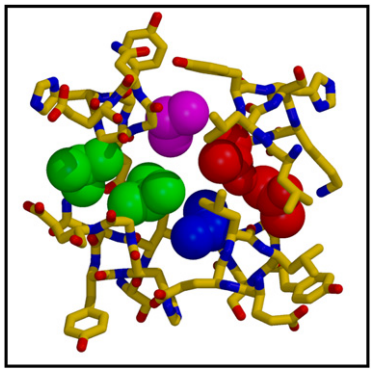
In This Issue

When an Insect Gets a Bug

PAGE 898

Fungal cyclooligomer depsipeptides exhibit antibiotic, antifungal, herbicidal, insecticidal, and nematicidal activities and inhibit multidrug-resistance, cancer cell proliferation, and cell motility. In this work, Xu et al. identify the gene encoding the nonribosomal peptide synthetase (NRPS) for beauvericin biosynthesis from the entomopathogen *Beauveria bassiana* and show that this NRPS uses unconventional biosynthetic mechanisms. Furthermore, heterologous expression of the beauvericin synthetase in *E. coli* demonstrates the feasibility of the heterologous production of a fully elaborated fungal cyclooligomer depsipeptide. Comparative insect infection assays using a beauvericin-nonproducer *B. bassiana* strain revealed that beauvericin is a bona fide virulence factor that plays a significant albeit not indispensable role in entomopathogenesis.

Zipping the Tetrameric Zipper



PAGE 908

Understanding the myriad ways in which proteins recognize and attach partner proteins is fundamental to understanding normal cellular processes as well as perturbed states such as Parkinson's or Alzheimer's disease. One common protein-protein interface involves thread-like "coiled coils," which form α helices that mutually intertwine. Coiled coils can include different numbers and sequences of helices. How is assembly with identical or different helices controlled? Deng et al. find here, using model helices that form tetrameric coiled coils, that the structure with different helices is more stable than the homogeneous alternatives. This heterospecific tetramer offers a powerful model for understanding an important protein-protein interface. (Figure credit: Deng et al.)

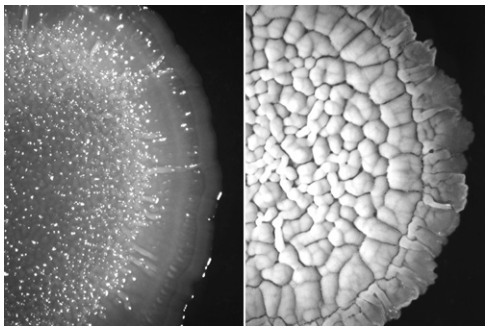
M1 Aminopeptidases Prototype Testing

PAGE 920

M1 aminopeptidases are widespread in nature and found in organisms ranging from bacteria and plants to vertebrates. Despite the fact that aminopeptidases remove only a single N-terminal residue, these enzymes exhibit a variety of important biological functions, e.g., processing of surface antigens, regulation of hypertension, inactivation of signaling peptides, and involvement in tumor angiogenesis. Tholander et al. use leukotriene A4 hydrolase/aminopeptidase as a prototype to study peptidolysis by these enzymes. The findings provide detailed insights to the active-site chemistry of M1 aminopeptidases and will aid in development of novel enzyme inhibitors.

Altered Cell Wall of *Mycobacterium smegmatis*

PAGE 930



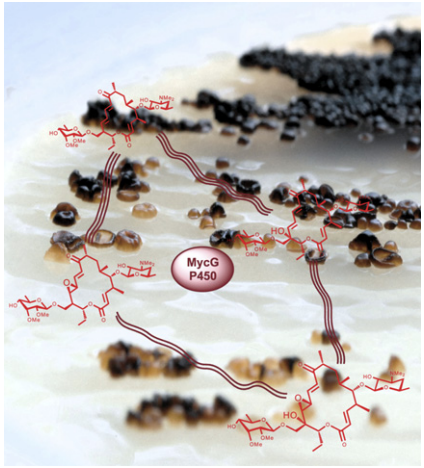
Mycolic acids are essential, virulence-associated components of the cell envelope of *Mycobacterium tuberculosis*, the causative agent of tuberculosis. In this issue, Bhatt et al. describe a mutant strain of *Mycobacterium smegmatis* that is defective in the final step of mycolic acid biosynthesis, namely, reduction of a β-oxo precursor. Surprisingly, this step was not necessary for subsequent processing and transport of mycolic acids, as the mutant strain incorporated the nonreduced precursor in the cell wall. Biological consequences included increased sensitivity to hydrophobic antibiotics like rifampicin and altered colony morphology with the implications for the reductase involved for use as a "secondary" drug target and a possible role in virulence in *M. tuberculosis*. (Figure-credits: Bhatt et al.)

