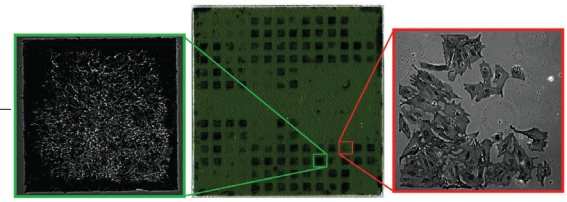
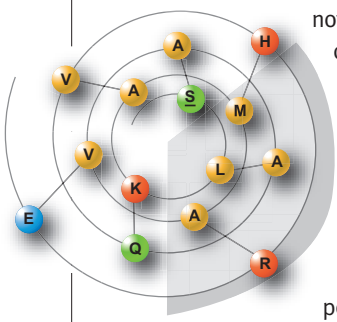
In this ISSUE



Transferring Labels

The thousands of proteins in the cell make it difficult to follow the activity of just one or two without some creative maneuvering. Several methods have been developed to label specific proteins in a cellular context. However, these methods are not without various shortcomings, including low efficiency or specificity of the labeling reaction, the labeling reaction itself perturbing the structure or function of the protein, or both. Zhou *et al.* (p 337) report the development of two site-specific protein labeling substrates that enable orthogonal labeling of proteins with various small-molecule tags.



TycC3-PCP, helix II

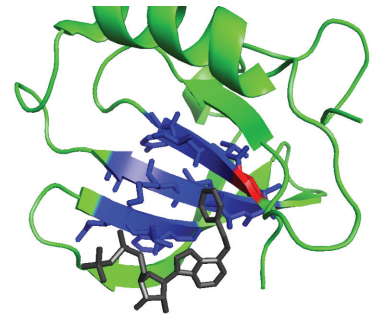
The labeling substrates, called S6 and A1, are based on peptidyl carrier protein or acyl carrier protein domains that serve as substrates for the phosphopantetheinyl transferases Sfp and AcpS. These enzymes transfer coenzyme A (CoA) to their native substrates, but their fortuitous promiscuity enables the transfer of a variety of small molecules to protein substrates, provided that the molecules are attached to CoA through its terminal thiol. Using phage display technology, the authors evolved the 12 residue peptides S6 and A1 to be specific substrates of Sfp and AcpS, respectively. Fusion of these peptides to various proteins at either the N- or C-terminus enabled specific, orthogonal labeling with various small molecules, including fluorescent tags and biotin, in cell lysates and on the cell surface.

Recipe for Self-Renewal

To date, finding growth conditions that support self-renewal of embryonic stem (ES) cells is akin to picking out the exact ingredients in your mother's famous chicken soup that make it taste just so. Few methods enable controlled investigations into specific factors required for maintaining ES cells in their undifferentiated, pluripotent state—the state that is brimming with potential in regenerative medicine. Now, Derda *et al.* (p 347) describe their recipe of self-assembled monolayers that contain defined surfaces in the search for "ingredients" that promote ES cell self-renewal.

The authors reasoned that surfaces that contain elements found in known substrates for ES cell growth that are likely to engage the cells in adhesive interactions and induce signaling pathways were logical candidates for promoting self-renewal. The protein laminin is one such element, and photolithographic methods were used to generate arrays of defined shape, size, and density of laminin-derived peptides. Cell staining for markers that indicate pluripotency enabled identification of several peptide sequences that promote ES cell growth in an undifferentiated state. Furthermore, the authors determined that the sequence and the density of the peptide were critical. It is important to note that the peptide identified in this study was also used to generate a 3D substrate that also supported self-renewal of ES cells. These studies provide a systematic method for identifying substrates that promote growth of ES cells and offer promising starting points for cooking up various biomaterials that support ES cell self-renewal.

Chemical Genetics and Kinases

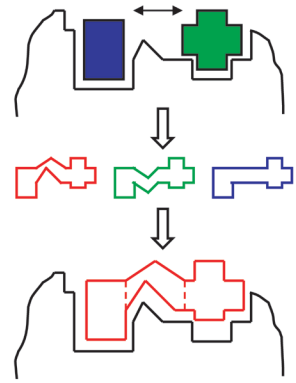


Tackling kinases is a formidable yet worthy cause. Kinases are instrumental in both normal and pathogenic processes, and dissecting their function and regulation will contribute greatly to our understanding of growth and development and help in the fight against a wide variety of diseases. Traditional approaches to kinase characterization, including the use of biochemical fractionation, small-molecule inhibitors, genetic manipulations, purified enzymes, kinase libraries, and mass spectrometry, have provided much information about kinase function and regulation, but the methods fall short in either specificity, speed, or sensitivity. Elphick *et al.* (p 299) review a chemical genetics approach in which various small molecules are used to probe kinase function. This technique encompasses many of the benefits of traditional kinase characterization methods and alleviates some of the drawbacks.

