In This Issue

Connecting the Quantum Dots

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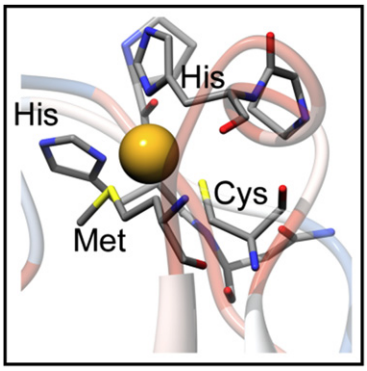
Quantum dots (QDs) are semiconductor crystals with sizes in low nanometer range. They have exceptional luminescent properties which form the basis for their use as imaging tools in biological systems. The biological applications for QDs range from fluorescence-based drug discovery assays and disease detection to intracellular reporting and they offer a unique set of tools for interrogation of biological function and mechanism. In this review, Rosenthal et al. introduce QDs, describe methods for the synthesis and design of biocompatible QDs, discuss several application to study of biologically relevant problems, point out some of the limitations of their use, and provide a future outlook of the field.



It's All in Local Geometry

PAGE 25

Identifying the factors that govern the thermal resistance of cupredoxins is essential for understanding their folding and stability and for designing novel, highly stable enzymes with biotechnological applications. Here Chaboy et al. show that the thermal unfolding of plastocyanins is closely related to the short-range structure around the copper centre. Cu K-edge X-ray absorption spectroscopy shows that the bond length between Cu and the S atom from the cysteine ligand is a key structural factor that correlates with the thermal stability of the cupredoxins in both oxidized and reduced states. Notably, this bond is modulated by long-range interactions within the protein matrix.

Genome Mining in *Streptomyces clavuligerus*

PAGE 32

The biochemical functions of two cryptic terpene synthases encoded by the sscg_02150 and sscg_03688 genes of *Streptomyces clavuligerus* ATCC 27074 have been assigned by Hu et al. via expression of the individual recombinant proteins in *Escherichia coli* using codon-optimized synthetic genes. Incubation of recombinant SSCG_02150 with farnesyl diphosphate (FPP) gave (-)-δ-cadinene while recombinant SSCG_03688 converted FPP to (+)-T-muurolol. Individual incubations of each recombinant terpene synthase with chirally deuterated samples of farnesyl diphosphate and NMR analysis of the resulting samples of deuterated (-)-δ-cadinene and (+)-T-muurolol supported a cyclization mechanism involving the intermediacy of nerolidyl diphosphate and a 1,3-hydride shift of the original H-1si of FPP.

Diacetylene Scaffold Overcomes the Resistance

PAGE 38

LpxC in lipid A biosynthesis is a novel antibiotic target for treating multi-drug-resistant Gram-negative infections. However, the majority of LpxC inhibitors display a limited range of antibiotic activity. Lee et al. show that compounds based on a diacetylene scaffold inhibit a wide range of Gram-negative pathogens by overcoming the resistance caused by sequence and conformational heterogeneity in the LpxC substrate-binding passage. The discovery of large, species-specific conformational differences in distinct LpxC enzymes and a relatively small scale of inhibitor-induced structural plasticity provides a molecular explanation for the limited efficacy of existing compounds and a clue for designing better inhibitors.

Peptide Phosphonates Protease Inhibitors

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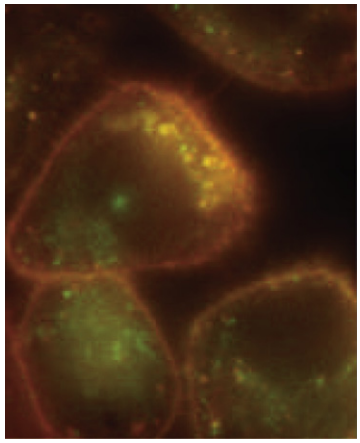


Serine proteases contain many important enzymes that contribute to numerous biological processes. Peptide phosphonates are activity-based probes (ABPs) that selectively bind active serine proteases in complex biological proteomes, allowing researchers to follow only those enzymes that are functionally relevant to a given biological process. In this work, Brown et al. improve upon the synthesis of peptide phosphonates and identify peptide length and prime-side sterics to be important determinants of potency for these molecules. The authors use this information to create an optimized ABP for trypsin-fold serine proteases and develop a novel cell surface labeling application to follow active proteases on live cancer cells.

Catalytically Active Transglutaminase 2 Clicks On

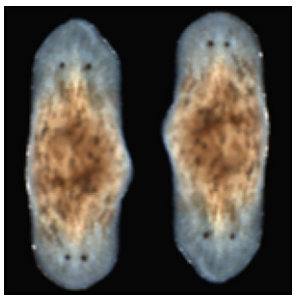
PAGE 58

Transglutaminase 2 (TG2), a ubiquitous member of the mammalian transglutaminases family, is found in intracellular and extracellular environments of many organs. In this study, Dafik and Khosla report the synthesis and preliminary biochemical and biological characterization of "clickable" inhibitors of TG2. These inhibitors possess the 3-halo-4,5-dihydroisoxazole warhead and bio-orthogonal groups such as azide or alkyne moieties that can be further covalently modified with fluorophores. The biological potential of these mechanism-based inhibitors is evaluated by their ability to inhibit and visualize transiently activated TG2 in a fibroblast scratch assay. These probes have the potential to serve as chemical tools to study allosteric regulation of this important multifunctional protein.

