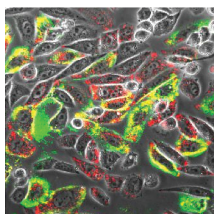
In this ISSUE

Sharpshooting Cells

Inflammatory macrophages in atherosclerotic lesions bear scavenger receptors (SRs) that take up many types of disease-associated lipoproteins. It would therefore be useful if these



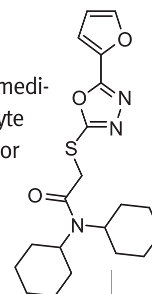
atherogenic macrophages could be selectively targeted as an initial step toward developing cell-specific therapeutics. However, SRs are also found in macrophages in normal cells and cannot be used alone as markers. Suzuki *et al.* (p 471) take advantage of the fact that both SRs and the collagen-degrading matrix metalloproteinase type 9 (MMP-9) are present in elevated levels in atherogenic macrophages to design a system that allows targeted delivery to SRs in these cells.

The authors design a system such that one domain of the complex has high affinity for the targeted SR. However, this domain cannot interact with the SR until a bacterial chaperone domain that is linked to it is removed. The chaperone domain is removed in the extracellular matrix by MMP-9, which recognizes a specific cleavage site between the two domains. After the inhibitor domain is cleaved, SR mediates the cellular uptake of the remaining protein product.

Specifically Nonspecific

Compounds identified as “hits” in high-throughput screens are unfortunately almost always accompanied by pesky “off-target” hits caused by artifacts in the assay. In an effort to identify the mechanism behind one of these artifacts, Auld *et al.* (p 463) report that inhibitors of luciferase, an enzyme commonly used in high-throughput screens, can counterintuitively masquerade as hitKnown luciferase inhibitors present in compound libraries were found to stabilize the enzyme and thus increase its concentration, albeit by a mechanism independent of the design of the assay. During the detection phase of the screen, the inhibitors are displaced by excess luciferase substrate, resulting in a deceptively elevated signal. This understanding of the source of such false positives will help researchers in the interpretation of their data, as well as in the design of appropriate follow-up studies.

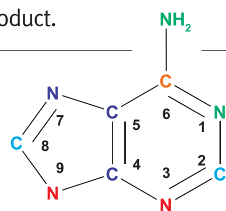
Picking the S1P Pocket



Interactions between sphingosine 1-phosphate and its five receptors, $S1P_{1-5}$, mediate many important physiological processes, including heart rate and lymphocyte trafficking. However, a lack of small-molecule agonists selective for each receptor has hampered our ability to probe the receptors’ individual roles. Searching for selective agonists of $S1P_1$ and $S1P_3$, Schürer *et al.* (p 486) perform systematic high-throughput screens using two structurally diverse compound libraries.

Reporter gene assays specific for either $S1P_1$ or $S1P_3$, followed by several secondary assays, enabled the identification of a number of selective $S1P_1$ and $S1P_3$ agonists. Structure–activity analysis, receptor modeling, and ligand docking studies revealed distinct differences in the receptor binding pockets that accounted for the selectivity of the compounds. The modeling studies also provided illuminating insights into why more hits were observed for $S1P_1$ than for $S1P_3$.

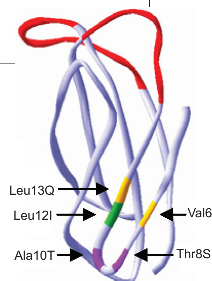
One-Pot Purines



Enzymatic syntheses of various complex biomolecules can be much more efficient and environmentally friendly than chemical syntheses. In particular, generation of isotopically labeled purines and pyrimidines, which can greatly facilitate structural characterization of nucleotide-containing compounds, is quite tedious using chemical methods. Schultheisz *et al.* (p 499 and Point of View p 460) engineer a new method for the total enzymatic synthesis of the purine nucleotides ATP and GTP.

A total of 28 enzymes from the pentose phosphate and *de novo* purine synthesis pathways were used in the single-pot reaction, along with the precursors glucose, glutamine, serine, ammonia, and CO_2. As these precursors are all available in various isotopically labeled forms, two variations each of ATP and GTP, each with distinct isotope patterns, were generated. Subsequent synthesis and NMR characterization of an RNA oligomer incorporating the labeled purine bases illustrated a key application of such compounds.

An Impressive Display



Compared with the scant 25,000 genes encoded by the human genome, the proteome boasts ~1 million proteins when alternative splicing and post-translational modifications are counted. Affinity reagents that enable the detection and manipulation of these proteins are powerful molecular tools, yet challenging to generate. Olson *et al.* (p 480) demonstrate how messenger RNA display can facilitate creation of just such tools.

Derivatives of the 10th fibronectin type III domain (10FnIII) of human fibronectin are attractive starting points for generating novel protein affinity reagents because of their stability and ease of production. *In vitro* selection using a combinatorial library based on 10FnIII was used to create reagents specific for the phosphorylated form of the NF-κB regulator IκBα. Selection resulted in a 10FnIII variant that was highly specific for phospho-IκBα and stabilized IκBα in cells, which could be used as a FRET indicator.

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