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Editorial overview: Catalysis and regulation: Enzyme structure, mechanism, and biosynthetic pathways

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The roots of enzyme structural biology can be traced back to 1926, when Professor James B. Sumner of Cornell University reported “a new protein which crystallizes beautifully and whose solutions possess to an extraordinary degree the ability to decompose urea into ammonium carbonate”.¹ The implications of Sumner’s discovery of urease were profound – up until then, it was not known that enzymes were proteins. Twenty years later, Sumner shared the 1946 Nobel Prize in Chemistry “for his discovery that enzymes can be crystallized”,² a discovery that set the stage for the first X-ray crystal structure of an enzyme, hen egg-white lysozyme, in 1965.³

Now, on the 90th anniversary of Sumner’s seminal discovery and the 51st anniversary of the lysozyme crystal structure, this volume presents diverse examples of enzyme structure-function studies that decipher and illuminate fundamental chemical mechanisms of catalysis. From enzymes that catalyze one-carbon reactions to enzymes that catalyze multi-carbon cascade reactions, X-ray crystal structures – and now, cryoelectron microscopy (“cryo-EM”) structures – lead the way.

Strikingly, recent technological advances in microscope and detector technologies enable atomic resolution structure determinations using cryo-EM, and *Subramaniam et al.* review recent advances in the field. Impressively, the 1.8 Å resolution structure of glutamate dehydrogenase reveals that the quality of a cryo-EM map can rival that of an electron density map calculated from X-ray diffraction data.

Even so, X-ray crystallography remains a powerful structure determination technique that is well suited for the study of multiple enzyme complexes to probe structure-function relationships. Among enzymes that catalyze one-carbon chemistry, the *N*-formyltransferases are particularly intriguing in that they transfer formyl groups from the cofactor *N*¹⁰-formyltetrahydrofolate to glucosamine substrates in the biosynthesis of the O-antigen of Gram-negative bacteria. *Holden et al.* review X-ray crystal structures and biochemical data and also provide the first view of a catalytically productive cofactor binding conformation. In another example of one-carbon chemistry, *Drennan et al.* review structure-function studies of 2-oxoacid:ferredoxin oxidoreductases (OFORs), which utilize cofactor thiamine

pyrophosphate. These enzymes catalyze the oxidation of α -ketoacids to generate acyl-CoA, carbon dioxide, and two electrons. The most recently studied member of the OFOR family is oxalate oxidoreductase, and structural analysis reveals intriguing domain architectures and iron-sulfur clusters that facilitate novel redox chemistry. The complexities of enzyme-substrate recognition and catalysis are indeed striking in one-carbon metabolism.

X-ray crystal structure analysis highlights exquisite functional adaptability in the superfamily of alpha/beta hydrolases as outlined by *Mindrebo et al.*, in which catalysis of diverse chemical reactions far beyond simple hydrolysis reactions is achieved in a common protein fold. In contrast, *Allen and Dunaway-Mariano* show how catalysis of a common chemical reaction, phosphate hydrolysis, is accomplished in enzyme families with diverse folds.

The past two decades have witnessed a renaissance in natural products biosynthesis, fueled in large part by the availability of crystal structures that provide foundations for understanding some of the most complex chemical mechanisms in biology. *Chen et al.* review the family of terpenoid cyclases, focusing on multi-domain and multi-subunit enzymes that catalyze sequential reactions in terpenoid biosynthesis. Such bifunctional synthases may enjoy proximity effects in catalysis to enhance product flux, especially in oligomeric systems. The terpene theme is extended by *Syren et al.* in their review of the squalene-hopene cyclases. Here, a water-assisted entropy-driven catalytic mechanism is emphasized. Strategies for expanding to novel reactions by active site modifications are also discussed. Precision coding and catalysis is the chemical strategy of polyketide biosynthesis, and *Robbins et al.* review key structure-function relationships in assembly line polyketide synthases. Intriguingly, a molecular turnstile has recently been proposed to regulate the proper channeling of reaction intermediates, so these megasynthases continue to yield magnificent insight on biological catalysis and regulation. *Peschke et al.* highlight the challenges of forming specific amino acid side chain cross-links in the biosynthesis of another group of natural products – the glycopeptide antibiotics. These cross-links are required to define three-dimensional shape essential for glycopeptide antibiotic activity. Elegant solutions have emerged from new protein structures that indicate how peptide products bound to non-ribosomal peptide synthetases recruit P450s to catalyze cross-link formation.

