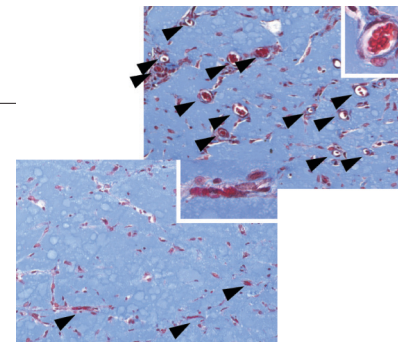
In this ISSUE

acs chemical biology

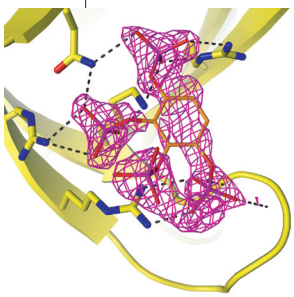


New Tricks for an Old Antifungal

New blood-vessel formation, or angiogenesis, is critical for proper growth, development, and wound healing, but it has rapidly become notorious for its role in many diseases, including cancer and rheumatoid arthritis. The therapeutic potential of anti-angiogenic agents has spurred intense research in this area, and several drugs currently used to treat other ailments have unexpectedly been found to possess anti-angiogenic properties. Treading further down this path, Chong *et al.* (p 263) screened a library of approved drugs for angiogenesis inhibitors and discovered that the antifungal drug itraconazole has potent anti-angiogenic activity.

Itraconazole functions as an antifungal agent by inhibiting 14α-demethylase (14DM), an enzyme involved in the biosynthesis of sterols in both fungi and humans. It is thus tempting to speculate that itraconazole similarly prevents angiogenesis by inhibiting 14DM. The authors provide compelling evidence that supports this hypothesis, such as phenotypic response, cholesterol sensitivity, and RNA knockdown data, although more research is needed to determine whether other mechanisms are also at play. It is important to note that itraconazole retained its anti-angiogenic activity *in vivo* in a mouse model, an exciting sign of its potential for the treatment of angiogenesis-related diseases.

The Binding Site Less Traveled



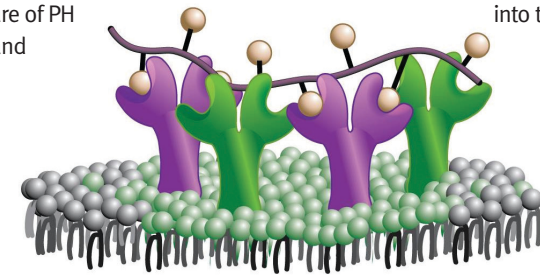
Protein kinase B (PKB) is an important cell signaling molecule, and its misregulation has been implicated in the development of certain cancers. In addition to its ATP-binding kinase domain, PKB contains a pleckstrin homology (PH) domain, whose interaction with phosphatidylinositol 3,4,5-triphosphate [PtdIns(3,4,5)P_3] leads to PKB activation. Most efforts to design PKB inhibitors have focused on the ATP-binding site, but the sheer number of ATP-binding proteins in the cells makes it exceptionally challenging to create specific kinase inhibitors. Mills *et al.* (p 242) have traveled down a different path, solving the crystal structure of the PH domain of PKB complexed with a small-molecule mimic of [PtdIns(3,4,5)P_3].

Benzene polyphosphates are enticing mimics of inositol polyphosphates because the phosphate pattern of the natural compound can be effectively displayed while attached to the more rigid benzene scaffold. Comparison of the crystal structure of PH with benzene 1,2,3,4-tetrakisphosphate and [PtdIns(3,4,5)P_3] showed similar binding interactions and highlighted the importance of the phosphate at the 4 position. In addition, molecular modeling studies suggested that other compounds could be designed and evaluated *in silico* as potential inhibitors of PKB function.

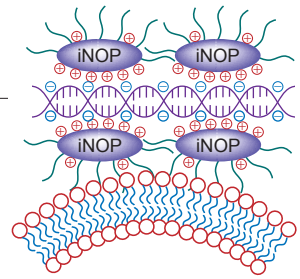
More is Better: Multivalent Ligands

Just imagining how cells are able to control their incredibly complex signaling processes to achieve the multitude of tasks required of them can boggle the mind. For example, the various antigens encountered by B cells trigger specific signaling events that can lead to the dramatically different responses of either immunity or tolerance. Antigen valency, or the number of antigens displayed by a given scaffold, is thought to be important in this process, but few tools exist to systematically explore this factor. Puffer *et al.* (p 252) now tackle the mechanisms behind B cell signaling by using innovative synthetic multivalent ligands for the B cell receptor (BCR).

