In This Issue

Axonal Chemorepulsion Blocked!

PAGE 691

In the adult central nervous system, lesioned axons fail to regenerate. The failure of axon regeneration is attributable to axonal growth inhibitors encountered by injured axons, including semaphorins. By using a combinatorial screening strategy, Montolio et al. have identified a stable peptoid (SICHI) that specifically blocks semaphorin 3A (Sema 3A) biological functions, including chemorepulsion, in both the developing and the adult brain. Moreover, SICHI enhanced the regeneration of lesioned axons in slice cultures. Given the involvement of Sema 3A in CNS regeneration and other human pathologies, the authors suggest that SICHI offers great potential for therapeutic approaches for diseases related to semaphorin function and axonal regeneration.

LXXLL-Peptides Perturb ERα Localization

PAGE 702

Synthetic peptide probes featuring specific estrogen receptor α (ERα) interaction motifs and visualization and localization tags, developed here by Carraz et al., allow probing the surface of the full-length ERα in cells. The LXXLL-peptide probes disrupt ERα–coactivator interactions, which can be monitored by confocal scanning microscopy. A successful recognition of the molecular surface of the ERα by the peptide probes results in the displacement of the ERα from the DNA and coactivator and leads to the ERα translocation into the nucleoli. The method enables quantification of the ERα nucleoli recruitment and can be used to assess the affinity of peptide sequences for ERα in the physiological context of a cell.

Morphological Screening for MOA and Off-Target Effects

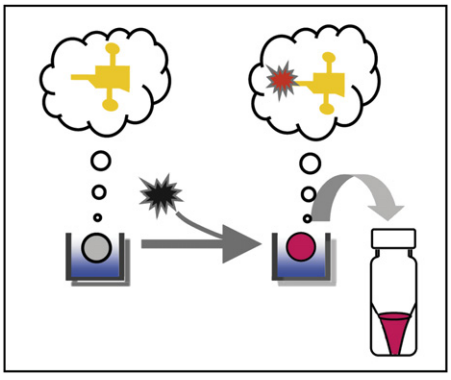
PAGE 712

Interaction of drugs with unintended targets is a major source of concern in drug discovery and development and one of the reasons for high attrition rates. Now, Abassi et al. describe an approach that utilizes microelectronics to monitor both the short-term and long-term kinetics of cellular responses to drugs and experimental compounds. The authors demonstrate the utility of this approach by highlighting several examples in which they have identified either new mechanisms or additional mechanisms for existing drugs and experimental compounds.

OBOC Comes to Focus

PAGE 724

The need to resynthesize primary hit compounds before determining their affinities in homogenous solution is an essential bottleneck in on-bead screening of large one-bead, one-compound libraries. Hintersteiner et al. use a single-bead labeling and analysis method for linking on-bead screening by automated confocal nanoscanning and bead picking, (CONA), with quantitative affinity measurements in solution by single molecule spectroscopy, compound quality control by HPLC, and structure determination by MS. This method is embedded into an integrated screening process spanning from design to single-cell validation of small molecular binders and inhibitors. (Figure adapted from Hintersteiner et al.)



Enzymes in Cyclooctatin Biosynthesis

PAGE 736

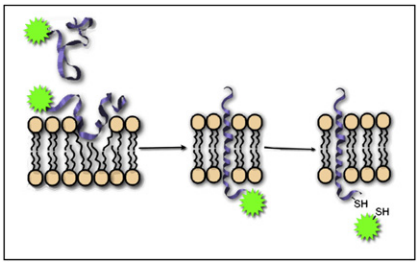
Cyclooctatin, a diterpene characterized by a 5-8-5 fused ring system, is a potent inhibitor of lysophospholipase. Kim et al. report the cloning and characterization of a complete cyclooctatin biosynthetic gene cluster consisting of four *cotB* genes from *Streptomyces melanosporofaciens* MI614-43F2 and heterologous production of cyclooctatin in *S. albus*. Incubation of the recombinant CotB2 enzyme with geranylgeranyl diphosphate resulted in the formation of an unprecedented tricyclic diterpene alcohol. The present study establishes the complete biosynthetic pathway of cyclooctatin and provides insights into both the stereo-specific diterpene cyclization mechanism of the geranylgeranyl diphosphate cyclase and the molecular bases for the stereo- and regiospecific hydroxylation.

