In This Issue

Pyrazinone Natural Products in *Staphylococcus aureus*

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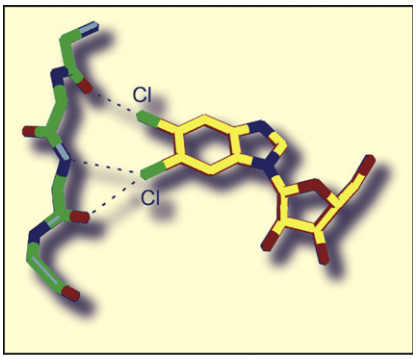
Each year in the U.S., methicillin-resistant *Staphylococcus aureus* infections are responsible for ~19,000 deaths. Here, Zimmermann and Fischbach report that a nonribosomal peptide synthetase conserved across *S. aureus* and other skin-associated staphylococci encodes a family of three pyrazinone natural products. As an unexpected family of small molecule natural products from the pathogen *S. aureus*, the pyrazinones may open a new window into the interspecies interactions that underlie the poorly understood process of skin colonization.

Bonds Halogenated



PAGE 931

P-TEFb inhibition contributes to the anticancer activity of many Cdk inhibitors under clinical investigation. Baumli et al. report a crystal structure of active P-TEFb subunit Cdk9 in complex with a selective inhibitor, DRB, and compare it to a Cdk2/DRB structure. The authors find that DRB forms Cdk9 specific halogen bonds to the kinase hinge region and that these are the molecular basis for the DRB selectivity towards Cdk9. These results open the possibility to exploit halogen atoms in inhibitor design to specifically target Cdk9.

No Insulin Resistance When O-GlcNAc Goes Up, Local View

PAGE 937

Macauley et al. find 6-acetamido-6-deoxy-castanospermine (6-Ac-Cas) to be a potent inhibitor of O-GlcNAcase (OGA). The structure of 6-Ac-Cas bound in the active site of an OGA homolog reveals features contributing to its potency. Treatment of 3T3-L1 adipocytes with 6-Ac-Cas increases O-GlcNAc levels; however, these increased O-GlcNAc levels do not induce insulin resistance either functionally or at the molecular level. These findings offer a blueprint for creating improved inhibitors and suggest pharmacological increases in O-GlcNAc levels do not, on their own, cause insulin resistance in 3T3-L1 adipocytes.

No Insulin Resistance When O-GlcNAc Goes Up, Global View

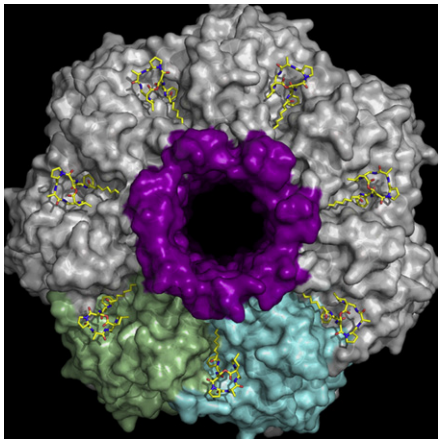
PAGE 949

Posttranslational O-GlcNAc modification of proteins is proposed to be a nutrient sensor. Studies suggest increases in O-GlcNAc levels cause insulin resistance and impairs glucohomeostasis. Macauley et al. address this hypothesis in rodent models using a selective inhibitor of O-GlcNAcase. Treatment with inhibitor increases O-GlcNAc but does not perturb insulin sensitivity or alter glucohomeostasis. Increased O-GlcNAc also does not affect the onset of insulin resistance induced by a high-fat diet. These results suggest that pharmacologically increased O-GlcNAc levels do not cause insulin resistance nor do they disrupt glucohomeostasis. Therefore, the protective benefits of increased O-GlcNAc may be achieved without deleteriously affecting glucohomeostasis.

ClpX/ClpA Bound State of ClpP Channeled Axially

PAGE 959

Identification of the ClpP protease, a key bacterial enzyme for maintenance of cellular protein homeostasis, as the target for the new class of antibiotics acyldepsipeptides has been heralded as a major advance in the search for new drug leads. The study by Li et al. presents a crystal structure of *Escherichia coli* ClpP complexed with acyldepsipeptide-1 and reveals that antibiotic binding opens a structured 20 Å diameter axial pore in the ClpP tetradecamer through which extended polypeptides can be threaded. Acyldepsipeptide-1 mimics the interaction of ClpP with its ATPases. Consequently, this structure provides a model for ClpP in the ClpAP and ClpXP holo-complexes.



