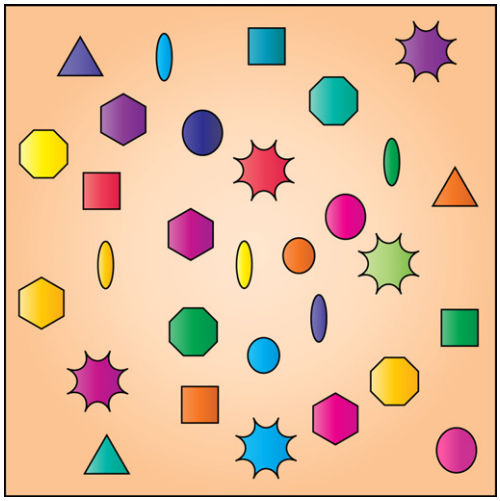
In This Issue

Activity-Based Metabolomic Profiling Hits TB



PAGE 323

Activity-based metabolomic profiling (ABMP) is a new and valuable tool with which to discover unannotated enzymatic activities. ABMP utilizes the cell's own metabolome as a highly dense and physiologically relevant library of potential substrates and products suitable for interrogation by a purified recombinant protein followed by untargeted detection and quantitation by liquid chromatography-coupled, time-of-flight mass spectrometry. The data by de Carvalho et al. demonstrate that Rv 1248c of *Mycobacterium tuberculosis* encodes a 2-hydroxy-3-oxoadipate synthase (HOAS) and not its annotated function as an α-ketoglutarate decarboxylase. ABMP is thus a highly valuable addition to the current functional genomics toolbox.

Deeper into the Blue and Red Chromophores

PAGE 333

Maturation of red fluorescent proteins has been shown to proceed through a blue intermediate. The chemical nature of this intermediate and the mechanism of red chromophore formation, however, remained unknown. To elucidate this mechanism, Subach et al. determined crystal structures of the red fluorescent protein TagRFP and its derivative, the blue fluorescent protein mTagBFP. Based on the crystallographic and mass spectrometry data, the authors have found that mTagBFP has a novel type of the blue chromophore. Moreover, the data allowed for the proposal of a chemical mechanism for the formation of the mTagBFP chromophore and its transformation into the TagRFP red chromophore.

Making of an Anti-TB Macrolide

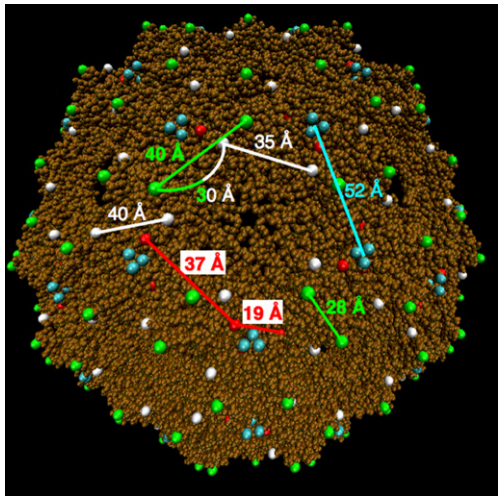
PAGE 342

The thuggacin secondary metabolites exhibit promising activity against Mycobacterium tuberculosis, the causative agent of tuberculosis. Several variants of these structures are biosynthesized by the myxobacterial strains *Sorangium cellulosum* So ce895 and *Chondromyces crocatus* Cm c5. By comparative analysis of the biosynthetic gene clusters in the two bacteria, coupled with gene inactivation, Buntin et al. have determined the mechanistic basis for these architectural differences. In addition, the authors have identified a new reductive carboxylase enzyme that likely participates in biosynthesis of an unusual polyketide extender unit hexylmalonyl-CoA.

Immunogens on Virus Capsid Scaffolds

PAGE 357

The surface of HIV-1 virus is covered in a dense array of host-derived carbohydrates. The broadly neutralizing HIV-1 antibody 2G12 binds a cluster of high-mannose carbohydrates on the surface glycoprotein gp120. Astronomo et al. chemically linked oligomannose motifs to the surface of icosahedral virus capsids to mimic the carbohydrates on the surface of HIV and showed these glycoconjugates to be good 2G12 antigenic mimics. Although authors were able to elicit high titres of mannose specific antibodies, these antibodies did not show HIV reactivity. These studies provided novel insights into the influence of carbohydrate linkage on mannose antigenicity and immunogenicity as well as the impact of epitope heterogeneity on carbohydrate vaccine design for HIV. (Figure adapted from Astronomo et al.)

