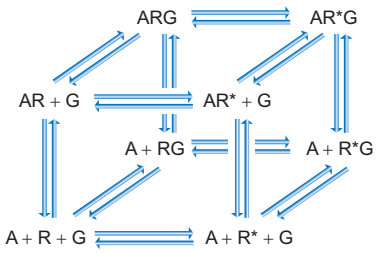
In this ISSUE

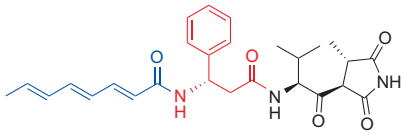
acs chemical biology



Allosteric GPCR Modulation

Heterotrimeric G-protein-coupled receptors (GPCRs) are a ubiquitous class of proteins involved in signaling. Because of their vital physiological roles, these proteins are the subject of substantial research in biological sciences and in drug discovery. A wealth of recent data indicates that GPCRs bind allosteric ligands that interact with receptor sites. Now, Bridges and Lindsley (p 530) review allosteric GPCR modulation by small-molecule ligands.

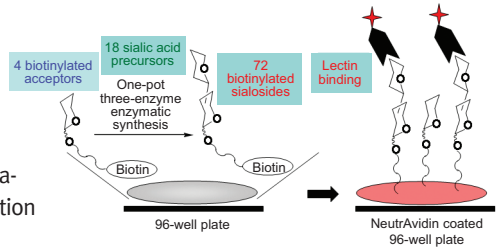
Biosynthetic Gatekeepers



Andrimid, an antibiotic that targets membrane biosynthesis, has a unique biosynthetic pathway that mingles unusual amino acids with a hybrid concoction of polyketide synthases and nonribosomal peptide synthetases. In an effort to decipher the steps involved in andrimid biosynthesis, Magarvey *et al.* (p 542) characterize five enzymes, AdmA, F, I, J, and H, that participate at the front end of the andrimid assembly line.

Using heterologously expressed proteins and various assays, the authors uncovered numerous gatekeeping roles for the Adm proteins. These included creating enantiopure amino acid substrates, overseeing stereo- and enantioselective substrate recognition, and ensuring substrate delivery to the appropriate module in the assembly line. These insights contribute to our understanding of this intriguing pathway and also facilitate its exploitation for the creation of novel bacterial fatty acid biosynthesis inhibitors.

Combinatorics and Chemoenzymatics



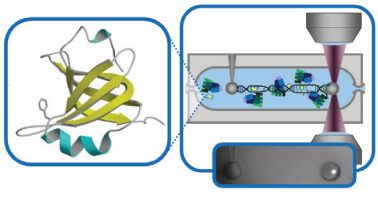
Sialic acids, which reside on the surface of many cell types, mediate various types of cell–cell interactions, including those involved in the immune response, host–pathogen interactions, and cancer metastasis. The numerous modifications found on sialic acids, such as acetylation and sulfation, coupled with challenges associated with oligosaccharide synthesis, complicate investigations into how sialic-acid-containing glycans interact with their various receptors. Chokhawala *et al.* (p 567) now present a combinatorial chemoenzymatic method that facilitates the creation and characterization of sialic-acid-containing biomolecules.

Sialosides were first synthesized in a one-pot amalgamation concocted of a sialic acid precursor, three enzymes, and a biotinylated sialyltransferase acceptor. The biotinylated, sialylated products were subsequently transferred to avidin-coated plates, where they were assayed for their reactivity with various sialic-acid-binding proteins. This clever strategy simultaneously enables access to and high-throughput screening of these important biomolecules.

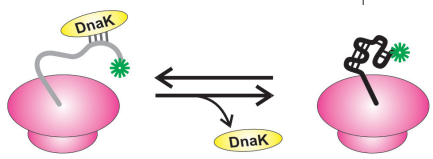
Stretching a Polymerase

Of the 10 subunits that comprise *E. coli* DNA polymerase III, the α subunit is the one responsible for its polymerase activity. Sequence analysis suggests that two domains in the α subunit, a helix-hairpin-helix $(HhH)_2$ domain and an oligonucleotide binding domain (OB), bind DNA, but this has not been directly demonstrated. McCauley *et al.* (p 577) use single-molecule force spectroscopy to explore the DNA binding properties of the α subunit.

DNA stretching experiments, which conveniently distinguish between dsDNA and ssDNA binding, revealed that the $(HhH)_2$ domain binds to dsDNA, whereas the OB domain binds to ssDNA. Interestingly, ssDNA binding occurred without actively melting the DNA, suggesting that the OB domain only passively binds ssDNA. DNA stretching measurements also enabled determination of the association constants between the α subunit and dsDNA and ssDNA.



Nascent Chain Dynamics



As a polypeptide chain materializes from the ribosome, a functional protein results only if the polypeptide folds correctly. Little is known, however, about the dynamics of nascent chains while they are still bound to the ribosome. Ellis *et al.* (p 555 and Point of View p 527) use dynamic fluorescence depolarization, a technique that enables discrimination of different types of molecular motion, to explore movement of nascent chains as they emerge from the ribosomal tunnel.

The globular protein apomyoglobin was selected for examination, and polypeptides of increasing lengths were selectively labeled at the N-terminal methionine in an *Escherichia coli* cell-free system. Dynamic fluorescence polarization revealed that independent nascent chain motions occurred during the intermediate and late stages of apomyoglobin elongation, but not during those of a natively unfolded control protein. These illuminating results suggest that only sequences capable of folding into functional structures exhibit such nascent chain dynamics.

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