In Review: Nitric Oxide in Living Systems

PAGE 1211

Nitric oxide (NO) is a gaseous diatomic radical that mediates both physiological and pathological functions. Tennyson and Lippard now review three stages of NO biochemistry: (1) generation, (2) translocation, and (3) action. In Stage 1, nitric oxide synthase generates NO (g) from L-arginine. Subsequent reactions can convert NO to a different nitrogen oxide (NO_x), an organonitrosyl, or a metal–nitrosyl complex, for transportation in Stage 2, wherein each derivative features unique chemical reactivity and biological activity. Upon delivery in Stage 3, NO exerts its physiological or pathological function by reaction with biomolecules containing redox-active metals or other residues.

L-Doping t-RNA

PAGE 1221

Three existing, structurally different phenylalanyl-tRNA synthetases, human mitochondrial (*Hsmt*PheRS), human cytoplasmic (*Hsct*PheRS), and eubacterial from *Thermus thermophilus* (*Tt*PheRS), catalyze mischarging of $tRNA^{Phe}$ with an oxidized analog of tyrosine, L-dopa. Moor et al. examine this phenomenon, and discover that the lowest level of L-dopa discrimination over phenylalanine exhibited by *Hsmt*PheRS is comparable to that of TyrRS. *Hsct*PheRS and *Tt*PheRS are capable of hydrolyzing the exogenous L-dopa-$tRNA^{Phe}$ as efficiently as Tyr-$tRNA^{Phe}$. However, editing activity of PheRS does not guarantee reduction of the aminoacylation error rate to escape misincorporation of L-dopa into polypeptide chains.

Breaking the C-P Bond

PAGE 1230

Phosphonates can be used as a nutrient source for phosphorus by some bacterial species that evolved enzymes able to cleave the inert C-P bond. Agarwal et al. now describe a structure of PhnA, a *Sinorhizobium meliloti* enzyme, and provide insights into the mechanism of C-P bond cleavage, a poorly studied activity within the catalytic repertoire of the alkaline phosphatase superfamily. The authors show that PhnA contains an atypical bimetallic center, which might be the key in accommodating specific distance requirements in the transition state during C-P bond cleavage.

Photoconvertible and Biphotochromic Fluorescent Proteins

PAGE 1241

Fluorescent proteins that can change their spectroscopic properties upon light irradiation are indispensible tools for certain protein tracking experiments and diffraction-unlimited light microscopy using PALM. In this work, Adam et al. describe rational design of fluorescent proteins that combine irreversible green-to-red photoconversion with on/off photochromism in both green and red state. Insight into the mutants' properties was gained through biochemical and in-depth spectroscopic characterization complemented with modeling data. The most interesting mutant, called NijiFP, was shown to perform excellently in confocal microscopy and is suitable to use in PALM imaging in both the green and the red form.

Dechlorinase: Cleaning Up Polution

PAGE 1252

Aromatic halogenated compounds are among the most important pollutants; they persist in the environment, causing serious problems. Dechlorintation is the key step in their degradation pathway but known dechlorinating enzymes are inefficient. In this work, Velazquez et al. have identified and characterized DrcA, a reductive dechlorinase enzyme from *Dictyostelium discoideum*. DrcA displays increased efficiency towards its natural substrate and may therefore inform design and development of dechlorinases with improved properties.

Let There Be Light: Uncaging of Biotin

PAGE 1261

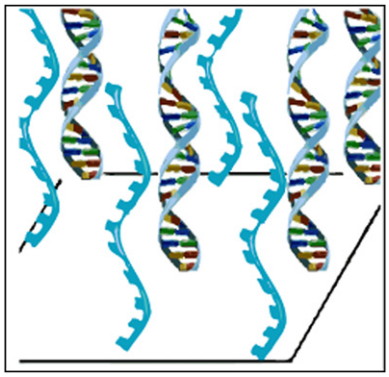
Biotin-(strept)avidin complex is widely used in biotechnology due to its extremely high binding constant; however, spatiotemporally controlled formation of the complex in live cells has not been previously examined. In this study, Terai et al. developed photoreleasable caged biotins, with low baseline affinity for (strept)avidin, which are activated to native affinity upon UV irradiation. Specific fluorescence labeling of live cells was accomplished using fluorophore-conjugated streptavidin, in combination with photoreleasable caged-biotin. The work also demonstrates the feasibility of irradiated-cell-specific drug delivery using caged-biotin-labeled cells, a prodrug, and streptavidin conjugated with prodrug-cleaving enzyme.