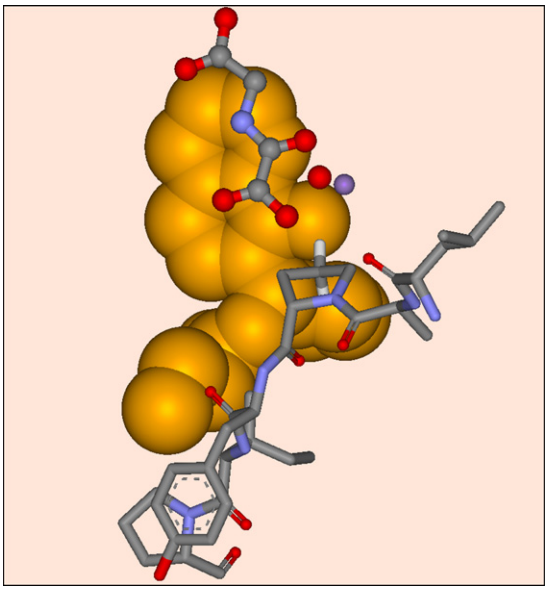


Monomeric or Oligomers? Not a Question Any Longer

PAGE 371

Submicroscopic oligomers of misfolded proteins are implicated as a source of toxicity to cells in several neurodegenerative diseases such as Huntington's. With the combinatorial use of tetracysteine tags, biarsenical dyes, and fluorescent proteins, Ramdzan et al. developed sensors for visually demarcating the localization of monomers of mutant huntingtin from all other oligomeric forms in live cells. The sensors showed individual cells to have a substantial proportion of oligomers not otherwise discernable from monomers by standard confocal microscopy. The approaches shown here are anticipated to have more general utility for the study of protein self-association directly in live cells.

Potent Activators of Hypoxic Adaptation



PAGE 380

Hypoxia is a biological stress relevant to diverse processes in development, tissue maintenance, and disease. Surprisingly, a comprehensive set of chemical probes has not been available to manipulate hypoxia signaling in living cells. Smirnova et al. harnessed a reporter of a hypoxia sensitive transcription factor for high throughput screening and identified potent activators of hypoxic adaptation that induce erythropoietin and vascular endothelial growth factor gene expression in human cells and protect neurons from oxidative death. These compounds hold promise as exciting therapeutics for diseases characterized by tissue hypoxia or metabolic imbalance such as anemia, stroke, myocardial infarction, and Alzheimer's disease. (Figure credit: Smirnova t al.)

Polyphenols with Altered ER Binding Affinity

PAGE 392

Polyphenols such as isoflavonoids display significant potential benefits for human health with promising effects in heart disease and cancer. Notably, some display striking affinities for steroid receptors. Chemler et al. describe the assembly of a modular plant-derived biosynthetic pathway in brewer's yeast capable of efficiently producing estrogen-like isoflavonoids. Based on the determination of the substrate requirements of isoflavone synthase, a series of natural and nonnatural isoflavones were prepared and their binding affinities for the human estrogen receptors (ERα and ERβ) were determined. Structure activity relationships are suggested based on changes to binding affinities related to small structure variations.

Dehydrophos Biosynthetic Gene Cluster

PAGE 402

The work by Circello et al. addresses the issue of bacterial resistance by exploring the biosynthesis of the antibiotic dehydrophos. Research focusing on the biosynthesis of phosphonates as secondary metabolites is limited and the data may be of use in deciphering future phosphonate gene clusters. Although dehydrophos is a tripeptide, no identifiable nonribosomal peptide synthetases are present within the minimal cluster, indicating an alternate mode of peptide bond formation. Similarly, dehydrophos contains an unusual vinylphosphonate moiety with no obvious mechanism for formation. Understanding these transformations could reveal unique chemistry and aid in combinatorial biosynthesis of phosphonates.