Using the ring-opening metathesis polymerization, the authors created several synthetic polymers of defined length, narrow polydispersity, and valency. The polymers ranged from 10 to 500 monomer units in length and displayed multiple copies of a 2,4-dinitrophenyl group, which is known to elicit an immune response through a specific BCR. Exposure of these multivalent ligands to specific B cells provided insights into the effects of ligand valency on BCR clustering, localization, internalization, signal amplification, and antibody production.



Published online April 20, 2007 • 10.1021/cb700069q CCC: $37.00



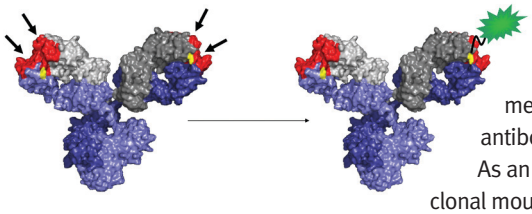
Introducing iNOPs

RNA interference, in which genes are turned off by short RNA sequences, is an invaluable tool for biological discovery and a promising therapeutic strategy for a number of diseases with genetic origins. However, challenges in the generation of chemically stable and efficient short interfering RNA (siRNA) sequences that can be administered in clinically effective doses have hampered the development of siRNA-based therapies. Baigude *et al.* (p 237) now present the creation of interfering nanoparticles (iNOPs) as effective siRNA delivery agents.

The iNOPs were designed to target the mouse gene that encodes apolipoprotein B (apoB), a protein involved in cholesterol metabolism. In an effort to maximize the iNOPs' pharmacogenetic properties and minimize their toxicity, the authors generated the iNOPs with lysines modified at the surface with lipid chains and chemically modified the siRNA component for enhanced stability. Mice treated with the iNOPs exhibited reduced apoB messenger RNA and protein levels in the liver and lower plasma concentrations of apoB and cholesterol. Moreover, treatment with the iNOPs did not elicit an immune response, was not toxic, and displayed favorable pharmacokinetic properties. These results present a hopeful strategy for the design of effective siRNA-based therapeutics.

Removing the Randomness

The uniquely specific binding properties of antibodies enable their use for biomolecule visualization, targeting, diagnostic applications, and catalytic activity. These applications often require labeling of the antibodies with various functionalities that range from fluorescent groups or toxins to enzymes or nanoparticles. Typical methods for installing these groups employ the amino acid side chains of the antibody, resulting in the random placement of the desired functionality on the antibody surface and potential complications in future experiments.



Scheck and Francis (p 247) now describe a general method for selectively labeling antibodies at their N-termini. As an initial test case, a monoclonal mouse anti-FLAG antibody was reacted with pyridoxal 5′-phosphate. This reaction presumably results in selective derivatization at the N-terminus to a pyruvamide functionality, which can then be selectively reacted with functionalized alkoxyamines. Indeed, the selectivity of the reactions was confirmed upon characterization with mass spectrometry and proteolytic digestion, and the integrity of the antigen recognition properties of the labeled antibodies was verified *via* various immunoassays. This method was used to label an assortment of monoclonal and polyclonal antibodies, an indication of the generality of this innovative approach.

Make No Bones about It

The outcome of a high-throughput screen (HTS) is only as good as the model system on which it is based. It is relatively straightforward to set up screens that use purified proteins or cultured cells, but the biological relevance of these systems is often lacking. Invertebrate animal models such as the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* may provide more relevant biological settings, and remarkable technology advances have enabled HTS in these animals. Ségalat (p 231) reviews the pros and cons of using such animal models in HTS.

A major advantage of HTS in invertebrate animal models is to harness the physiological context that they provide. This is especially enticing when a pertinent disease model in the animal exists and in the particularly challenging situations of targeting diseases for which the molecular causes are unknown or that have exceptionally complex biology such as neurodegenerative or muscle disorders. However, the differences between invertebrates and mammals are significant, and thus, some diseases, such as cardiovascular diseases and behavioral disorders, may not be effectively modeled in these organisms. Nonetheless, in the right circumstances, using invertebrate animals for HTS may indeed someday lead to just what the doctor ordered.