Kinase Inhibition and Alternative Splicing

PAGE 67

Fedorov et al. report the discovery and characterization of a highly potent and selective inhibitor (KH-CB19) for the CDC2-like kinase isoforms 1 and 4 (CLK1/CLK4). The high selectivity of the inhibitor is explained by cocrystal structures which revealed a non-ATP mimetic binding mode and a halogen bond to the kinase hinge region. At low nanomolar concentration, KH-CB19 effectively suppressed phosphorylation of SR (serine/arginine) proteins in cells, generated a unique pattern of splicing factor dephosphorylation, and had a profound effect on splicing of tissue factor isoforms.



Regrowing a Head with a Little Help

PAGE 77

The work by Beane et al. identifies a physiological property, membrane voltage, as a novel regulator of head-tail polarity during regeneration in adult planarians. This exciting discovery reveals an innovative and useful method for controlling patterning in adult stem cell-derived tissues, including brain. The authors show that pharmacologically induced changes in membrane voltage are sufficient to completely initiate head regeneration. As demonstrated here, chemical modulation of ion transport using known drugs (which are already approved for human use) could be a powerful tool to induce the regrowth of lost tissues (organs and limbs) that are correctly patterned and thus functional.

Lantibiotics from *Bacillus* to *Escherichia coli*

PAGE 90

The lichenicidin gene cluster was successfully expressed in *Escherichia coli* by Caetano et al., thus constituting the report of a full reconstitution of a lantibiotic biosynthetic pathway in vivo by a Gram-negative host. This system was further exploited to characterize and assign the function of proteins encoded in the biosynthetic gene cluster in the maturation of lichenicidin peptides. Moreover, a trans complementation system was developed for expression of Bliα and Bliβ lantibiotic variants in vivo. This contribution will spur future studies in the heterologous expression and engineering of lantibiotics.

Tautomycetin Blocks SHP2

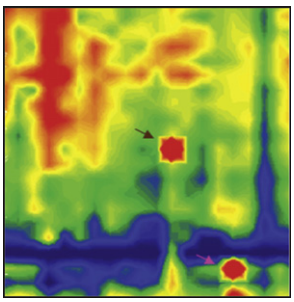
PAGE 101

SHP2, a positive transducer of growth factor and cytokine signaling and a bona fide oncogene tyrosine phosphatase associated with multiple forms of leukemia and solid tumors, is identified as a cellular target for natural product tautomycetin (TTN), an antifungal agent with novel immunosuppressive activity, by Liu et al. TTN and its engineered analog TTN D-1 block both SHP2-mediated signaling in T cell and activating SHP2-induced hematopoietic progenitor hyperproliferation and monocytic differentiation. Crystal structure of SHP2 in complex with TTN D-1 reveals molecular insights upon which novel therapeutics targeting SHP2 can be developed based on the TTN polyketide scaffold.

Finding New Methylation Targets

PAGE 111

Lysine methylation is an important regulatory posttranslational modification of proteins. Dhayalan et al. apply peptide array methylation to determine an optimized target sequence for the SET7/9 (KMT7) protein lysine methyltransferase. Based on this, the authors identify 91 peptide substrates from human proteome and confirm methylation of corresponding protein domains for 11 examples, which more than doubles the number of known SET7/9 targets. They show that the degree of methylation, i.e., whether monomethyllysine or dimethyllysine products are formed by SET7/9, depends on the substrate. SET7/9 was found to be interconnected with other posttranslational modifications, as the phosphorylation of its substrate proteins was found to inhibit the enzyme.



Arginyltransferase Mystery Is Out

PAGE 121

Posttranslational arginylation mediated by arginyltransferase (ATE1) plays an important role in cardiovascular development, cell motility, and regulation of cytoskeleton and metabolic enzymes. Arginylation was discovered decades ago; however, its molecular mechanisms remained poorly understood due to the lack of good biochemical models. Here, Wang et al. report the development of an in vitro arginylation system, in which ATE1 function and molecular requirements can be tested with a controlled number of components. Using this system, the authors discovered important molecular properties of ATE1 enzymes and gained mechanistic insights into the arginylation reaction that explain some of the mysteries surrounding this enigmatic posttranslational modification.

Aurilide Prefers Prohibitin

PAGE 131

Aurilide is a potent cytotoxic marine natural product that kills human cells at the picomolar to nanomolar range; however, its mechanism of action has been unknown. Here, Sato et al. show that aurilide selectively binds to prohibitin 1 (PHB1) in the mitochondria, activating the proteolytic processing of optic atrophy 1 (OPA1), and resulting in mitochondria-induced apoptosis. The mechanism of aurilide cytotoxicity suggests that PHB1 is an apoptosis-regulating protein amenable to modulation by small molecules. Aurilide may serve as a small molecule tool for studies of mitochondrion-induced apoptosis.

