In This Issue

Chemical Modifications of Proteins

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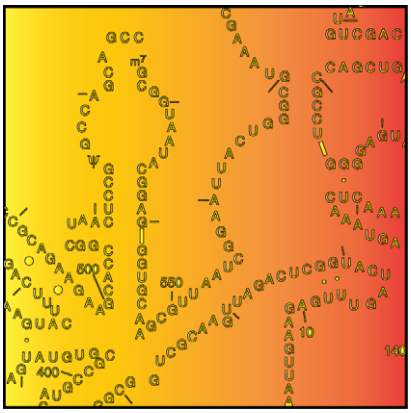
In vivo proteins exist in number of different posttranslationally modified forms. Since the patterns of posttranslational modification are sometimes difficult to control under physiological conditions, there is an increasing need to introduce these modifications, and others, chemically. The review article by Baslé et al. explains the process of chemical modification and discusses a number of recently developed methods for introducing them by carefully balancing reactivity and selectivity. The authors describe a wide selection of diverse tools that are now available for both those working in chemistry and those working in biology that can be used to achieve the desired modification. The review also touches on application aspects of this work, as well as providing a well-rounded discussion.

16S Ribosomal RNA Regulated by an RNA Switch



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Artificial genetic switches for controlling gene expression via an external stimulus are important tools in chemical and synthetic biology. Here, Wieland et al. expand the application range of RNA switches to the regulation of 16S rRNA function in *Escherichia coli*. The authors incorporated hammerhead ribozymes at several positions into orthogonalized 16S rRNA. Ribosomal function is remarkably tolerant towards the incorporation of large additional RNA fragments. However, ligand-dependent ribozyme-mediated cleavage results in severe reduction of 16S rRNA stability. In addition to expanding the regulatory toolbox, the presented artificial riboswitches should prove valuable in order to study aspects of rRNA folding and stability in bacteria.

Enzyme HTS in a Droplet

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Granieri et al. describe a microfluidic approach for the screening of structurally complex enzyme variants. Similar to phage display, the enzyme variants are displayed on the surface of viral particles. However, by using a mammalian expression system and encapsulating the resulting viral particles into tiny droplets (picoliter volumes), the approach enables the screening of structurally complex enzymes (e.g., requiring glycosylation and/or membrane anchorage) under multiple turnover conditions. In comparison to conventional screening formats such as microtiter plates, the system described here allows more than 100-fold increased throughput and almost one million-fold reduced consumables costs, due to the tiny assay volumes.

Microtubule Pore Lights Up

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Microtubules are cytoskeleton components that are formed by polymerization of α- and β-tubulin that assemble to form an elongated tube with an inner diameter of about 25 nm. Barasoain et al. now design a fluorescent probe derived from the paclitaxel structure Hexaflutax that binds the pore and stabilizes microtubules. The authors describe an interesting biphasic association of Hexaflutax, suggesting the existence of two different binding sites and two different modes of binding that the authors confirm arise from two different poses of the taxane moiety.

Uncultured Bacteria Get a Siderophore Boost

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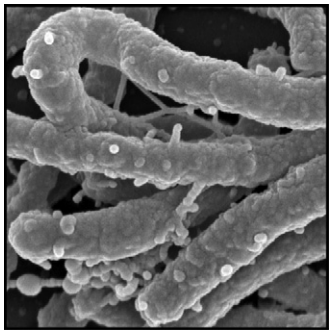
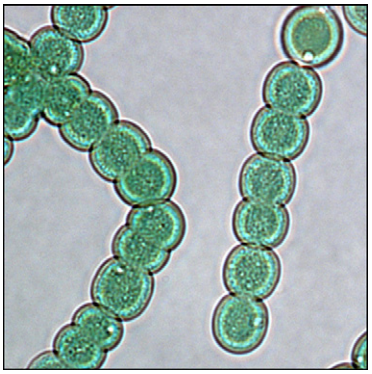
The ''Great Plate Count Anomaly'' is an unsolved problem in microbiology referring to the difference between cells counted from an environmental sample and the number of colonies that form on solid media. In this study, D'Onofrio et al. demonstrate that several otherwise uncultured bacteria from intertidal sediment can grow in the presence of siderophores, chelators of insoluble iron, that were produced by microbes from the same environment. A model helper, related to *Micrococcus luteus*, was shown to produce five new siderophores, which induced the growth of several uncultured bacteria. Further experiments based on this observation resulted in the isolation of rare bacterial species. (Figure credit: D'Onforio et al.)



