In This Issue

Murine Thymoproteasome-Specific Subunit β5t by ABP

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Peptides generated by the thymus-specific proteasome and presented by MHC class I molecules on the surface of cortical thymic epithelial cells play an important role in the positive selection of thymocytes. The thymoproteasome uniquely contains the β5 t subunit. In this short communication, the Overkleeft group shows by using a panel of activity-based probes (APB), affinity purification, and active-site determination by LC-MS^3 that the β5 t is indeed an active thymo-proteasome subunit. Interestingly, ABP's with a Thr at P2 react with β5 t, suggesting a substrate preference different from the β5 and β5i subunits.

Marine Macrolide Captures Actins Attention

PAGE 802

Many forms of marine macrolides exhibit toxicity toward the actin cytoskeleton and thus are promising templates from which to design anticancer and antimicrobial therapeutics. Lobophorolide is a marine macrolide derived from the common tropical seaweed *Lobophora variegata* that disrupts the actin cytoskeleton using a mechanism that is different from many other macrolides. Blain et al. now show that it stabilizes a polymerization-deficient actin dimer in which two lobophorolide molecules cooperate to form a dimerization interface that is composed entirely of the small molecule. This binding mode holds important implications for development of actin-targeting drugs and the evolution of macrolide biosynthetic enzymes.

Fighting Malaria with Small Molecule Inhibitors and Active Site Probes

PAGE 808

Malaria remains one of the most devastating infectious diseases, causing close to a million deaths per year. The emergence of drug resistance to all affordable drugs continues to be the main hurdle to treat this disease. It is therefore urgent to identify new drug targets to expand the portfolio of antimalaria drugs. In this work, Deu et al. describe the use of small molecule inhibitors and use activity-based probes to demonstrate that inhibition of dipeptidyl aminopeptidase 1 impairs parasite growth both in vitro and in a mouse model of malaria. Overall, the study validates this cysteine protease as an antimalarial target and identifies several potentially valuable inhibitor scaffolds for this protease.

Metabolomic View of Microbial World

PAGE 820

Liebeke et al. now report comparison of quantitative metabolite levels of *Staphylococcus aureus* grown in complex LB-Broth and in minimal medium with that of wild-type *S. aureus* strain 8325 and the isogenic eukaryotic-like protein serine/threonine kinase (Δ*pknB*) and phosphatase (Δ*stp*) deletion mutants. Detection of several remarkable differences, e.g., in nucleotide metabolism and especially cell wall precursor metabolites, indicates a previously unreported importance of serine/threonine kinase/phosphatase on peptidoglycan and wall teichoic acid biosynthesis. These findings may lead to new insights into the regulation of staphylococcal cell wall metabolism.

Brain Metabolomics of GDE1(−/−) Mice

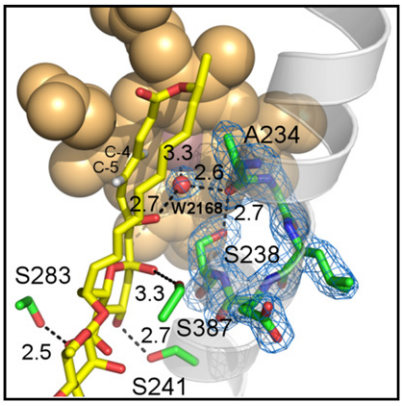
PAGE 831

The function of glycerophosphodiesterase (GDE) enzymes in mammalian organisms is largely unknown. Here, Kopp et al. show, using mouse genetics and untargeted metabolomics, that GDE1 regulates a set of glycerophospho (GroP) metabolites in brain, including both known (GroP-inositol) and novel (GroP-serine and GroP-glycerate) natural products. The metabolomic profiles also revealed that serine was significantly reduced in brains of GDE1-disrupted mice, thus designating GroPSer as a reservoir for free serine in the nervous system. Taken together, these findings indicate that the mammalian "GroP-metabolome" is quite diverse in structure and function and designate GDE1 as one of its principal enzymatic regulators in vivo.

Hydroperoxoferric Intermediate in Cytochrome P450 Epoxidation

PAGE 841

Kells et al. present an X-ray structure of PimD both substrate-free and in complex with 4,5-deepoxypimaricin. PimD is a cytochrome P450 monooxygenase with native epoxidase activity that is critical in the biosynthesis of the polyene macrolide antibiotic pimaricin. Epoxidation by P450 typically includes formation of a charge-transfer complex between an oxoferryl π-cation radical species (Compound I) and the olefin π-bond as the initial intermediate. Evidence presented here suggest that epoxidation of 4,5-deepoxipimaricin proceeds via a hydroperoxoferric intermediate (Compound 0). The oxygen atom of Compound 0 distal to the heme iron may insert into the double bond of the substrate to make an epoxide ring.

