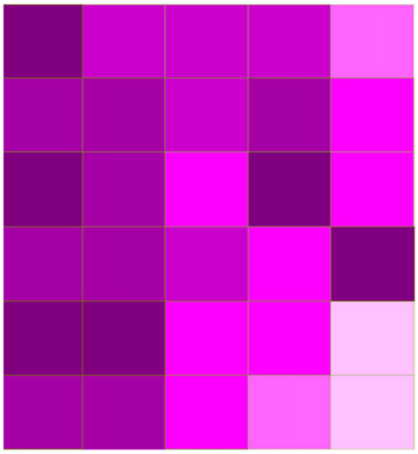
Chemistry & Biology

In This Issue

Introducing Glycobioarray

PAGE 553

A key goal of glycomics is large-scale integrated approaches to study structure-function relationships of glycans, eventually at the level of whole systems. Glycoarrays are powerful tools for the interrogation of glycan-protein interactions but have lacked the ability to generate cell-response data. Puvirajesinghe et al. describe a ‘‘glycobioarray’’ platform that measures activation of fibroblast growth factor signaling by heparin saccharides and that has a potential for functional glycomics screening and for high-throughput assessment of a range of living cell responses.



Staphylococcus Gives In to the Hijacker

PAGE 559

The natural product CJ-15,801 is an inhibitor of *Staphylococcus aureus*, but not other bacteria. It bears close structural resemblance to pantothenic acid, the vitamin precursor of coenzyme A (CoA), which suggests that its biological target is an enzyme involved in CoA biosynthesis or utilization. Van der Westhuyzen et al. demonstrate that CJ-15,801 is transformed by the uniquely selective *S. aureus* pantothenate kinase, the first CoA biosynthetic enzyme, into a substrate for the next enzyme, phosphopantothenoylcysteine synthetase, which is inhibited through formation of a tight-binding structural mimic of its native reaction intermediate. These findings reveal that CJ-15,801 (like the sulfonamide antibiotics) acts as a vitamin biosynthetic pathway antimetabolite.

How Do Hemimethylated CpG Sites Get More Methyls

PAGE 572

The specific methylation of hemimethylated CpG sites by Dnmt1 is important for the inheritance of DNA methylation patterns. Recently, an autoinhibition model for the specificity of Dnmt1 was proposed based on truncated Dnmt1 analysis, in which unmethylated DNA binds to Dnmt1 CXXC domain, which prevents methylation. Here, Bashtrykov et al. show that the autoinhibition model does not apply for full-length Dnmt1. The authors demonstrate that a Dnmt1, a variant which carries an exchange in the catalytic domain, displays a reduction in specificity, indicating that recognition of hemimethylated CpG sites resides within the catalytic domain.

Hitting It Off with MAGL

PAGE 579

Monoacylglycerol lipase (MAGL) serves as a key metabolic hub connecting endocannabinoid and eicosanoid signaling networks. Selective MAGL inhibitors are needed to functionally probe endocannabinoid and eicosanoid systems and hold potential for treating human disorders, including pain and inflammation. Chang et al. report a different class of MAGL inhibitors, O-hexafluoroisopropyl (HFIP) carbamates, that display excellent potency, selectivity, and in vivo activity. That HFIP carbamates appear to achieve their potency and selectivity at least in part because of the bioisosteric nature of their leaving group leads the authors to speculate that this type of substrate-mimetic strategy could be generalized to develop inhibitors for many serine hydrolases.

Aloha to Honaucins A–C

PAGE 589

Three structurally novel natural products, honaucins A–C, were isolated from the Hawaiian bloom-forming cyanobacterium *Leptolyngbya crossbyana*, as described by Choi et al. All three metabolites exhibited potent inhibition of a quorum sensing-dependent phenotype in *Vibrio harveyi* BB120 as well as of lipopolysaccharide-stimulated nitric oxide production in the murine macrophage cell line RAW264.7. Synthesis of honaucin A as well as a number of analogs and subsequent evaluation in biological assays revealed the essential structural features for activity in this chemical class and provided analogs with greater potency in both the anti-inflammatory and quorum sensing inhibitory assays.

