

## COVER STORY

The discovery of ribozymes demonstrated that RNA molecules, like proteins, catalyze important biochemical reactions. Given the major structural differences between nucleic acids and proteins, it has been unclear whether RNA enzymes could employ the same catalytic strategies that are used effectively by proteins. In this issue, Das and Piccirilli show that a hepatitis delta virus (HDV) ribozyme uses an active-site general acid to catalyze phosphodiester

cleavage. The three-dimensional structure of an HDV ribozyme previously showed that a key cytosine (C76) residue was positioned near the scissile phosphate bond, but its catalytic role had remained unclear. To address the function of the conserved C76, Das and Piccirilli synthesized HDV ribozymes that contained a 'hyperactivated' phosphorothiolate bond at the cleavage site. Using enzyme kinetics and precise functional group mutations of C76, the authors implicated C76 as the general acid responsible for protonation of the leaving group during the cleavage reaction. These studies suggest that general acid catalysis may be a common feature of RNA catalysis. The single-atom substitution and ribozyme kinetics of Das and Piccirilli may provide the tools necessary to investigate this intriguing hypothesis in other ribozymes. [Articles, p. 45; News & Views, p. 5]

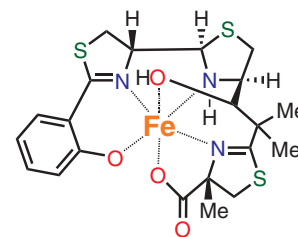
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obtained during agonist activation. They observed that inverse agonist binding induces a conformational change that is distinct from agonist-mediated GPCR activation. The authors suggest that GPCRs may respond to diverse ligands by accessing distinct conformational states with unique kinetic profiles, rather than by acting as binary switches. [Letters, p. 25]

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## Blocking iron acquisition

New tools are needed in the antibiotic arsenal to combat increasing bacterial resistance to known drugs. One approach to identifying new antibiotics is to inhibit processes essential for bacterial infection. Siderophores, chemicals that are secreted by bacteria to capture the necessary iron to grow during infection, offer an untapped target. Now Quadri, Tan and colleagues have synthesized the first inhibitor of siderophore biosynthesis. *In vitro*, this inhibitor prevented siderophore biosynthesis in *Mycobacterium tuberculosis* and *Yersinia pestis*.

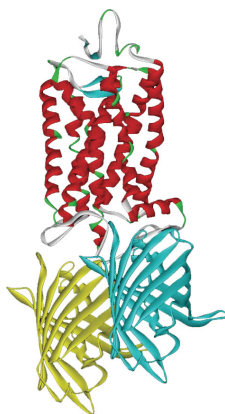


Blocking siderophore production prevented *M. tuberculosis* and *Y. pestis* growth under iron-limited conditions. This small molecule represents a potential new therapeutic lead against tuberculosis and plague, and a chemical probe for further investigating the role of siderophores during infection. [Letters, p. 29]

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## Flicking the inverse switch

G protein-coupled receptor (GPCR) activation initiates numerous signal-transduction pathways. Ligand binding to a GPCR alters the receptor's conformation and thereby modulates its interactions with downstream effectors. 'Agonist' ligands bind selectively to a target GPCR and activate signaling in a process that has been likened to an 'off-on' switch. In contrast, binding of 'inverse agonist' ligands to GPCRs lowers receptor signaling activity, particularly in constitutively active receptors. Vilardarga and coworkers provide new insight into how inverse agonist binding alters GPCR receptor conformation. Based on earlier studies of GPCR activation in living cells, the authors constructed a conformational sensor consisting of the  $\alpha_{2A}$ -adrenergic receptor ( $\alpha_{2A}$ AR) coupled to cyan and yellow fluorescent proteins (CFP and YFP). The authors measured the rate of fluorescence resonance energy transfer (FRET) between CFP and YFP induced by inverse agonist binding and compared it to results



## Protein cutting probed

Proteases are important players in biological processes from viral packaging to programmed cell death. However, dissecting out the unique biological roles of this large family of closely related proteins remains a substantial challenge. Small molecules that target and label only active forms of enzymes, called activity-based probes, offer one means for looking directly at endogenous enzyme activity. But highly specific molecules are necessary to investigate the roles of closely related enzymes *in vivo*. Bogoy and colleagues have now generated probes with improved specificity for members of the cysteine protease family, including caspases and cathepsins. Application of these probes reveals that only the fully cleaved form of legumain, a key player in antigen presentation, is active in cells and tissues. [Letters, p. 33]

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## Chemistry alive

Green fluorescent protein is a powerful label for cellular imaging of proteins, but it cannot be easily extended to other biomolecules. In their review, Prescher and Bertozzi describe the recent development of bioorthogonal chemical reporters as an alternative for tagging biomolecules. In this approach, a small label, with chemical reactivity distinct from that of biologically occurring molecules, is attached to the desired biomolecule by cellular machinery and detected by selective reaction with a probe. [Review Article, p. 13]

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