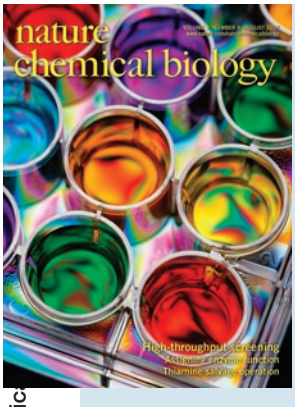
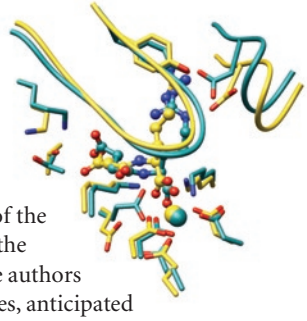


High-throughput screening

High-throughput screening (HTS) of small-molecule libraries has become the predominant strategy to look for new drug leads. Beyond its importance in drug discovery, HTS is being increasingly used by academic scientists to look for chemical probes of biological processes. In this issue, we present a collection of articles designed to reflect the current state of the art in HTS, with a particular emphasis on practical considerations that will be important to investigators new to the field. Inglese and colleagues provide an overview of the ins and outs of choosing and conducting an HTS assay, whether with purified proteins or in a cell-based screen [**Review, p. 466**]. Shelat and Guy provide insight into the chemical scaffold composition of commercial screening libraries and ideas for how to select an appropriate library from among the increasing number of commercial options [**Commentary, p. 442**]. Carpenter provides a guide to conducting microscopy-based primary chemical screens, which have opened the door to screening for subtle cellular phenotypes [**Perspective, p. 461**]. To increase the transparency of the screening process and enable comparisons between the numerous published screens, Inglese, Shamu and Guy provide some guidelines for information to include when reporting small-molecule screens [**Commentary, p. 438**]. Oprea and colleagues propose a cheminformatic web interface that would bring together the cheminformatic and computational biology tools that will be necessary for analyzing the large amount of screening data that is being generated [**Commentary, p. 447**]. Overall, the increasing sophistication of chemical screening promises to broaden our understanding of the biological activities of small molecules and dramatically expand the range of biological processes that can be chemically modulated. *JK*

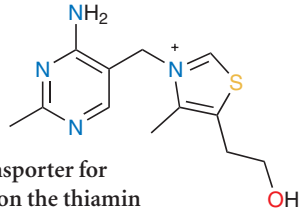
On the search for function



Assigning function to the growing number of putative polypeptides in a robust way is an inherent challenge. In particular, Song *et al.* suspected that the high homology of BC0371 to an epimerase in the enolase superfamily resulted in its misassignment, as the low turnover of the prospective substrate suggested that the enzyme had a different function. The authors performed docking of likely substrates, anticipated by the well-defined chemistry of the superfamily, into a homology model in which multiple rotamer conformations of important active site residues were sampled to identify superior substrates. Kinetic and crystallographic verification of these substrates, and in particular the near perfect overlay of the optimal homology model with the experimentally derived structure, allowed them to confidently reassign the protein as an *N*-succinyl arginine/lysine racemase. [**Letters, p. 486; News & Views, p. 452**] *CG*

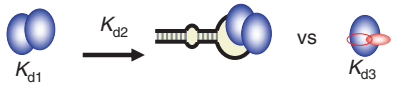
Written by Mirella Bucci, Catherine Goodman & Joanne Kotz

Thiamin comes full circle



Bacterial thiaminase II is encoded by TenA and has long been thought to be responsible for hydrolysis of thiamin. In *Bacillus halodurans*, TenA clusters with genes for the protease YlmB and a putative transporter for hydroxypyrimidine, an intermediate on the thiamin biosynthetic pathway. This led Haas Jenkins *et al.* to propose that rather than being involved in thiamin hydrolysis, thiaminase II is involved in a salvage pathway in which thiamin formed from the decay of various organisms is degraded by the basic soil in which the bacteria live. When the authors added purified recombinant YlmB and TenA proteins to a solution of soil-degraded thiamin, a new product, formylaminopyrimidine, was identified. Transport of formylaminopyrimidine to the cell interior and deformylation by YlmB yielded aminopyrimidine. TenA could then convert aminopyrimidine to hydroxypyrimidine for incorporation into the thiamin biosynthetic cycle, thus completing the recycling of this important cofactor. [Letters, p. 492; News & Views, p. 454] *MB*

Hu are you?



mRNAs with (A+U)-rich elements in their 3′ untranslated regions are short-lived unless they are bound and stabilized by Hu proteins, a family of post-transcriptional gene regulators. To begin to understand an emerging relationship between HuR and cancer, Meisner *et al.* screened approximately 50,000 natural product extracts for compounds that would inhibit the interaction of HuR with a target RNA. After mapping the binding site of the compounds to two RNA recognition motif domains of HuR, the authors proposed nine mechanisms to explain the inhibition by the compounds. Using a mathematical analysis, they determined that the model that fits equilibrium binding and competition data most reliably is the one in which the compounds inhibit the RNA-protein interaction by interfering with HuR homodimerization. This demonstration of the stoichiometry of HuR-RNA binding and identification of a Hu-targeted compound should help elucidate the role of Hu proteins in the stabilization of mRNAs involved in cancer. [**Articles, p. 508**] *MB*

PTT biosynthesis redefined

Phosphonothricin tripeptide (PTT) is an unusual natural product in that it contains a phosphinic acid group, which incorporates a C-P bond. While some intermediates of the biosynthetic pathway were known, a recent report of the full gene cluster indicated that there are more proteins acting in the pathway than expected. Using a variety of *in vivo* and *in vitro* assays, Blodgett *et al.* now report an expanded pathway that firmly establishes hydroxyethylphosphonate as an on-pathway intermediate and further identifies a new intermediate, CMP-5′-phosphonoformate, as a precursor to the antibiotic. Similarly, these new insights into the biochemical process allowed the authors to reassign the putative function of the enzyme encoded by *phpH*. The increased understanding of PTT biosynthesis now opens the door to the detailed enzymatic characterization of these interesting reactions. [Letters, p. 480] *CG*