In This Issue

Chemical Probe Discovery Goes Bam!

PAGE 1273

Pharmaceutical companies are producing only a handful of drugs each year while costs per drug have soared into the billions. Novel approaches to drug discovery are sorely needed. Academic laboratories, including those studying model organisms, are rising to meet this challenge. To increase the likelihood of identifying promising lead compounds, Wallace et al. designed a scheme whereby compounds are preselected based on empirical and computational data. By screening a library of ~80,000 compounds in yeast, the authors identified growth inhibitors that proved effective in other model organisms that span great evolutionary distances from yeast to worm to human cells. These methods and the resulting molecules provide a guide for academic drug discovery.



PNA Encoded Peptide Library

PAGE 1284

Svensen et al. describe results of treating human cells overexpressing either integrins or the CCR6 receptor with a 10,000 member PNA-encoded peptide library. Extraction of the PNA tags from the surface of the cells was followed by a PNA tag to DNA translation and PCR amplification. This enabled decoding of the tags via microarray hybridization and allowed identification of previously unreported peptide ligands for αvβ3 and αvβ5 integrins and CCR6.

Evolving the Gatekeeper

PAGE 1290

The entry points for assembly line biosynthesis are gatekeeper adenylation domains. Villiers and Hollfelder now report altering their specificity by directed evolution. Randomization of active site residues leads to a dramatic specificity change of five orders of magnitude. By manipulation of a steric exclusion mechanism, the authors achieve a 170-fold improvement in processing of a new substrate (Ala), accompanied by a 1000-fold decrease in handling of the original substrate (Phe), resulting in a dramatic change.

Small G-Protein, Large Effects

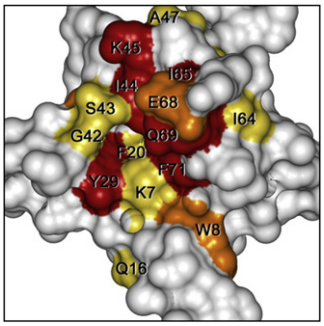
PAGE 1300

Inhibition of methione aminopeptidase-2 (MetAP-2) by the antiangiogenic compound TNP-470 disrupts Wnt planar cell polarity (PCP) signaling in endothelial cells; however, a substrate(s) whose activity is altered upon MetAP-2 inhibition, resulting in loss of Wnt PCP signaling, is not known. Here, Sundberg et al. utilize N-terminal proteomics to identify the small G-protein Rab37 as a novel MetAP-2 substrate that accumulates in the presence of TNP-470. A functional role for aberrant Rab37 accumulation in TNP-470's mode-of-action is demonstrated using a MetAP-2-resistant point-mutant.

Entering the Trans-Golgi Network

PAGE 1312

In this study, He et al. report synthesis and biochemical and functional characterization of phosphorylated inositol lipid derivatives. The authors demonstrate that the synthetic analogs retain the natural lipid binding and membrane deformation properties and therefore can replace the natural lipid in biological and biochemical applications. These synthetic compounds could be instrumental for examining the cell processes occurring within the trans-Golgi network.



Aspirin for *Pseudomonas*

PAGE 1320

Salicylic acid (SA) is a well-known plant-defense signaling molecule and is incorporated by some bacteria in iron-chelating molecules. Here, Li et al. report on a novel SA-containing compound from a plant-associated *Pseudomonas putida* that serves as a colonization factor based on a conquer-and-kill strategy: promysalin promotes movement over a solid surface and biofilm formation by its producer and selectively antagonizes growth of kin bacterial species, including the human pathogen *Pseudomonas aeruginosa*. In addition to SA biosynthesis, the unique promysalin biosynthetic gene cluster mediates incorporation of two unusual building blocks, the amino acid derivative 2-pyrroline-5-carboxylic acid and fatty acid-derived dihydroxymyristamide.

Diketide Chiral Building Blocks and PKS Modules

PAGE 1331

The ketoreductases (KRs) of modular polyketide synthases (PKSs) naturally reduce polyketide intermediates in the context of large, enzymatic assembly lines. Piasecki et al. sought to determine whether isolated ketoreductases could biocatalyze the stereospecific reduction of a panel of unnatural substrates. Reactions of eleven KRs on five diketide substrates were analyzed by chiral chromatography. Biocatalytically robust KRs were identified for each KR-type, and reactions were scaled up to yield over 100 mg of stereopure diketide. Thus, "green reactions" driven by glucose in an aqueous medium helped generate chiral diketides, valuable as substrates in PKS enzymology and as building blocks in natural product synthesis.