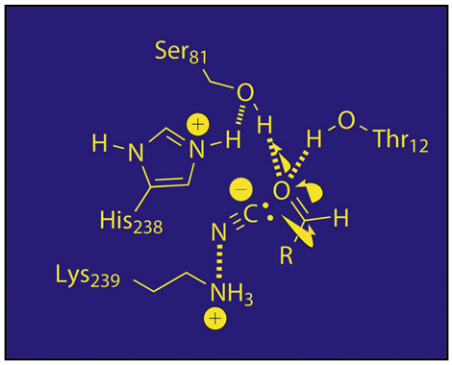


Chemical-Chemical Interaction Profiling

PAGE 852

Farha and Brown tackle the problem of understanding the mechanism of action of growth perturbing small molecules that have been discovered in compound screening. Here, the authors probed the mode of action of uncharacterized inhibitors of the growth of *Escherichia coli* with careful investigations of the interactions with compounds of known biological activity. Specifically, they examined growth inhibition by a collection of 200 novel antibacterial compounds when combined with a panel of 14 known antibiotics of diverse mechanism and chemical class. The chemical-chemical interaction data provide a fingerprint of biological activity and testable hypotheses regarding the mechanism of action of novel bioactive molecules, which was used to determine the mode of action of an inhibitor of folate biosynthesis and a DNA gyrase inhibitor and identify eight membrane-active compounds, found to be promiscuously synergistic with known bioactives.

From an Esterase to a Hydroxynitrile Lyase



PAGE 863

Proteins with similar structure can catalyze different chemical reactions. To learn how enzymes catalyze reactions, evolve to catalyze new reactions, and create new enzymes for synthesis, researchers engineer new types of catalytic activity into enzymes. The closest structural relatives of SABP2, a plant esterase, are not other esterases, but hydroxynitrile lyases (HNLs), which catalyze a different reaction. Previous structural and mechanistic studies identified two amino acid differences in the active site of HNLs as compared to esterases. Padhi et al. now show that making those two substitutions in SABP2 switches its esterase activity to HNL activity.

Surfactin Synthetase Initiation Module

PAGE 872

The fatty acid moiety is a key structural element of nonribosomally assembled lipopeptide antibiotics, but little is known about the mechanism of lipid transfer during the initial step of biosynthesis. In this study, Kraas et al. dissect the initiation condensation domain of the surfactin synthetase SrfAA and identify that it catalyzes amide bond formation between the CoA-activated fatty acid and the N-terminal amino acid of surfactin. Biochemical and gene disruption studies of four fatty acyl coA ligases of *Bacillus subtilis* confirm the suggestion that the activated fatty acids for surfactin biosynthesis are provided in *trans* by enzymes from biological pathways of the primary metabolism.

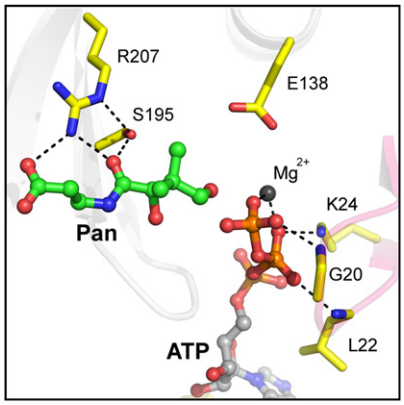
Targeting Integral Membrane Proteins

PAGE 881

Membrane proteins are important pharmaceutical targets, but they pose challenges for fragment-based drug discovery. Here, Früh et al. present the first successful use of biophysical methods to screen for ligands to an integral membrane protein. The *E. coli* membrane protein DsbB was solubilized in detergent micelles and lipid bilayer nanodiscs. DsbB was immobilized with retention of functionality and used to screen 1,071 drug fragments for binding using target immobilized NMR screening (TINS). The ability to insert a broad array of membrane proteins into nanodiscs, combined with the efficiency of TINS, demonstrates its potential as a generic approach for finding fragments targeting membrane proteins.

Pantothenate Kinase 3 Regulation by Small Molecules

PAGE 892



Pantothenate kinase (PanK) catalyzes the rate-controlling step in coenzyme A (CoA) biosynthesis. Biochemical analysis of site-directed mutants, reported by Leonardi et al., indicates that pantothenate binds in a tunnel adjacent to the active site that is occupied by the pantothenate moiety of the acetyl-CoA regulator. A high-throughput screen for PanK3 inhibitors and activators identified thiazolidinediones, sulfonylureas, and steroids as inhibitors and fatty acyl-amides and tamoxifen as activators. The PanK3 activators and inhibitors either stimulated or repressed CoA biosynthesis in cultured cells. The flexible acetyl-CoA regulatory domain of PanK3 also binds the substrate, pantothenate, and small molecule inhibitors and activators.

A HAT Inhibitor Keeps Oral Cancer in Check

PAGE 903

Altered histone acetylation is associated with several diseases, including cancer. In the present work, Arif et al. found that histones are hyperacetylated in oral cancer patient samples. Mechanistically, overexpression, as well as enhanced autoacetylation, of p300 induced by NPM1 and GAPDH proteins causes the hyperacetylation, which is nitric oxide (NO) signal dependent. Inhibition of the histone acetyltransferase (HAT) activity of p300 by a water-soluble, small molecule inhibitor, Hydrazinocurcumin (CTK7A), reduced the xenografted oral tumor growth in mice. This could be a potential antineoplastic therapeutic strategy, particularly against oral cancer.

