is often important for further work. Methods identifying targets have been previously reviewed [25] and include total sequencing of resistant mutants in *M. tuberculosis* [26^{••}], transposon mutagenesis [27] in suppressor and enhancer screens, and affinity-based assays such as three-hybrid systems [28,29], changes in 2-D electrophoretic mobility [30], affinity purification [31], and biotinylation with or without cross-linking [32]. The rapidly expanding repertoire of genomic and proteomic tools is likely to have a significant impact on target identification, with the ability to match gene expression patterns [33] or assay direct binding in protein chips [34]. However, even in the absence of direct target identification, small-molecule candidates can serve as tools to understand biology through indirect means. For example, because they cause conditional blocks, other regulators of phenotype can be identified by genetic suppressor or enhancer screens, even in the absence of specific target identification. In the case of brefeldin, the compound was used to study protein secretion long before its actual target was identified [35].

Compounds that are used as drugs must generally be specific and highly avid. However, even agents with less than optimal properties can be quite useful experimentally. Many probes and 'proof of concept' inhibitors can be used to preliminarily define phenotypes and validate targets without having a high binding affinity [11^{••}]. These can then serve as starting points for further experiments and, possibly, chemical optimization.

The last major challenge is that existing compound libraries are not ideally suited for use in bacterial chemical genetic screens. Much debate exists over the two types of libraries that currently exist: 'drug-like' and 'natural product-like' libraries. Which is better for screening prokaryotic systems? Large commercial compound libraries are available that have been generated to fit a set of physicochemical criteria that describe existing drugs, creating a set of 'drug-like' molecules [36]. The difficulty with these collections in application to bacteria is twofold. First, although libraries contain enormous numbers of compounds, many of these chemicals were originally synthesized with specific targets in mind (typically eukaryotic enzymes), and they are therefore biased toward a small number of enzymes, such as protein kinase inhibitors. Thus, these libraries actually have limited diversity.

Secondly, current compound libraries consist largely of chemicals that have 'drug-like properties' as defined for eukaryotic systems [36]. In contrast, most known antibiotics violate these properties, with higher molecular weights, greater rigidity and fewer degrees of freedom, more stereogenic centers, larger or more complex ring scaffolds, fewer nitrogen, sulfur and halogen atoms with more oxygen atoms, and more hydrogen bonding elements [37]. In fact, most current antibiotics are natural

products or 'natural product-like', with the fluoroquinolones and linezolid as the marked synthetic exceptions.

Is nature trying to teach us something? Unlike humans who are perhaps the accidental tourist in the biosphere, bacteria have existed millions of years in the environment, co-evolving with other organisms that produce antibacterial, natural products (for example, *Streptomyces* strains, marine organisms, plants, fungi, and other bacteria [38,39]). This evolution of natural products most certainly did not occur in the setting of selection pressure from humans, but more likely from bacteria. In the words of Samuel Danishefsky, "There are major teachings in these natural products that we would do well to consider. They may be reflecting eons of wisdom and refinement" [40].

Because of the co-evolution of natural products with bacterial targets, even more than eukaryotic ones, natural product or 'natural product-like' libraries may be optimal for screening bacterial systems. Such libraries can be constructed using strategies such as diversity-oriented synthesis (DOS) [41]. Whether these DOS libraries are generated based on a natural product scaffold [42], or generating some configuration of 'natural product-like' elements [43], or a hybrid of the two [44], these libraries will probably be critical to the success of the marriage of chemical genetics and prokaryotic biology.

Conclusion

In the end, does chemical genetics have any relevance to therapeutics and drug development? Is it merely an academic exercise that, at best, generates powerful tools for biological investigation and, at worst, results in a collection of 'hits' relegated to some shelf in a laboratory? We believe that one of the great strengths of chemical genetics is exactly that it is based on small molecules and thus a step closer to drug development than alternative genetic approaches. This does not imply that every hit obtained in such chemical screens is a candidate drug. Though all hits have the potential to be so, it would be naïve to have such expectations. Instead, we would argue that every hit can serve another important function.

