In This Issue

NRPS under Close Surveillance

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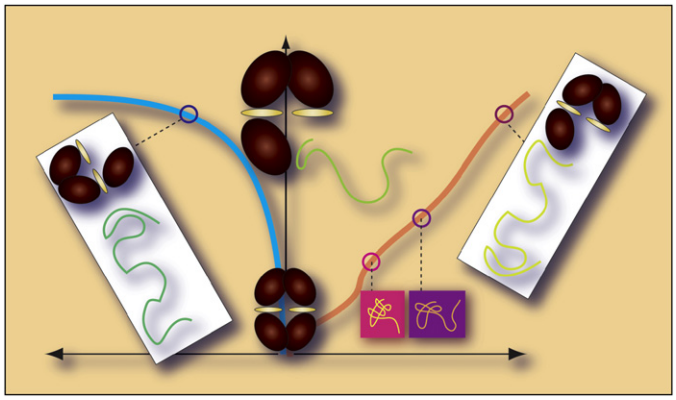
Past examinations of protein interactions within NRPSs have established the COM^D and COM^A domains of cognate NRPSs to be responsible for the selective interactions necessary for proper processing of NRP intermediates. Hur et al. have developed bioorthogonal cross-linking probes that are compatible with carrier protein modification and sensitive to the selective protein interactions of the COM domains between cognate NRPSs. Based on these results, the authors aim to apply these tools towards structural studies of the interactions that occur during selective communication between NRPS modules and to gain further insight into the cryptic mechanisms employed by multifunctional biosynthetic assembly lines.

Ion Mobility to the Rescue



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Frequently, subtle differences in the stability afforded by ligand binding to protein assemblies cannot be detected by mass spectrometry (MS). Here, Hyung et al. show that monitoring the unfolding of protein subunits, using ion mobility-MS (IM-MS), allows differentiation of the effects of ligand binding not normally observed by MS alone. Using wild-type and disease-associated variants of tetrameric transthyretin, MS data indicate that populations of the variant protein are less stable than wild type. IM-MS, however, is able to show that the natural ligand of transthyretin, thyroxine, provides a larger stability increase to the tetramer composed of variant subunits than to the wild-type protein-ligand complex.

Oncogene Fighting Peptides

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Rho GTPases are activated by RhoGEFs, many of which have been isolated as oncogenes. Strategies to inhibit their activity are therefore actively being sought. Here Bouquier et al. devise a peptide inhibitor screening strategy to target the activity of the oncogenic RhoGEF Tgat. Using this strategy, the authors identify a peptide that specifically inhibits Tgat GEF activity in vitro and strongly reduces its oncogenic properties in vivo, most remarkably by decreasing tumor development in nude mice. They demonstrate that small peptides are potent inhibitors that can interfere with RhoGEF functions in vivo, which represents a promising alternative for the discovery of leads for new therapeutic drugs.

N-methyltransferase MtfA for Modifying Glycopeptide Antibiotics

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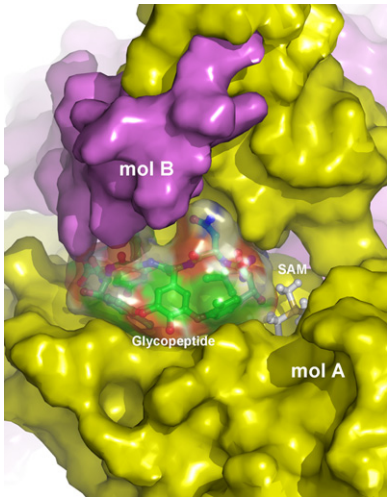

Glycopeptide antibiotics (GPAs) comprise a class of anti-infective agents with activity against serious Gram-positive bacterial pathogens. However, resistance to these antibiotics is becoming a serious and growing threat. Shi et al. have shown that the N-methyltransferase MtfA from the vancomycin-class GPA biosynthetic pathway can be used in vitro and in vivo to expand the chemical diversity of teicoplanin-like GPAs by additional methylation of the peptide scaffold. The crystal structure of MtfA together with computational docking and molecular dynamics simulations provided a model of demethyl-vancomycin aglycone binding to MtfA. Enzyme-catalyzed methyl transfer can be explored as another approach to generate methylated GPA derivatives. (Figure credit: Shi et al.)