Importin α/β Pathway Maxed Out

PAGE 940

Nuclear import of many nuclear proteins is mediated through the classical importin α/β pathway. Kosugi et al. report the development of efficient and specific inhibitors for this transport pathway using a novel method of peptide inhibitor design. Amino acid sequence design using an activity-based profile of a nuclear localization signal generated two peptides that bound to the full-length importin α with K_d values in the picomolar range. The high affinity of these peptides, called bimax1 and bimax2, led to formation of a stable complex with importin α, which was resistant to cargo release activities in the nucleus, and resulted in specific inhibition of the importin α/β pathway.

MycCI and MycG, Cytochrome P450 Enzymes



PAGE 950

Macrolide antibiotics are a class of valuable anti-infective agents that include a macrolactone ring, at least one appended sugar unit, and additional hydroxyl and/or epoxide groups installed by cytochrome P450 enzymes. These functional groups not only contribute to structural diversification, but often improve the bioactivity of resulting products. Herein, Anzai et al. have characterized in vitro two important P450 enzymes from the mycinamicin biosynthetic pathway of *Micromonospora griseorubida*. The work described enhances understanding of macrolide antibiotic production by microorganisms and benefits future work on creating novel antibiotics to fight against emerging infectious diseases with multi-drug-resistance pathogens. (Figure credits: Anzai et al.)

Cystatin C Hijacked by IdeS

PAGE 960

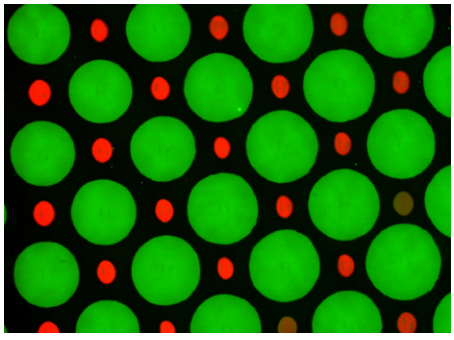
The protease inhibitor cystatin C is considered the physiologically most important emergency inhibitor of cysteine protease activity. The present study by Vincents et al. reveals a novel and unexpected role for cystatin C in stimulating, rather than counteracting, protease activity. Kinetic studies show that cystatin C efficiently accelerates the enzymatic velocity of the streptococcal papain-like cysteine protease IdeS. This finding is the first report of a protease inhibitor that accelerates the activity of its putative target protease and a unique example of how a host protease inhibitor is "hijacked" by a bacterial protease to increase its activity.

Human TGM-2 under Attack

PAGE 969

A tissue transglutaminase (TGM-2) catalyzes the formation of either inter- or intramolecular covalent bonds between a protein-bound glutamine and lysine residues. The TGM-2 crosslinking reaction contributes to neurodegeneration in polyglutamine repeat disorders. Lai et al. identified chemical inhibitors to TGM-2 and found them to be active in treating polyglutamine-mediated neurodegeneration disease in *Drosophila*. These inhibitors serve as valuable lead compounds for the development of orally active TGM-2 inhibitors. Since these inhibitors have been approved for human use, expectation is that they will accelerate the development of TGM-2 inhibitors.

Evolving Tight Couples



PAGE 979

The protein streptavidin (SA) is one of the most widely used proteins in molecular biology, biotechnology, and, most recently, nanotechnology. Levy and Ellington have developed and implemented an in vitro compartmentalization (IVC) selection scheme for the identification of streptavidin variants with altered specificities for the biotin analogs. These methods should prove useful for generating a variety of novel SA reagents and for evolving other extremely high-affinity protein:ligand couples. (Figure adapted from Levy and Ellington)

Efficacious Antifungal Agents Targeting DHFR

PAGE 990

Candida glabrata is a lethal fungal pathogen that is resistant to many clinically available antifungal agents. In order to discover drug leads for *C. glabrata*, Liu et al. screened a class of propargyl-linked antifolates that are inhibitors of the essential enzyme dihydrofolate reductase (DHFR) and identified several with modest potency. Additionally, the authors determined a high resolution structure of *C. glabrata* DHFR bound to one of these antifolates. Using the structure, they designed and synthesized second generation inhibitors with high affinity and selectivity for the pathogenic enzyme. The new compounds potently inhibit the growth of the fungus without overt toxicity to human cells.

