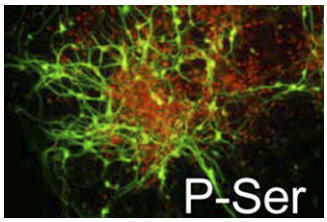
# In This Issue

## Membrane Attachment Slows Down Prion Protein Aggregation

PAGE 994

Toxic conformations of proteins are the cause of many severe diseases in humans. The prion protein (PrP) is special among these proteins because it is an infectious agent that can convert native PrP into its toxic isoform. This process most likely takes place in the natural environment of PrP, the outer leaflet of the cell membrane. To elucidate this process, Olschewski et al. have established two semisynthetic strategies to obtain recombinant PrP equipped with a C-terminal GPI anchor mimic. These strategies allowed the study of membrane-attached PrP and led to the findings that membrane attachment slows down formation of PrP aggregates. An important step towards elucidating prion conversion and turn-over was achieved by transfer of semisynthetic PrP into the plasma membrane of cells.

## Regulation of Neural Stem Cell Differentiation




PAGE 1019

Neural stem cells (NSCs) hold great promise for repair of the nervous system following injury or disease. Small molecules that specifically enhance the production of neurons from NSCs are highly sought after. Saxe et al. found that in cell-based phenotypic screening with primary cultured murine NSCs, a serine metabolite, phosphoserine, promotes the differentiation and survival of neurons produced from NSCs. Target identification studies demonstrated a role for metabotropic glutamate receptor 4 in NSC biology and revealed novel receptor coupling to the mTor signaling pathway. Phosphoserine was also found to be an efficient means to enhance neuron production from human ES cells.

## Probing G Protein Signaling

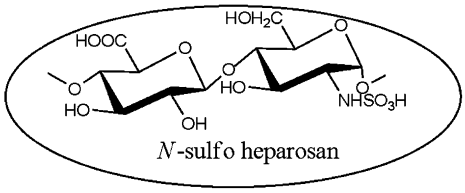
PAGE 1007

There exists a lack of small molecule modulators for G proteins due to their extremely tight affinity towards GDP and GTP. In this issue, Vincent et al. report small molecules developed for the highly specific inhibition or activation of the engineered G protein. The rational design preserved binding of the natural substrates to the G protein, and the mutations were functionally silent in a cellular context. This novel tool can be used for isolating specific G protein effectors as the authors demonstrate with the identification of Nol1 as a putative novel effector of H-Ras. Finally, the generalization of this system was confirmed by applying it to Rap1B, suggesting that this method will be applicable to other G proteins.

## Harvesting the Power of Heparan Biosynthetic Enzymes

PAGE 986

Heparan sulfate (HS) is a highly sulfated polysaccharide with a wide range of biological activities. Heparin, an analog of HS, is a commonly used anticoagulant drug. Chemical synthesis of HS with specific sulfation patterns is difficult, especially for those molecules larger than a hexasaccharide. Furthermore, these hexasaccharides may not fully represent the functions of the polysaccharide. As reported in this issue, Chen et al. utilized an array of heparan biosynthetic enzymes to synthesize a small library with eight unique polysaccharides. The authors discovered novel structures with anticoagulant activity. In general, this enzymatic approach could be used to develop any polysaccharide-based therapeutic reagents.



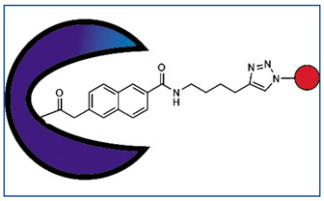

## Reaching the Target: Reversibly Modified Hydrophobic Prodrugs

PAGE 1065

Monahan et al. have developed the use of reversibly modified hydrophobic prodrugs for first-pass targeting of liver tumors in loco-regional therapy. In this study, authors demonstrate that hydrophobicity dramatically enhances tumor cell targeting in vitro and in vivo. Incorporating rapid lability to the hydrophobic moiety protects nontargeted tissues from exposure to the highly active agent. Utilizing this methodology, authors demonstrate that a cell-impermeable compound can be converted into an active therapeutic, thereby potentially leading to the development of therapeutics with low systemic toxicity. This approach is particularly applicable to first-pass treatment strategies involving liver tumors or ovarian cancer.