Window into the Small Heat-Shock Proteins

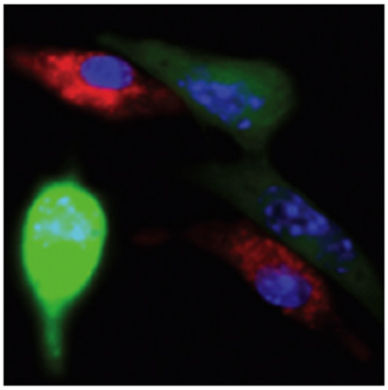
PAGE 599

The intrinsic heterogeneity of proteins is one of the greatest challenges facing structural biologists today. Stengel et al. present a mass spectrometry analysis algorithm, CHAMP, for estimating the distribution of stoichiometries comprising a polydisperse ensemble. The authors apply their approach to elucidate the organization of complexes formed by a small heat-shock protein with different target proteins. They find that these molecular chaperone interactions are mass dependent and the native organization of the targets is retained. This indicates that protection happens early in the denaturation process and explains how variable complex morphologies result from the generic mechanism of protection afforded by these molecular chaperones.

Untangling Kinase Preference

PAGE 608

Kettenbach and colleagues describe the development and application of a mass spectrometry-based approach to identify the amino acid preferences in substrate sequences that are preferred by a given kinase. The method relies on using libraries of naturally occurring peptides that are desphosphorylated prior to treatment with purified kinase, followed by identification by tandem mass spectrometry. In this study, the authors demonstrate that specific, individual motifs are preferred by kinases that are frequently not well represented by a single consensus sequence alone. In addition, the authors discover that some kinases prefer to phosphorylate substrates at peptide termini and validate some of these findings in cells in culture.



Reporting on Tumor-Associated Macrophages

PAGE 619

Verdoes et al. describe the synthesis and characterization of a cathepsin S-directed, quenched activity-based probe (qABP), BMV083. The use of an optimized nonpeptidic scaffold resulted in enhanced in vivo properties relative to previously reported peptide-based probes. In a mouse breast cancer model, BMV083 provided high tumor specific fluorescence that could be visualized using noninvasive optical imaging methods. Furthermore, analysis of probe-labeled cells demonstrated that the probe primarily targets macrophages with an M2 phenotype. Thus, BMV083 is a potential valuable in vivo reporter for tumor-associated macrophages that could greatly facilitate the future studies of macrophage function during the process of tumorigenesis.

Drugs and Degrons

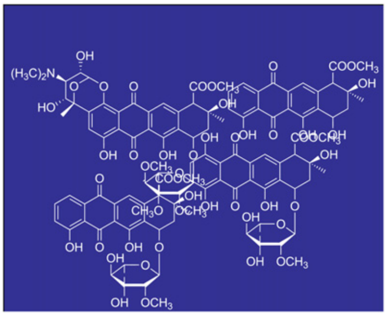
PAGE 629

Several drugs induce the degradation of their target proteins. The discovery of compounds with this property has been largely serendipitous, and a general strategy for the design of such compounds has yet to emerge. Long et al. have discovered that Boc_3Arg-linked inhibitors induce degradation of their target proteins. Robust degradation was observed with the Boc_3Arg moiety in the context of three different inhibitor scaffolds and with three different target proteins, including an abundant endogenous protein. Importantly, the inhibitors can be covalent or noncovalent. Thus, Boc_3Arg is a “degron” that will be useful in the design of drugs and chemical tools.

SnoaW/SnoaL2 Two-Component Monooxygenase

PAGE 638

Nogalamycin is an anthracycline antibiotic that harbors significant biological activity. The metabolite is hydroxylated at C-1 during the biosynthesis, but the gene cluster does not encode classical monooxygenases that could be responsible for the reaction. Here, Siitonen et al. demonstrate in vivo and in vitro that the hydroxylation is performed by a two-component monooxygenase SnoaW/SnoaL2 in the presence of NAD(P)H. The authors propose a model where the atypical short-chain alcohol dehydrogenase (SDR) SnoaW reduces the anthraquinone using NADPH, which enables activation of molecular oxygen and formation of a hydroperoxy intermediate. The 1-hydroxylated product may be formed through protonation of the intermediate by SnoaL2.



Tailoring Enzymes with Hidden Ancestral Functions

PAGE 647

Patrikainen et al. have compared homologous angucycline modification enzymes from five closely related *Streptomyces* pathways, which allowed them to deduce the biosynthetic steps responsible for three alternative products, gaudimycin C, dehydrorabelomycin, and 11- deoxylandomycinone. The data suggests that the C-12 β-hydroxylated urdamycin and gaudimycin metabolites appear to be the ancestral representatives from which landomycins and jadomysins have evolved as a result of functional divergence of the ketoreductase LanV and hydroxylase JadH, respectively. Importantly, the study reveals that many of the modification enzymes carry several alternative, hidden, or ancestral catalytic functions, which are strictly dependent on the biosynthetic context.