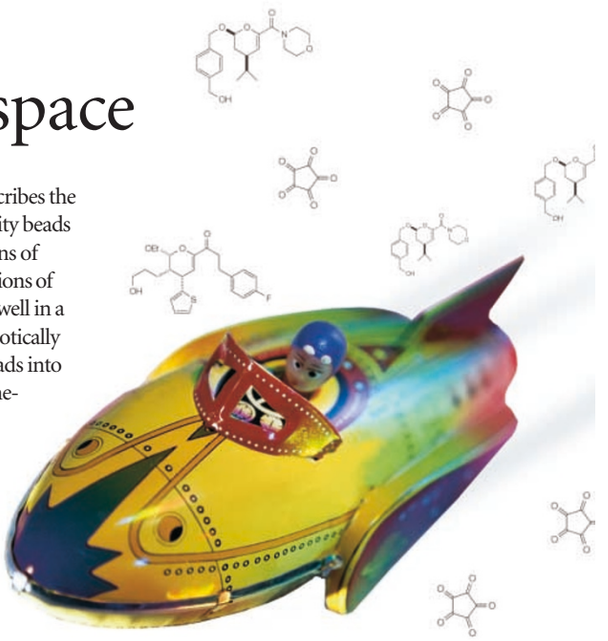
CHEMICAL GENETICS

Exploring chemical space



In the field of chemical genetics, small molecules are used in place of genetic modification to modulate the functions of proteins. If your aim is to boldly go into this strange new world, you first need to equip yourself with a potentially interesting set of chemical modulators. In back-to-back articles in December's *Chemistry & Biology*, Stuart Schreiber and colleagues describe a general method to synthesize large and diverse chemical libraries for use in chemical genetics, and a technology platform for the automated delivery of those libraries into multiple screening assays.

In the main, the described method relies on improving and optimizing many existing techniques. The first paper describes the synthesis of a 4,320-member library of dihydropyrancarboxamides by split-pool synthesis, a combinatorial strategy that is designed to give the greatest chemical diversity for the smallest number of synthetic steps. The chemical reactions were carried out on building blocks that were linked to 500–600-μm polystyrene macrobeads by very stable silicon-based linkers, which were shown to withstand a remarkably varied array of reaction conditions. The relatively large size of the beads had two distinct advantages: they were large enough to be automatically arrayed into standard 384-well plates at a density of one bead per well, and each bead gives rise to around 100 nmoles of synthetic material, potentially enough for thousands of assays.

The second paper describes the conversion of high-capacity beads into arrayed stock solutions of compounds at concentrations of approximately 5 mM per well in a microtitre plate. After robotically transferring the macrobeads into individual wells, the synthesized compounds were cleaved from their linkers by treatment with hydrogen fluoride/pyridine, eluted, and then resuspended as stock solutions. These plated stocks were then automatically diluted into 'daughter plates' for use in several chemical genetic assays as proof of principle.

Compounds were identified by keeping track of the synthetic history of each bead. Every reaction step and added building block is encoded on the bead with a chloro-aromatic tag, and the binary sequence of tags can then be read by electron-capture gas chromatography once the compound is cleaved from the bead. By good fortune, the authors found that a small amount of tag also inserted itself into the compounds, but not at sufficient levels to alter their chemical genetic behaviour. The levels of tag were, however, high enough for the history of each compound to be decoded by subjecting a few microlitres of the stock solution to the decoding procedure, obviating the need to analyse the bead after cleavage of the compound. Automated decoding of stock solutions should prove to be a very useful way of determining the structure of 'hits' from chemical genetic assays.

Schreiber and colleagues coin the term 'annotation screening' to describe the collection of multiple data sets of biological information by comprehensively screening new chemical libraries using this highly automated approach. Their technology platform uses only commercially available reagents, which might encourage others to explore this final frontier.

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References and links

ORIGINAL RESEARCH PAPERS Blackwell, H. E. *et al.* A one-bed, one-stock solution approach to chemical genetics: part 1. *Chemistry & Biology* **8**, 1167–1182 (2001) | Clemons, P. A. *et al.* A one-bed, one-stock solution approach to chemical genetics: part 2. *Chemistry & Biology* **8**, 1183–1195 (2001)
WEB SITE Schreiber's lab: http://www.schreiber.chem.harvard.edu/index.html