It Takes Two Starter Modules to Biosynthesize



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Nonribosomal peptides are a chemically diverse family of compounds for which important clinical and industrial applications are found. Anabaenopeptins are a family of hexapeptide protease inhibitors that contain an array of proteinogenic and nonproteinogenic amino acids as well as a conserved ureido bond. Here, Rouhiainen et al. show that these peptides are assembled on a nonribosomal peptide synthetase enzyme complex in the bloom-forming cyanobacterium *Anabaena*. Surprisingly, anabaenopeptin structural variants are produced by *Anabaena* simultaneously through the use of two separate starter modules. Anabaenopeptin synthesis constitutes a novel exception to the colinearity rule of nonribosomal peptide biosynthesis. (Figure credit: Rouhiainen et al.)

Unnatural Lysine Derivatives in Src SH2

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Src SH2 domain inhibitor development has proven to be challenging due to the broad specificity of the SH2 domain and the poor pharmacokinetic profile of the invariant phosphate mimics. In this study, Virdee et al. use semisynthesis to introduce unnatural lysine derivatives into the specificity-determining region of the Src SH2 domain. Protein containing the derivative diaminobutyric acid (Dab) demonstrates altered binding characteristics such that the authors observe enhanced affinity for the pYDEI phosphopeptide at the expense of affinity for the canonical pYEEI peptide. Such reengineered SH2 domains with altered specificity may find roles as research tools and therapeutics.

Covalent FGFR Inhibitors

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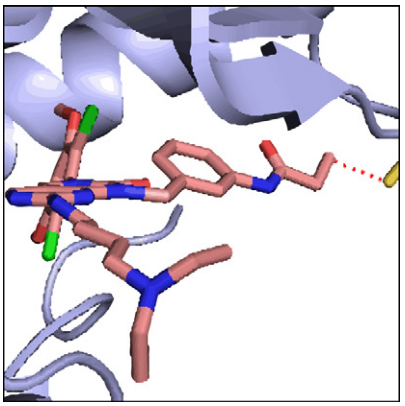
All FGFR inhibitors that have been developed for clinical use target FGFRs in a reversible manner. Here, Zhou et al. report the first irreversible inhibitor (FIIN-1) that inhibits FGFRs with a nanomolar potency and a high selectivity. FIIN-1 reacts with Cys486 positioned at the P-loop of FGFRs, and the concomitant tight ATP-competitive binding within the active site leads to a potent irreversible inhibition of the kinases. Comparable analysis with a reversible inhibitor revealed that the covalent modification not only increases the potency of the inhibitor, but also brings up a moderate inhibition of the drug-resistant gatekeeper mutant (V561 M) of FGFR1. (Figure credit: Zhou et al.)



23 Natural Tubulysins and Counting

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The tubulysins are a family of complex peptides that exhibit cytotoxic activity against cancer cells. Chai et al. now apply comparative analysis of the tubulysin gene clusters from two strains of myxobacteria and couple it with in vitro assays to reveal significant insights into the underlying biosynthetic pathway. In addition, the authors show that the strains make 23 novel tubulysins in total, reflecting the inherently diversity-oriented nature of the biosynthesis. These new compounds are targets for chemical synthesis, with the aim of increasing our knowledge of structure-activity relationships in this promising class of secondary metabolites.

