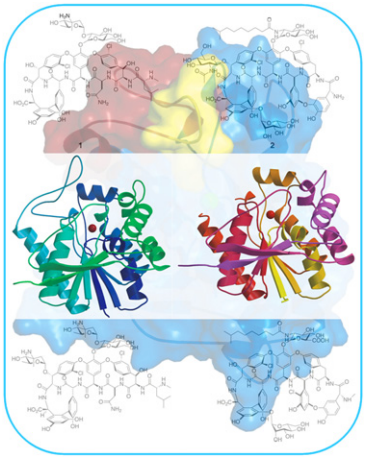
In This Issue

Lipoglycopeptide Antibiotic Deacetylases Full Speed Ahead



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Lipoglycopeptide antibiotics are particularly effective against Gram-positive, drug-resistant bacteria in part because of a fatty acid modification of a sugar residue on the molecule. Prior to the addition of these fatty acids, dedicated enzymes must remove an acetyl group on the sugar. Here, Zou et al. describe the structures of enzymes that carry out this task during the production of the lipoglycopeptide antibiotics teicoplanin and A40926 and show how these enzymes carry out removal of the acetyl group. The work might aid efforts aimed towards the production of novel lipoglycopeptide antibiotics. (Figure credits: Zou et al.)

Making Carbs, Using HTS, Seeing HF

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The synthesis of oligosaccharides remains a chemical challenge due to the need to control stero- and regioselectivity. Glycosynthases are mutant glycosidases that are capable of catalyzing the formation of a glycosidic linkage between activated glycosyl fluoride donors and suitable acceptor sugar. Ben-David et al. developed a simple universal high-throughput screening procedure for monitoring glycosynthase activity. The method relays on detection of release of hydrofluoric acid, a by-product of all glycosynthase reactions. The authors applied their method for the directed evolution of a glycosynthase, which led to the isolation of improved variants. Analysis of these variants suggested a general path for rational optimization of glycosynthases.

Aurora B Kinase: Resistance is Futile

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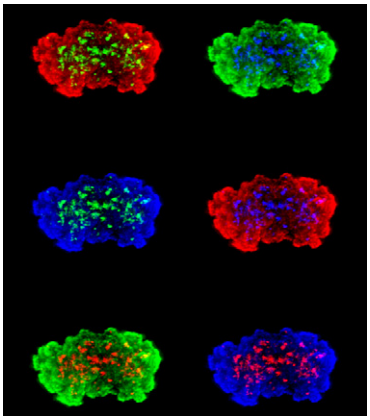

The Aurora kinases are serine/threonine kinases important for regulation of mitotic functions. They have emerged as anticancer drug targets and several inhibitors are in clinical trials. However, a growing concern is whether tumors will acquire drug resistance. Girdler et al. now investigate this issue by focusing on cancer cells resistant to a selective Aurora B inhibitor. The authors develop a screen to identify those cells and demonstrate that all the resistant clones contained mutations in Aurora B which either block inhibitor binding or hyperactivate the kinase. These mutants are also resistant to other classes of Aurora inhibitors. Therefore, limiting clinical drug resistance will require identification of novel chemical scaffolds which inhibit the mutants described here. (Figure credits: Girdler et al.)

Orphan Enzymes Find their Substrates

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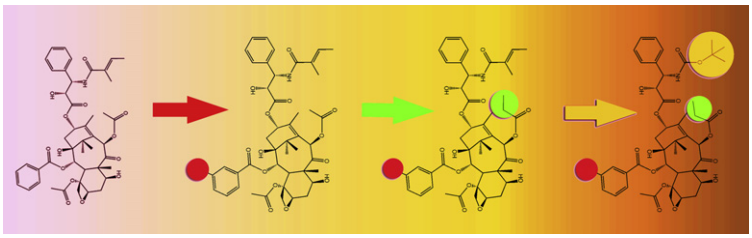
Characterization of orphan enzymes, for which the catalytic functions and substrates are unknown, is a significant challenge in the postgenomic era. Furuya et al. describe a general method for exploring the catalytic potentials of orphan monooxygenases, enzymes that catalyze addition of a single oxygen atom from molecular oxygen into a substrate. The authors employ Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR/MS) that offers unique advantages due to its remarkable mass resolution and accuracy. Their analysis functionally assigned two putative cytochrome P450s from *Bacillus subtilis.* In general, FT-ICR/MS-based approach streamlines the process of screening for substrates and can be adapted to large-scale screening needed in the genome-wide characterization.

Taxane Gets a Makeover



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Multidrug resistance of tumor cells is a major obstacle in chemotherapy. The predominant cause is the overexpression of the P-glycoprotein pump. In resistant cells exposed to taxanes (paclitaxel, docetaxel), two opposing forces control antitumor drug uptake: binding to target microtubules, keeping the drug inside the cell, and binding to P-glycoprotein, pumping it out. Matesanz et al. now design novel taxanes with very high affinity for microtubules, and thus the ability to overcome resistance, by determining the effect on the binding affinity of modifications in different positions of the drug molecule and combining the most favorable ones in a single drug molecule. (Figure adopted from figure provided by Matesanz et al.)

