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Combinatorial chemistry and molecular diversity
Tools for molecular diversification and their applications in chemical biology

Editorial overview

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Prof Silverman received his B.S. degree in chemistry from UCLA in 1991. He received his Ph.D. degree in chemistry with Dennis A Dougherty at Caltech in 1997, and he performed postdoctoral research with Thomas R Cech at the University of Colorado at Boulder. In 2000, he joined the Department of Chemistry at the University of Illinois at Urbana-Champaign, where his research focuses on fundamental and applied studies of nucleic acids. This includes investigations of RNA folding and catalysis as well as studies of DNA as a catalyst, conformational constraint and sensor.

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Prof Hergenrother received his B.S. in chemistry from the University of Notre Dame, graduating in 1994. He went on to the University of Texas at Austin, where he received his Ph.D. in chemistry in 1999 working with Stephen F Martin. After a postdoctoral stint with Stuart L Schreiber at Harvard University, he joined the Department of Chemistry at the University of Illinois at Urbana-Champaign in 2001. His research is focused on using small molecules to identify novel targets for the treatment of cancer, neurodegeneration and drug-resistant bacteria.

Combinatorial techniques are now firmly implanted in the mainstream of chemical biology, and every passing year brings exciting new applications of developed combinatorial strategies. As the limits of high-throughput approaches to molecular diversity are pushed, there is a constant need for the development of novel technologies that will enable next-generation approaches. The reviews in this issue of *Current Opinion in Chemical Biology* cover recent advances in many areas of chemical biology, and they describe developments in both *tools* for combinatorial synthesis and analysis as well as *applications* of molecular diversity to solve important problems in chemical biology. The tools that are covered include computational design, accessing compound collections, and novel supports for compound synthesis, as well as NMR spectroscopy, microfluidics and fluorescence microscopy. The applications range from novel nanomaterials, glycosides, and fluorophores to aptamer sensors and therapeutics. In some cases, the distinction between tools and applications is blurry, as new tools are a prerequisite for many state-of-the-art applications. Implicit in our selection of topics is the notion that 'combinatorial chemistry' is no longer defined by the traditional bead-linker-compound triumvirate, but rather encompasses numerous strategies for molecular diversification with broad impact on chemistry, biology, physics and materials science.

Although structure-based design and combinatorial chemistry traditionally have existed as separate (and sometimes rival) camps, it is increasingly recognized that together they make a powerful pair. **Rupasinghe and Spaller** illustrate the considerable benefits of searching for a small-molecule ligand for a particular receptor by first using structure-based design methods to narrow the chemical space, followed by combinatorial synthesis of a family of related small molecules. This approach acknowledges that structure-based design methods are not to the point where a single 'best' compound can be identified purely through computational methods. Instead, such methods should be viewed as a tool to inform the design of a focused combinatorial library.

A computational approach that has proven very useful in combinatorial chemistry is that of structure-based virtual screening. **Ghosh, Nie, An and Huang** describe several recent examples of this approach to generate lead compounds for further drug development. Although many challenges remain in such efforts, a tremendous advantage is the very large number of compounds that may be interrogated without the need for chemical synthesis or an actual high-throughput screen.

Traditionally, combinatorial libraries have been either made on solid support, or they have been synthesized in solution and solid-supported reagents have been used to facilitate purification. However, it is now becoming clear that the direct synthesis of combinatorial libraries as arrays on planar solid supports offers considerable advantages. For this purpose **Blackwell** details the use of the SPOT technique, in which macroarrays of small molecules are constructed on cellulose paper. This method offers advantages for both synthesis and screening of focused combinatorial libraries containing approximately 50–200 members.

Scientists seeking to address biological questions are often keenly interested in screening collections of small molecules. However, many biologically oriented scientists often have limited access to such compounds, along with little or no training in the construction of combinatorial libraries. Fortunately, for many purposes the fastest means by which to identify a biologically active compound is not through synthesis of a new combinatorial library, but rather by screening a pre-existing set of compounds. Where can one obtain such collections? **Hergenrother** addresses this question by providing a practical guide to those seeking access to compound collections. His review details private, public, commercial and free sources of small molecules. In many cases, the most difficult part of constructing a compound collection — the actual small-molecule synthesis — has already been performed. As such, the notion to harness the existing power (and existing compounds) within any chemistry department is discussed. A model is proposed for how to formalize a relationship between chemists and biologists to make optimal use of compounds that have already been synthesized.

In the drug discovery process, methodological advances can assist every step of the process, from identifying and validating targets to assaying the *in vivo* properties of drug candidates. **Betz, Saxena and Schwalbe** provide an update on the use of NMR spectroscopy through all stages of drug discovery and development. They show how NMR can be judiciously applied to advance essentially all aspects of the chemistry and biology of identifying novel pharmaceutically active compounds.