Much effort is currently invested in identifying essential genes in bacteria using classical genetics and genomics. These genes are then designated as 'potential drug targets', without any true sense of how 'drug-targetable' they are. Although essential gene products may appear to be good targets, partial inhibition by a compound may not result in death. In addition, the function of a putative target might overlap enough with that of another protein so that inhibition might produce little or no phenotypic change. The not infrequent failure of candidates identified in reverse chemical genetic screens (which target a preselected gene product) to generate the desired phenotype is testament to the dangers of such an approach,

exacerbated by the uncertainty of the cell-permeability of any given small-molecule inhibitor.

In contrast, a forward chemical genetic approach guarantees that hits define good drug targets. This strategy allows the small molecule to identify the 'Achilles heel' of the cellular pathway without relying on *a priori* assumptions. Thus, this strategy allows us to identify and focus on reasonable biological candidates for drug development, rather than to be caught in random, *a la carte* selection from long lists of essential genes.

The field of chemical genetics as applied to bacteria is currently wide open. We have the opportunity to reap the benefits from decades of microbiological discovery that have taken us far beyond the idea that the only phenotype of interest in bacteria is alive or dead. This approach should allow us to considerably expand the limited repertoire of functions targeted by current antibiotics and provide important weapons to help turn the tide in the war of resistance that we are currently losing against pathogens.

