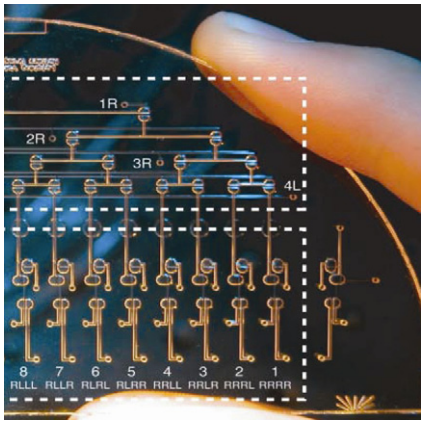
In This Issue

In Review: Microfluidic Devices



PAGE 1052

Microfluidic systems are an attractive solution for the miniaturization of biological and chemical assays. The typical sample volume can be reduced up to one million-fold and a superb level of spatiotemporal control is possible, facilitating highly parallelized assays with drastically increased throughput and reduced cost. In this review, Vyawahare et al. focus on systems in which multiple reactions are spatially separated by immobilization of reagents on two-dimensional arrays, or by compartmentalization in microfabricated reaction chambers or droplets. The authors discuss both current applications and likely future impacts in areas such as next generation sequencing, single cells / single organism assays and high-throughput screening.

In Brief: FASII Pathway and Self-Resistance

PAGE 1067

Kalimantacin/batumin antibiotics are emerging as potential treatment option for combating pathogenic bacteria with high resistance to currently available treatments, such as methicilin resistant *Staphylococcus aureus* (MRSA). Here, Mattheus et al. show that one of the gene products from the kal/bat gene cluster, *batG*, is not essential for antibiotic biosynthesis but rather encodes a functional FabI isozyme that confers full resistance to kalimantacin/batumin. Thus, kalimantacin antibiotics inhibit bacterial fatty acid synthesis (FASII pathway), which has significant implications for the clinical potential of these antibiotics, since the essential nature of the FASII pathway in Gram-positive pathogens is currently under debate.

In Brief: Genetically Directing ε-N, N-Dimethyl-L-Lysine

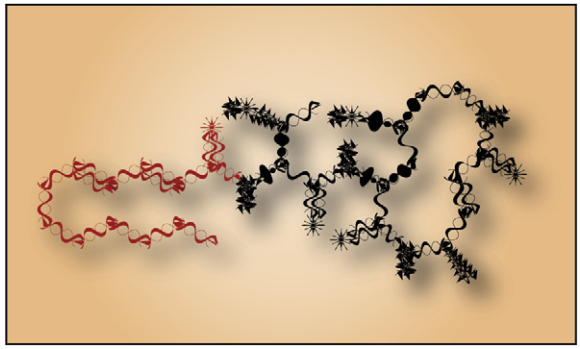
PAGE 1072

A molecular understanding of the biological phenomena orchestrated by lysine Nε-methylation is impeded by the challenge of producing site specifically and quantitatively methylated histones. Here, Nguyen et al. report a general method that combines genetic code expansion and chemoselective reactions for the quantitative, site-specific installation of dimethyl-lysine in recombinant histones. The authors demonstrate the utility of this method by preparing H3K9me2 and show that this modified histone is specifically recognized by heterochromatin protein 1 beta. Extensions of the strategy reported here will allow a range of chemoselective reactions (which have be used for residue-selective but not site-selective protein modification) to be leveraged for site-specific protein modification.

In Brief: N-Acylation in Glidobactin Biosynthesis

PAGE 1077

Glidobactins are a group of N-acylated cyclic tripeptides secreted by a species of *Burkholderia* that are known to act as potent and selective proteasome inhibitors. N-acylation is a common modification in peptidyl natural products and frequently has a profound impact efficiency. In an effort to understand glidobactin biosynthesis and N-acylation better, Imker et al. have carried out an in vitro investigation of the N-acylation reaction with heterologously expressed proteins from the glidobactin gene cluster. The authors found that N-acylation initiates glidobactin biosynthesis by condensation between fatty acyl-CoA donors and the first amino acid prior to elongation with the second and third amino acids.



Inhibitor Selectivity in Prokaryotic IMP Dehydrogenases

PAGE 1084

The rise of multiply drug resistant bacteria creates an urgent need for new antibiotics and novel antibiotic targets. Inosine 5′-monophosphate dehydrogenase (IMPDH), a key enzyme in the biosynthesis of RNA/DNA precursors, is a target for cancer therapy that has not been exploited in antibiotic development. Here, Gollapalli et al. report selective inhibitors of IMPDH from the protozoan parasite *Cryptosporidium parvum* also exhibit antibacterial activity. Susceptible enzymes are defined by a structural motif that is found in IMPDHs from a wide variety of pathogenic bacteria, including seven select agents, suggesting that IMPDH-targeted inhibitors can be developed into a new class of broader spectrum antibiotics.