MAGL: Supreme Controller of 2-AG Signaling

PAGE 744

Endocannabinoids are signaling lipids that regulate a broad spectrum of mammalian physiological and behavioral processes. Monoacylglycerol lipase (MAGL) is a principal degradative enzyme for the endocannabinoid 2-arachidonoylglycerol (2-AG). Here, Long et al. use the selective inhibitor JZL184 to globally assess the contribution that MAGL makes to the metabolism of 2-AG. These studies identify organs, such as brain, where MAGL exerts profound control over 2-AG signaling and, conversely, tissues where other enzymes might contribute to 2-AG and monoglyceride metabolism. This knowledge should guide the advancement of MAGL inhibitors as potential pharmaceutical agents for human disorders that would benefit from heightened endocannabinoid signaling.

pHLIP Brings Molecules into Cells



PAGE 754

A broad range of cell-impermeable molecules is excluded from discovery efforts because they cannot traverse membranes on their own. In their study, Thévenin et al. explore the properties of the peptide pHLIP, which can target tumors based on their acidity, as a delivery system by defining some of the properties of molecules that can be translocated into cells. The authors show that pHLIP can efficiently deliver membrane-impermeable peptides into cancer cells and that the translocation is pH dependent and mediated by transmembrane helix formation. Thus, pHLIP might have the potential to expand therapeutic options for acidic tissues such as tumors and sites of inflammation and to mitigate the side effects associated with conventional chemotherapy. (Figure provided by Thévenin et al.)

Acylhydrazone-Based Cleavable Linkers

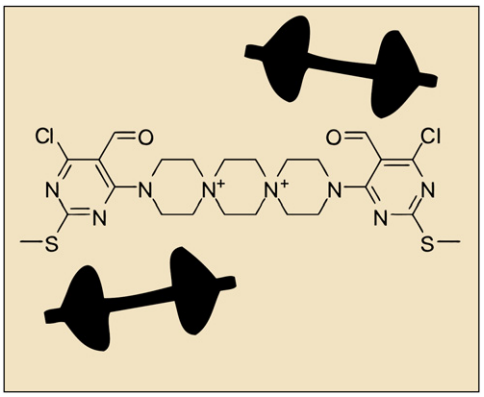
PAGE 763

Proteomic searches using affinity-based chromatography [e.g., biotin-(strept)avidin] have been severely hampered by low-protein recovery yields, protein destruction and denaturation, and the release of background proteins from the support. These limitations have confounded protein identification. Park et al. now describe an acylhydrazone-based cleavable linker that permits the efficient isolation of proteins with a traceable tag (i.e., isotopic, fluorescent) allowing detection and identification under mild conditions. Protein release proceeded with significantly reduced levels of background proteins. The use of acylhydrazone linkers is expected to be generalized allowing for the selective release of tagged molecules from noncovalent and covalently tagged supports.

Dumbbell-Shaped Cell Adhesion and Growth Promoter

PAGE 773

During an image-based phenotype screening of a chemical library, Yamazoe et al. made a discovery of a small molecule that boosts the adhesion and growth of human cells. Follow-up chemical and cell biological experiments suggested that the diaryldispirotripiperazine derivative (adhesamine) targets selective cell-surface glycosaminoglycans, especially heparan sulfate, and increases cell adhesion and growth. Adhesamine induces apparently normal cell adhesion accompanied with organized actin structures and activation of FAK and ERK. Additionally, adhesamine was shown to enable the adhesion of floating lymphocytes to cell culture plates, thus facilitating the lymphocytes' microinjection process. Therefore, adhesamine may find its use as a cell-attaching reagent for cell engineering and basic cell biology.



IL-6 Inhibitor by Fusion of Receptor Fragments

PAGE 783

Inhibitors of proinflammatory cytokines are effective drugs in the treatment of chronic inflammatory diseases. Here, Wiesinger et al. present the development of an inhibitor of murine IL-6, mIL-6-RFP, which is based on the fusion of the IL-6 receptor proteins IL-6Rα and gp130. The concept of inline fusion of receptor fragments has the great advantage that the inhibitor is encoded by a single gene, as compared to antibodies or cytokine traps, where two subunits have to be expressed in parallel. This strategy offers numerous possibilities for specific cytokine inhibition in gene delivery approaches based on viral vectors, transgenic animals and finally gene therapy.