Peptide with a Direct Punch

PAGE 970

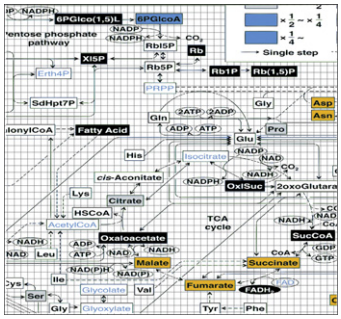
The structure and function of the innate defense regulator 1018, a 12 residue peptide, was investigated by Wieczorek et al., and the peptide was shown to have enhanced innate immune regulatory and moderate direct antibacterial activities. Structurally, 1018 was shown to adopt a variety of folds tailored to its different functions. The structural data is discussed in light of the ability of 1018 to potently induce chemokine responses, suppress the LPS-induced TNF-α response, and directly kill both Gram-positive and -negative bacteria.

Regulating Protein Stability in CNS

PAGE 981

Iwamoto et al. developed a general technique in which the stability of a specific protein is regulated by a commercially available small molecule. Mutants of *E. coli* DHFR were engineered to be unstable in the absence of its high-affinity ligand, trimethoprim (TMP). When this domain is fused to a protein of interest, its instability is conferred to the fused protein, resulting in rapid degradation of the entire fusion protein. TMP stabilizes the domain in a rapid, reversible, and dose-dependent manner and has the ability to cross the blood-brain barrier, enabling the regulation of proteins expressed in the mammalian central nervous system.

Metabolomics to the Rescue: Finding the Target



PAGE 989

In this report, Kitagawa et al. discuss efforts to identify the cellular target of the unique natural bioactive product glucopiericidin A (GPA) as a prelude to development of chemical genetic studies. The report describes the natural product screening to obtain the unique bioactive compounds. Next, the authors develop the use of CE-MS metabolomic analysis to identify glucose transporter as the target of the GPA, which demonstrates utility of the approach to identify specific molecular target of an important natural product.

Bioluminescent Activity-Based Probe for Caspase-1

PAGE 999

The role of caspase-1 in inflammation has been studied intensely over recent years. However, the field lacks sensitive and selective tools to monitor not only its abundance, but also its activity. Here, Kindermann et al. present a bioluminescent activity-based probe (ABP) for caspase-1, developed by the Reverse Design concept, where chemically optimized protease inhibitors are turned into selective substrate ABPs. The probe exhibits excellent selectivity and ~1000-fold increase in sensitivity compared to existing peptidic caspase-1 substrates. Moreover, the authors could monitor and quantify caspase-1 activity directly in cell lysates. The activity correlated well with processing of prointerleukin-1β and prointerleukin-18 in PMA-stimulated cells.

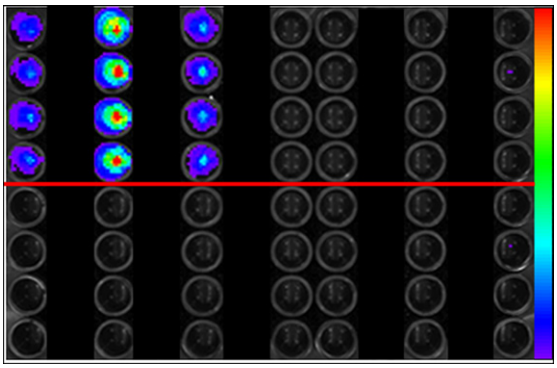
Thermal Regulation of HSP26

PAGE 1008

Benesch et al. have employed a range of biophysical techniques to investigate the structure and dynamics of the molecular chaperone HSP26 from *Saccharamoyces cerevisiae*. The hybrid approach that combines multiangle light scattering, fluorescence spectroscopy, NMR spectroscopy, and mass spectrometry uncovers the remarkable thermally regulated conformational and oligomerization transitions of this protein in vitro, which likely enables its protective function in vivo.

Fragment Complementation Assays All Aglow

PAGE 1018



Understanding the functional complexity of protein interactions requires mapping biomolecular complexes within the cellular environment over biologically relevant time scales. Villalobos et al. describe a set of reversible, multicolored heteroprotein complementation fragments based on various firefly and click beetle luciferases that utilize the same substrate, D-luciferin. Luciferase heteroprotein fragment complementation systems enabled dual-color quantification of two discreet pairs of interacting proteins simultaneously or two distinct proteins interacting with a third shared protein in live cells. These dual-color protein interaction switches may enable directed dynamic analysis of a variety of protein interactions in living cells.

High Redox Potential Laccases Evolved in a Lab

PAGE 1030

Maté et al. describe eight rounds of directed evolution of the fusion gene formed by the alpha-factor prepro-leader and the high-redox potential laccase from basidiomycete PM1. After screening over 50,000 clones generated by random mutagenesis, in vivo DNA shuffling, IvAM, and/or site directed mutagenesis, the laccase activity was enhanced up to 34,000 fold, the largest improvement ever reported for this kind of system. The ultimate variant obtained through this evolutionary process is readily secreted by *S. cerevisiae* in a soluble, very active, and very stable form, particularly in terms of temperature, pH, and cosolvents.