Lock the Morpheein Down

PAGE 586

Morpheeins are homo-oligomeric proteins that reversibly dissociate, change shape, and reassemble differently as an allosteric control mechanism. Small molecules that specifically bind to one oligomeric assembly are proposed as allosteric drugs. Lawrence et al. now use in silico and in vitro techniques to discover an allosteric inhibitor that traps porphobilinogen synthase in inactive hexameric form. The inhibitor, morphlock-1, binding site is phylogenetically variable and inhibition is species specific, thus identifying this universally essential protein as a target for antibiotic development. The authors suggest application of this novel inhibitor discovery strategy to other proteins that function as morpheeins.

SARS-Coronavirus Main Proteinase Structure with Suicide Inhibitor

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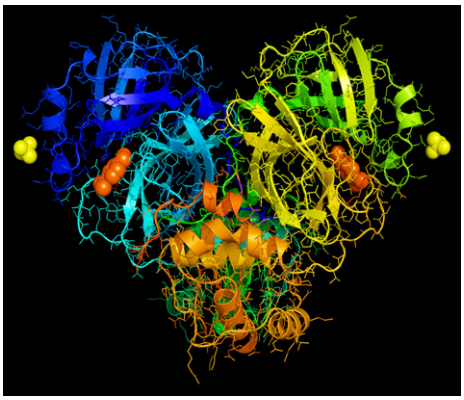


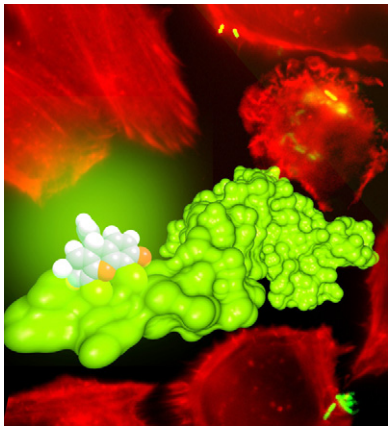
The functional importance of the SARS coronavirus main proteinase (M^{pro}) in the viral life cycle makes this enzyme an attractive target for the development of anti-coronaviral therapeutics. Verschueren et al. present crystal structures of the SARS-CoV M^{pro}, the active-site cysteine of which has been acylated by benzotriazole esters that act as suicide inhibitors. Interestingly, in one of the structures, the thioester product has been hydrolyzed and benzoic acid is observed to bind to the hydrophobic S2 pocket. The results further understanding of the important role of the aminoterminus for catalysis as well as the design of benzotriazole inhibitors with improved specificity. (Figure credit: Verschueren et al.)

Adaptive Evolution Applied to Engineering Synthetic Metabolic Pathways

PAGE 607

Nature has balanced most metabolic pathways such that no one enzyme in the pathway controls the flux through that pathway. However, unnatural or nonnative, constructed metabolic pathways may have limited production due to unfavorable properties of one or more enzymes in the pathway. Yoshikuni et al. have used a probable mechanism of adaptive evolution to engineer the in vivo properties of two enzymes in a constructed metabolic pathway, minimize the bottlenecks in the pathway, and improve the productivity of the desired final product. This strategy might find broad applicability in the functional construction of biological pathways in heterologous hosts.

See the Pattern of Salmonella Effector Protein Release



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Here, VanEngelenburg and Palmer develop an experimental approach for imaging direct secretion of bacterial effector proteins into mammalian cells in real time. The authors use the FlAsH/tetracysteine in situ chemical labeling technology to detect genomically tagged effectors. Effectors are under control of endogenous promoters, thus minimally perturbing the delicate balance of Type-III secretion. The authors detected a difference in the secretion rate of two *Salmonella* effector proteins, SopE2 and SptP. The differential secretion rate suggests there is a temporal hierarchy of effector delivery that is directly related to effector function. This ''preprogrammed pattern'' of release has important implications for understanding bacterial invasion. (Figure credit: VanEngelenburg and Palmer)

Improving Glycosylated Polyenes

PAGE 629

Glycosylated polyenes (GPs) represent a major class of antifungal agents, within which candicidin appeared as the earliest biosynthesis model of this family. GPs are usually produced in vivo as a complex of derivative compounds. Detailed understanding of the control mechanisms of their structural variations and conversions might be of practical significance for structural diversification and obtaining derivatives with improved pharmacological and/or physicochemical properties. Additionally, generation of strains with predominant production of unique and superior compounds is also desirable. Zhou et al. now describe results of this approach by using the FR-008/candicidin complex as a model.