Acknowledgements

DTH is supported by NIH grant AI060708. EJR is supported in part by NIH grant AI51929 and by the Investigators in Pathogenesis of Infectious Disease Award from the Burroughs Wellcome Fund.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Freedman LP: **Increasing the complexity of coactivation in nuclear receptor signaling.** *Cell* 1999, **97**:5-8.
2. Camilli A, Bassler BL: **Bacterial small-molecule signaling pathways.** *Science* 2006, **311**:1113-1116.
3. Mitchison TJ: **Towards a pharmacological genetics.** *Chem Biol* 1994, **1**:3-6.
4. Weisenberg RC, Borisy GG, Taylor EW: **The colchicine-binding protein of mammalian brain and its relation to microtubules.** *Biochemistry* 1968, **7**:4466-4479.
5. Schreiber SL, Liu J, Albers MW, Karmacharya R, Koh E, Martin PK, Rosen MK, Standaert RF, Wandless TJ: **Immunophilin-ligand complexes as probes of intracellular signaling pathways.** *Transplant Proc* 1991, **23**:2839-2844.
6. Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ: **Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen.** *Science* 1999, **286**:971-974.
7. Straight AF, Cheung A, Limouze J, Chen I, Westwood NJ, Sellers JR, Mitchison TJ: **Dissecting temporal and spatial control of cytokinesis with a myosin II inhibitor.** *Science* 2003, **299**:1743-1747.
8. Peterson JR, Mitchison TJ: **Small molecules, big impact: a history of chemical inhibitors and the cytoskeleton.** *Chem Biol* 2002, **9**:1275-1285.
9. Turk BE, Wong TY, Schwarzenbacher R, Jarrell ET, Leppla SH, Collier RJ, Liddington RC, Cantley LC: **The structural basis for substrate and inhibitor selectivity of the anthrax lethal factor.** *Nat Struct Mol Biol* 2004, **11**:60-66.
10. Kauppi AM, Nordfelth R, Uvell H, Wolf-Watz H, Elofsson M: **Targeting bacterial virulence: inhibitors of type III secretion in yersinia.** *Chem Biol* 2003, **10**:241-249.
11. Hung DT, Shakhnovich EA, Pierson E, Mekalanos JJ: **Small-molecule inhibitor of *Vibrio cholerae* virulence and intestinal colonization.** *Science* 2005, **310**:670-674.
Describes a chemical genetic approach to virulence expression in *Vibrio cholerae* through a high-throughput chemical screen to identify virstatin, a small-molecule inhibitor of cholera toxin and TCP expression, by inhibition of the transcriptional regulator ToxT, which has efficacy in an intestinal mouse model for cholera, thus demonstrating the potential for chemical genetic *in vivo* studies.
12. Nicolaou KC, Roecker AJ, Barluenga S, Pfefferkorn JA, Cao GQ: **Discovery of novel antibacterial agents active against methicillin-resistant *Staphylococcus aureus* from combinatorial benzopyran libraries.** *ChemBioChem* 2001, **2**:460-465.
13. Gauthier A, Robertson ML, Lowden M, Ibarra JA, Puente JL, Finlay BB: **Transcriptional inhibitor of virulence factors in enteropathogenic *Escherichia coli*.** *Antimicrob Agents Chemother* 2005, **49**:4101-4109.
14. Nordfelth R, Kauppi AM, Norberg HA, Wolf-Watz H, Elofsson M: **Small-molecule inhibitors specifically targeting type III secretion.** *Infect Immun* 2005, **73**:3104-3114.
Describes small molecules (acetylated salicylates) that inhibit the type III secretion system in *Yersinia tuberculosis* and Yop secretion, resulting in attenuated infection of HeLa cells.
15. Eggert US, Ruiz N, Falcone BV, Branstrom AA, Goldman RC, Silhavy TJ, Kahne D: **Genetic basis for activity differences between vancomycin and glycolipid derivatives of vancomycin.** *Science* 2001, **294**:361-364.
16. Jacobs MA, Alwood A, Thaipisuttikul I, Spencer D, Haugen E, Ernst S, Will O, Kaul R, Raymond C, Levy R *et al.*: **Comprehensive transposon mutant library of *Pseudomonas aeruginosa*.** *Proc Natl Acad Sci USA* 2003, **100**:14339-14344.
17. Liberati NT, Urbach JM, Miyata S, Lee DG, Drenkard E, Wu G, Villanueva J, Wei T, Ausubel FM: **An ordered, nonredundant library of *Pseudomonas aeruginosa* strain PA14 transposon insertion mutants.** *Proc Natl Acad Sci USA* 2006, **103**:2833-2838.
18. Ji Y, Zhang B, Van SF *et al.*: **Identification of critical staphylococcal genes using conditional phenotypes generated by antisense RNA.** *Science* 2001, **293**:2266-2269.
19. Lamesch P, Milstein S, Hao T, Rosenberg J, Li N, Sequerra R, Bosak S, Doucette-Stamm L, Vandenhaute J, Hill DE *et al.*: **C. elegans ORFeome version 3.1: increasing the coverage of ORFeome resources with improved gene predictions.** *Genome Res* 2004, **14**:2064-2069.
20. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H *et al.*: **Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis.** *Science* 1999, **285**:901-906.
21. Gossen M, Bujard H: **Tight control of gene expression in mammalian cells by tetracycline-responsive promoters.** *Proc Natl Acad Sci USA* 1992, **89**:5547-5551.
22. Guzman LM, Belin D, Carson MJ, Beckwith J: **Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter.** *J Bacteriol* 1995, **177**:4121-4130.
23. Wong SM, Akerley BJ: **Inducible expression system and marker-linked mutagenesis approach for functional genomics of *Haemophilus influenzae*.** *Gene* 2003, **316**:177-186.
24. Wright JS III, Jin R, Novick RP: **Transient interference with staphylococcal quorum sensing blocks abscess formation.** *Proc Natl Acad Sci USA* 2005, **102**:1691-1696.
Describes the use of a small-molecule inhibitor (AIP variant) to inhibit quorum sensing in an *in vivo* model of staphylococcal abscess formation and shows the timing requirements for agr (global accessory gene regulator) during infection.
25. Tochtrop GP, King RW: **Target identification strategies in chemical genetics.** *Comb Chem High Throughput Screen* 2004, **7**:677-688.