The authors describe how the use of various ATP analogues and small-molecule kinase inhibitors coupled with genetically engineered kinases has enabled rapid and specific characterization of many aspects of kinase function. One significant accomplishment has been the determination of the substrates of many kinases, a significant challenge in this field given the numbers of kinases and kinase substrates present in the cell. Future applications of these methods, especially when combined with exciting new techniques in proteomic analyses, will further illuminate the characteristics of these challenging enzymes and potentially lead to new clinical agents for kinase-related disorders.

Published online May 18, 2007 • 10.1021/cb700090r CCC: $37.00

A Fragmented Approach



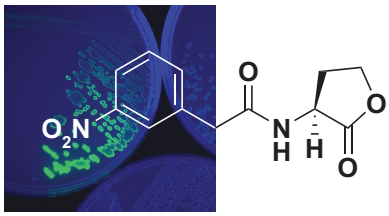
At first glance, breaking molecules into fragments for the purposes of drug discovery may seem like a step backward. Fragment-based approaches, however, in which small-molecule fragments are screened for binding and later optimized into lead compounds, have demonstrated promise in drug discovery efforts. Chen *et al.* (p 329) now describe a new twist on fragment-based screening with 2D NMR spectroscopy. They call the method pharmacophore by interligand nuclear Overhauser effect (ILOE).

This approach relies on the detection of protein-mediated ILOEs, or interactions between a pair of ligands that bind to adjacent pockets on a protein surface. Unlike other fragment-based screening approaches, however, pharmacophore by ILOE does not require detailed structural information of the components being investigated, thus loosening the requirements for its application. The authors screened 96 compound fragments against an inactive form of mitogen-activated protein kinase p38α, a protein involved in the inflammatory response. They found the strongest ILOEs between a fragment that contained an indole functionality and one that had a piperidine ring. Using various *in vitro* assays, they evaluated several commercially available compounds that contained these pharmacophores. This led to the identification of BI-12H11, a potent and remarkably selective inhibitor of p38α. Moreover, activated cells exposed to BI-12H11 exhibited a dose-dependent reduction in tumor necrosis factor-α production, evidence that the compound may indeed possess anti-inflammatory properties. This approach offers an enticing complement or even an alternative to existing high-throughput and fragment-based screening methods.

Population Control

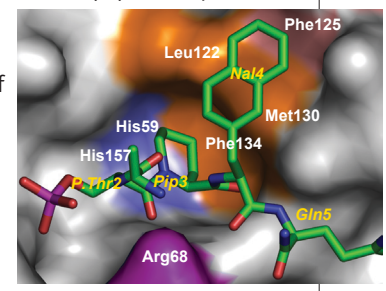
Bacteria have the remarkable ability to monitor the density of their population, a talent that enables them to establish mutually beneficial relationships with host organisms as well as initiate infections. This process, termed quorum sensing, is controlled by specific chemical signals, but detailed understanding of the signaling pathways and small molecules involved is lacking. Geske *et al.* (p 315 and Point of View p 293) now describe several small-molecule agonists and antagonists of quorum sensing in the marine symbiont *Vibrio fischeri*.

The quorum-sensing process in Gram-negative bacteria like *V. fischeri* employs diffusible N-acylated L-homoserine lactones that interact with receptors called R proteins, initiating signaling events that enable intercellular communication. On the basis of the structure of *N*-(4-bromophenylacetanoyl)-L-homoserine lactone (4-bromo-PHL), a known antagonist of quorum sensing in other Gram-negative bacteria, a small library of PHLs with varying phenylacetanoyl moieties was synthesized. The compounds were tested for their ability to either inhibit or induce receptor function by monitoring luminescence in an appropriate mutant *V. fischeri* strain. Several potent antagonists and agonists were discovered, and striking structure–activity relationships were revealed among the compounds. These novel molecules provide an important new set of chemical tools that will help decipher the fascinating yet enigmatic process of quorum sensing.



Pinning Down Pin1 Inhibition

Pin1 is a prolyl peptide isomerase (PPIase) that acts on several key regulators of the cell cycle, but unfortunately it also appears to play a sinister role in the development of cancer. Pin1 is distinguished from other PPIases, such as cyclophilins and FK506 binding proteins, in that its substrates are dipeptide sequences that contain a phosphorylated serine or threonine followed by a proline. They provide a jumping off point for the design of selective, effective inhibitors. To this end, Zhang *et al.* (p 320) have pinned down the crystal structures of two non-natural peptide inhibitors in complex with Pin1.



The Pin1 inhibitors were designed by borrowing an idea from nature. The non-natural amino acid pipecolic acid, which is found in the natural product PPIase inhibitors FK506 and rapamycin and is known to be an effective proline mimic, was incorporated into a combinatorial library. Two high-affinity inhibitors were discovered, and the crystal structures of the inhibitors bound to Pin1 offered much insight into how the compounds interact with the enzyme and provide hints about the design of future inhibitors with improved affinity and pharmacological properties. For example, a strategically placed intramolecular hydrogen bond was found to mimic the cyclic conformation of FK506 and rapamycin, an indication that cyclic compounds based on these Pin1 inhibitors could possess enhanced activity.