## Cytochrome P450 Profiling In Vivo



PAGE 1043

Cytochrome P450 enzymes, a large and diverse protein family in mammals, play key roles in the metabolism of endogenous signaling molecules, xenobiotics, and drugs. As Wright and Cravatt report, a broad-spectrum, mechanism-based P450 inhibitor, 2-ethynylnaphthalene, was converted into an activity-based chemical proteomics probe by derivatization with a versatile click chemistry handle that enabled the selective tagging, detection, enrichment, and identification of P450 enzymes in any biological system. The activity-based probe proved capable of monitoring both drug induction and inhibition of P450 enzymes in vivo. This chemical proteomic strategy offers a versatile method to monitor P450 activities and small molecule interactions and should facilitate the functional characterization of this large and diverse enzyme class.

## Evolving Enzymes with Retained Activity in Organic Cosolvents

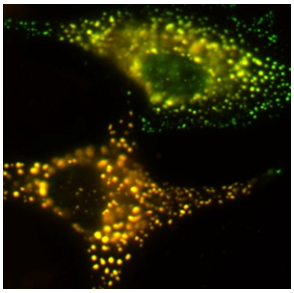
PAGE 1052

Mimicking the Darwinist algorithm of natural selection, directed molecular evolution allows the tailoring of enzymes to be more robust, stable, and in general exhibit many improved features, through several rounds of random mutagenesis and DNA recombination. Due to their high versatility, fungal laccases are considered among the most promising green catalysts of this century. In their current work, Zumarraga et al. tailored a fungal laccase that tolerates high concentrations of organic cosolvents, a property that is not required in this enzyme's natural environment, in five cycles of directed evolution. The expression of this enzyme in *Saccharomyces cerevisiae* allowed authors to use this organism as a DNA-recombination toolbox to generate diversity.

## Dye-Triggered Aggregation Reveals PKA Dynamics in Living Cells

PAGE 1031

The tetracysteine sequence YRECCPGCCMWR fused to the N-terminus of green fluorescent protein (GFP) self-aggregates upon biarsenical labeling in living cells or in vitro. Such dye-triggered aggregates form temperature-dependent morphologies and are dispersed by photobleaching. Fusion of the biarsenical aggregating GFP (BA-GFP) to the regulatory or catalytic subunit of PKA traps intact holoenzyme in compact fluorescent puncta upon biarsenical labeling. Surprisingly, Martin et al. show that elevated cAMP does not allow RI$\alpha$ and C$\alpha$ to diffuse far apart unless the pseudosubstrate inhibitor PKI or locally concentrated substrate is coexpressed. Overall, effective separation of type I PKA is substrate dependent, whereas type II PKA dissociation relies on autophosphorylation.



## Type-IV Glycopeptide Side-Chain Cyclization

PAGE 1078

The P450-type monooxygenases StaF, StaG, StaH, and StaJ of the A47934 producer *S. toyocaensis* catalyze crosslinking and form the linear precursors to a tetracyclic glycopeptide antibiotic scaffold that is the base for antibiotic activity. Hadatsch et al. used gene inactivation to correlate the position of biaryl- and biarylether-crosslinks in the peptide backbone and the catalyzing oxygenase. Functional assignment and deduction of the biosynthetic sequence for oxygenase steps was achieved only when the combination of the mass spectrometric analysis for both the gene inactivation mutants and the wild-type cultures was employed. This study sheds new light on our understanding of glycopeptide biosyntheses and reveals features unique to type-IV glycopeptide antibiotics.