As a relatively new and intriguing methodology, microfluidics has had a substantial impact on many scientific areas. Here, **Chen and Ismagilov** describe the use of nanoliter 'plugs' of compounds in microfluidic systems. Such systems could be valuable for screening small-molecule compound libraries without consuming large amounts of precious compounds. Microfluidics may also be applied for enzyme assays and protein crystallization trials, again with minimal usage of expensive samples. In some cases, the microfluidic systems may even be applied to organic synthesis. In these cases, the combinatorial facet of the experiment is not in the structure of the final product but the choice of reagents and reaction conditions.

The logistics of biological assays have traditionally limited high-throughput screens to readouts of the chromogenic, fluorescent or luminescent variety. As compounds with very specific phenotypic effects on cells are increasingly sought, such general assays become less useful. **Eggert and Mitchison** detail the use of sophisticated microscopy in the high-throughput screening of compound collections. Using such approaches, it is now possible to capture images of each and every well in a high-throughput screen, allowing rapid identification of hit compounds through computational image analysis. Recent advances that are addressed are the use of model organisms such as zebrafish and the development of automated methods for image analysis.

Imaging approaches often depend on the availability of organic moieties with useful fluorescent properties (i.e., organic fluorophores). **Finney** reviews the latest combinatorial approaches to new fluorophore synthesis with an eye on developing novel fluorescent probes. Such efforts promise to expand the methodological toolkit available for many applications in which an optical signal is the key to the experimental design.

Uses of phage for combinatorial materials chemistry are described by **Merzlyak and Lee**. By presenting up to 100 billion peptide sequences on the surface of viral particles, phage display can be used to identify peptides that bind to inorganic materials such as semiconductor substrates and carbon nanotubes. Phages can also be used to direct the assembly of nanomaterials; a particularly interesting example of this is described in bone mineralization.

For many applications throughout chemistry and biology, an unnatural chemical group must be attached to a biomolecule. **Antos and Francis** show how careful chemical design using transition metals can expand our ability to synthesize such bioconjugates. By focusing on the side chains of relatively rare amino acids such as tryptophan and tyrosine, efficient coupling of proteins to organic reagents can be achieved. In addition, when an unnatural amino acid can be incorporated into a protein of interest, a number of reactions have been developed to enable subsequent bioconjugation.

A natural type of bioconjugation is the attachment of sugars to small-molecule compounds, forming glycosides. **Blanchard and Thorson** describe how enzymes may be used for natural product glycosylation, thereby expanding our access to compounds that are likely to have biological activity. A key insight of the new approach termed 'combinatorial biosynthesis' is that a fundamental understanding of nucleotidesugar biosynthesis pathways

combined with discovery of useful glycosyltransferase enzymes can be applied experimentally for natural products chemical biology.

Many practical applications require sensing of a molecular species in combination with a method to report on the sensing event. Recent years have seen considerable advances in the use of nucleic acids for such purposes. **Navani and Li** outline a wealth of approaches along these lines, using either nucleic acid aptamers that bind to ligands or nucleic acid enzymes that both bind to ligands and catalyze subsequent chemical reactions. The multifaceted abilities of 'functional nucleic acids' (FNAs) show that nucleic acid platforms are particularly useful in assays for detecting targets as diverse as metal ions, small-molecule metabolites, proteins and nucleic acids. In some cases, nucleic acid sensors may also be used within drug discovery efforts.

Finally, nucleic acid aptamers may be applied directly as therapeutic compounds. By considering both *in vitro* selection technologies and questions of drug delivery, **Lee, Stovall and Ellington** review the latest advances in using aptamers as therapeutics. Several aptamers are in clinical trials and one is in clinical use; prospects are discussed for their future use against both extracellular and intracellular targets. In a nice illustration of the interplay between tools and applications, the desire for practical aptamers has spurred the development of technical advances in both selection methodology and chemical modification of nucleic acids. For clinical use, a major challenge for aptamers is to control aspects of their delivery and *in vivo* pharmacokinetics, and efforts in both areas are addressed.

Methods for 'traditional' combinatorial chemistry — the construction and screening of small-molecule libraries — have continued to advance as new tools have facilitated cleaner reactions, simpler purifications, and more sophisticated screens. The creative and successful application of these tools to the most pressing scientific problems of the day will ultimately validate these tools and advance not just the fields of combinatorial chemistry and molecular diversity but science as a whole. As editors of this issue, we are grateful to all of the authors for their time and effort in updating all of us on the leading edge of chemical biology research in combinatorial chemistry and molecular diversity.