26. Andries K, Verhasselt P, Guillemont J, Gohlmann HW, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E *et al.*: **A diarylquinoline drug active on the ATP synthase of mycobacterium tuberculosis.** *Science* 2005, **307**:223-227.
Describes the identification of a new drug against *Mycobacterium tuberculosis*, with demonstration of rapid killing and clearing in mice, and the possible characterization of its target through total genome sequencing of resistant mutants.
27. Moore JM, Salama NR: **Mutational analysis of metronidazole resistance in *Helicobacter pylori*.** *Antimicrob Agents Chemother* 2005, **49**:1236-1237.
28. Licitra EJ, Liu JO: **A three-hybrid system for detecting small ligand-protein receptor interactions.** *Proc Natl Acad Sci USA* 1996, **93**:12817-12821.
29. Becker F, Murthi K, Smith C, Come J, Costa-Roldan N, Kaufmann C, Hanke U, Degenhart C, Baumann S, Wallner W *et al.*: **A three-hybrid approach to scanning the proteome for targets of small molecule kinase inhibitors.** *Chem Biol* 2004, **11**:211-223.
30. Towbin H, Bair KW, DeCaprio JA, Eck MJ, Kim S, Kinder FR, Morollo A, Mueller DR, Schindler P, Song HK *et al.*: **Proteomics-based target identification: bengamides as a new class of methionine aminopeptidase inhibitors.** *J Biol Chem* 2003, **278**:52964-52971.
31. Harding MW, Galat A, Uehling DE, Schreiber SL: **A receptor for the immunosuppressant FK506 is a *cis-trans* peptidyl-prolyl isomerase.** *Nature* 1989, **341**:758-760.
32. Sin N, Meng L, Wang MQ, Wen JJ, Bornmann WG, Crews CM: **The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2.** *Proc Natl Acad Sci USA* 1997, **94**:6099-6103.
33. Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennett HA, Coffey E, Dai H, He YD *et al.*: **Functional discovery via a compendium of expression profiles.** *Cell* 2000, **102**:109-126.
34. MacBeath G, Schreiber SL: **Printing proteins as microarrays for high-throughput function determination.** *Science* 2000, **289**:1760-1763.
35. Morinaga N, Tsai SC, Moss J, Vaughan M: **Isolation of a brefeldin A-inhibited guanine nucleotide-exchange protein for ADP ribosylation factor (ARF) 1 and ARF3 that contains a Sec7-like domain.** *Proc Natl Acad Sci USA* 1996, **93**:12856-12860.
36. Ajay A, Walters WP, Murcko MA: **Can we learn to distinguish between 'drug-like' and 'nondrug-like' molecules?** *J Med Chem* 1998, **41**:3314-3324.
37. Feher M, Schmidt JM: **Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry.** *J Chem Inf Comput Sci* 2003, **43**:218-227.
38. Gillespie DE, Brady SF, Bettermann AD, Cianciotto NP, Liles MR, Rondon MR, Clardy J, Goodman RM, Handelsman J: **Isolation of antibiotics turbomycin A and B from a metagenomic library of soil microbial DNA.** *Appl Environ Microbiol* 2002, **68**:4301-4306.
39. Brady SF, Chao CJ, Clardy J: **New natural product families from an environmental DNA (eDNA) gene cluster.** *J Am Chem Soc* 2002, **124**:9968-9969.
40. Borman S: **Interview with Samuel Danishefsky.** *Chem Eng News* 2002, **80**:23.
41. Tan DS: **Current progress in natural product-like libraries for discovery screening.** *Comb Chem High Throughput Screen* 2004, **7**:631-643.
42. Pelish HE, Westwood NJ, Feng Y, Kirchhausen T, Shair MD: **Use of biomimetic diversity-oriented synthesis to discover galanthamine-like molecules with biological properties beyond those of the natural product.** *J Am Chem Soc* 2001, **123**:6740-6741.
43. Tan DS, Foley MA, Shair MD, Schreiber SL: **Stereoselective synthesis of over two million compounds having structural features both reminiscent of natural products and compatible with miniaturized cell-based assays.** *J Am Chem Soc* 1998, **120**:8565-8566.
44. Nicolaou KC, Pfefferkorn JA, Cao GQ: **Selenium-based solid-phase synthesis of benzopyrans I: applications to combinatorial synthesis of natural products.** *Angew Chem Int Ed Engl* 2000, **39**:734-739.