Making Pain Go Away

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Endocannabinoids are lipid signaling molecules that regulate a wide range of mammalian behaviors, including pain, inflammation, and cognitive/emotional state. The endocannabinoid anandamide is principally degraded by the integral membrane enzyme fatty acid amide hydrolase (FAAH), and there is currently much interest in developing FAAH inhibitors to augment endocannabinoid signaling in vivo. Here Ahn et al. report the discovery of an FAAH inhibitor PF-3845 that displays an unprecedented combination of potency, selectivity, and in vivo efficacy. PF-3845 selectively inhibits FAAH in vivo, raises brain anandamide levels for up to 24 hr, and produces profound cannabinoid-receptor-dependent reductions in inflammatory pain.

Pyrrole-Amide Antibiotic Assembly

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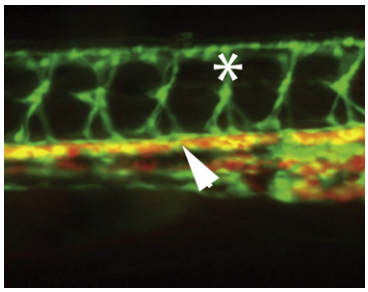
Pyrrole-amide antibiotics are DNA minor-groove binders. Juguet et al. report characterization of a gene cluster directing the biosynthesis of a pyrrole-amide antibiotic, congocidine (netropsin), from *S. ambofaciens* that was previously uncharacterized. They show that congocidine is assembled by an iterative nonribosomal peptide synthetase (NRPS) constituted of a free-standing module and several single-domain proteins and propose an assembly mechanism that provides a basis for the elucidation of the molecular principles of the biosynthesis of antibiotics of the pyrrole-amide family. Both the atypical organization and the mechanism of the NRPS illustrate the versatility of this family of enzymes.

Genetic Screen in Reverse and Chemical



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Inhibition of blood vessel growth in a tumor is an important mode of action for several oncology drugs used in clinical practice. However, new drugs are needed as existing ones are hampered by limited efficacy and side effects. Kalén et al. combined two different screening approaches, reverse- and chemical genetic screening, and identified 16 putative drug targets and 28 pharmacologically active compounds regulating blood vessel growth and function. Importantly, the study suggests that drug targets and compounds for complex biological processes, such as blood vessel growth, can be efficiently identified through combination of vertebrate screening models. (Figure from Kalén et al.)

ssDNA Aptamer Meets Serine Protease

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Activated protein C (APC) is a serine protease that plays a central role in the regulation of the hemostatic network and possesses anti-inflammatory and cytoprotective functions. Now, Müller et al. describe results of selection of a class of high-affinity ssDNA APC-aptamers (HS02) that selectively inhibit the anticoagulant activity of APC without affecting its cytoprotective functions. The ability to specifically inhibit APC's anticoagulant functions makes HS02 a potential therapeutic agent for the treatment of APC-related bleeding. Finally, the exosite specificity of the APC aptamers makes them interesting molecular tools to probe APC's interactions with several macromolecular substrates and cellular receptors.

Androgen Receptor Presents Its Surfaces

PAGE 452

Different nuclear receptors (NR) ligands exert different effects on NR structure, which leads to differential presentation of protein-protein interaction surfaces on NRs and binding of functionally distinct interaction partners. Norris et al. focus on androgen receptor (AR) to develop an approach to link ligand-induced changes in receptor structure to specific pharmacological responses by identifying over 150 proteins/polypeptides that interact with AR in a manner differentially affected by ligand binding. The accumulated information on protein-AR interactions was employed to develop a compound-profiling tool for separating ligands into functionally distinguishable classes.

ΔF508 CFTR Escapes Doom, SGC Mimics Implicated

PAGE 461

The glycosphingolipid, sulfogalactosyl ceramide (SGC) binds the N-terminal domain of all Hsp70s to inhibit the ATPase activity required for peptide binding and chaperone function. Park et al. test the effect of a soluble SGC mimic on Hsp70 chaperone-dependent, endoplasmic reticulum-associated degradation (ERAD) of misfolded ΔF508 cystic fibrosis transmembrane conductance regulator (ΔF508CFTR), an important factor in cystic fibrosis. AdamantylSGC reduced ΔF508CFTR ERAD, suggesting inhibition of Hsp70 chaperone function in cells. ERAD blockade alone was, however, insufficient for maturation of rescued ΔF508CFTR. The lower SGC level of cells and tissues defective in CFTR indicates a role in SGC synthesis and implies a potential "catch 22" cycle to promote ΔF508CFTR degradation.