Surrogate Acetyl ACP Donors for BryR

PAGE 1092

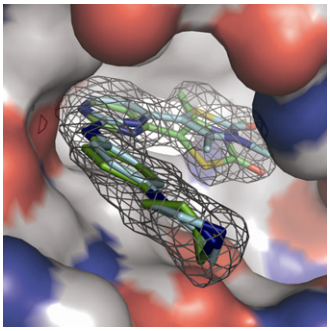
In vitro analysis of natural product biosynthetic gene products isolated from unculturable symbiotic bacteria is necessary to probe the functionalities of these enzymes. Herein, Buchholz et al. report a biochemical characterization of BryR, a key enzyme in the biosynthetic pathway for the medicinally important natural product bryostatin. BryR's role in polkyketide synthase β-branching was verified by radio-SDS PAGE, FTICR-MS, and SPR. Specificity for protein-bound acyl groups was examined with surrogate surrogate acetyl-ACP donors. These findings reveal an important example of molecular recognition between protein components that are essential for biosynthetic fidelity in natural product assembly and modification.

S. aureus and *B. subtilis*: You Go Your Own Way to WTA

PAGE 1101

Wall teichoic acids (WTA) are important cell surface polymers in many Gram-positive organisms. An understanding of WTA biosynthesis is important for conducting detailed studies on their cellular functions. Brown et al. have used a combination of in vitro reconstitution and genetics to elucidate the pathways for polyribitol-phosphate WTA biosynthesis in *B. subtilis* W23 and *S. aureus*. Although these organisms make similar WTA polymers, the biosynthetic pathways differ in several unexpected ways. This work highlights the enzymatic diversity of WTA biosynthetic enzymes and should facilitate continued efforts to predict teichoic acid gene functions.

Selective CDK Inhibitors as Anticancer Agents



PAGE 1111

A difficulty in the development of ATP antagonist kinase inhibitors is target specificity. Wang et al. introduce a strategy to identify compounds that block the cyclin-dependent kinases responsible for regulating transcription: CDK7 and especially CDK9. The screening cascade uses cellular assays based on mitotic index and nuclear p53. The authors classified compounds into several mechanistic groups and describe the transcriptional inhibitor class in terms of kinase inhibition and cellular mode of action. A structural selectivity rationale is presented that was used to optimize potency and biopharmaceutical properties and led to the development of a transcriptional inhibitor, 3,4-dimethyl-5-[2-(4-piperazin-1-yl-phenylamino)-pyrimidin-4-yl]-3H-thiazol-2-one, with anticancer activity in animal models.

Compartmentalized Co-Catabolism in *Mycobacterium tuberculosis*

PAGE 1122

Mycobacterium tuberculosis (Mtb) has evolved to survive in its human host environment by number of adaptations, including those relevant for central carbon metabolism. Although carbon metabolism plays a major role in Mtb's pathogenicity, biochemical knowledge and complete precursor-product relationships among metabolites are incomplete. To explore distinct adaptations of Mtb metabolism, de Carvalho et al. perform a metabolomic analysis of Mtb's central carbon metabolism and show that Mtb is capable of simultaneously co-catabolizing different carbon sources to maximize growth. Moreover, the authors show that during co-catabolism, a carbon source is catabolized through different pathways and has distinct metabolic fate.

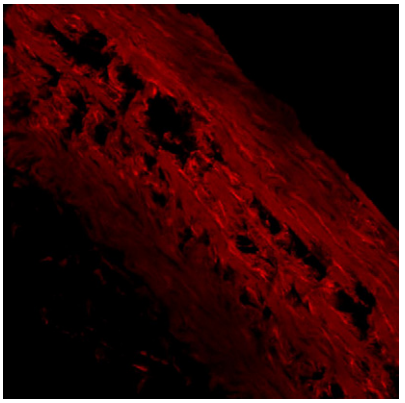
Painting the Human Cannabinoid 2 GPCR Ligand-Interaction Landscape

PAGE 1132

Activity of the CB2 cannabinoid receptor has been implicated in the pathogenesis of autoimmune and inflammatory diseases and allergic responses. Thus, small-molecule ligands that bind to and inhibit the CB2 receptor have promise as potential therapeutics for these and other disorders. In order to design such ligands that are potent and selective for the CB2 receptor without inducing off-target effects, it is important to understand the ligand-binding architecture of the CB2 receptor. By using a "ligand-assisted protein structure" experimental approach, the work by Mercier et al. has directly identified cysteine residues in the human CB2 receptor as being key to antagonist/inverse agonist ligand binding to and activity at the human CB2 receptor.

Tissue Transglutaminase Associated with Arterial Stiffening

PAGE 1143



Tissue transglutaminase (TG2) catalyzes the cross-linking of proteins and has been implicated in arterial stiffness. In order to interrogate this role, Chabot et al. have prepared a fluorescent irreversible inhibitor as a probe for TG2 activity. This probe was synthesized on solid support, characterized kinetically, and then used to stain the aorta from rats used as a model of isolated systolic hypertension. TG2 activity was thus shown to increase over 4 weeks of the hypertension model, corresponding with the previously observed increase in arterial stiffness and suggesting an association between TG2 and arterial rigidification.

(me)Lan Holds a Key to Lacticin 3147 Stability

PAGE 1151

The lantibiotics are an unusual category of antibiotics which differ due to the presence of structures known as lanthionines. These lanthionines come in two forms i.e. lanthionine (Lan) and β-methyllanthionine (meLan). In addition to being of key importance with respect to the structural integrity of lantibiotics, it is presumed that (me)Lan also contribute to the resistance of lantibiotics to thermal stress and proteolytic degradation. However, somewhat surprisingly, such a role has not been investigated in great depth. Using the lantibiotic lacticin 3147, and derivatives thereof, Suda et al. address this issue and highlight the extreme importance of (me)Lans in this regard.