Guo et al. continue the theme of multi-domain proteins. Mind Bomb 1 (Mib1) is an E3 ligase that plays a key role in regulating transcellular communication in the Notch signaling pathway. They show how full-length Mib1 resembles a multi-protein Cullin-RING ligase, except that the functionality of multiple subunits in a Cullin ligase corresponds to multiple domains in the single polypeptide chain of Mib1. Additionally intriguing is the bivalent interaction between the Notch ligand and Mib1, suggesting the possibility of dimerization in ligand molecular recognition and signal transduction.

The chemical repertoire of enzymes is expanded by cofactors and flavins have long been recognized as important in this regard. *Leys and Scrutton* review highlights from the recent flavin literature and reveal further diversity in reactions catalyzed by these remarkable cofactors. Prenylation and formation of superoxidized flavin-N5 oxide species are the latest

additions to the flavin reaction palette. *Marabelli et al.* review how flavin chemistry in LSD1 histone demethylase is being used to develop covalent inhibitors and how non-catalytic domains facilitate interactions with other chromatin-associated proteins. Knowledge of LSD1 catalytic activity, inhibition and protein interactions has advanced rapidly since the discovery of LSD1, primarily from structural investigations. *Markolovic et al.* discuss the importance of human JmjC oxygenases as demethylases and hydroxylases in the regulation of gene expression and protein biosynthesis from a structural perspective. Like LSD1, these are complex enzymes comprising catalytic and non-catalytic domains, but unlike LSD1 they operate as Fe (II) and 2-oxoglutarate dependent enzymes. JmjC oxygenases have attracted pharmaceutical interest in epigenetic regulation, especially in the treatment of cancer. Emerging structures provide new impetus in this area and assist in functional assignment of other JmjC oxygenases. The complex architecture of metalloproteins is exemplified further by the [FeFe]-hydrogenases. *Dinis et al.* review the latest advances relating to the assembly and chemistry of metallocofactors required to catalyze the reversible reduction of protons to form hydrogen. Here structure determination, biochemical and spectroscopic studies are beginning to reveal complex mechanisms of cofactor assembly but questions remain. The authors provide insight into the latest developments as a platform for future studies. In the final contribution we learn how Nature can also steer complex reaction chemistry in the absence of organic or metallocofactors. *Bui and Steiner* discuss oxygen activation in cofactor-free 2,4-dioxygenases. Oxygen activation would normally require the intervention of metals or organic cofactors to cope with the poor reactivity of triplet oxygen with organic substrates. Structure determination combined with spectroscopy and calculations now reveal how cofactor-free dioxygenases can perform these reactions in absence of additional cofactors.

Despite over half a century of intensive research on the structures of enzymes we are still uncovering exciting, and sometimes surprising, chemical strategies and complexity in their mode of action. The remarkable findings reflected in this review series is testimony to this claim and this augurs well for new advances in the years to come.

References

1. Sumner JB. The isolation and crystallization of the enzyme urease. *J Biol Chem.* 1926; 69:435–441.
2. The Nobel Prize in Chemistry. Nobelprize.org. Nobel Media AB 2014. 1946. Web. 1 Sep 2016; <http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1946/>
3. Blake CCF, Koenig DF, Mair GA, North ACT, Phillips DC, Sarma VR. Structure of hen egg-white lysozyme. *Nature.* 1965; 206:757–761. [PubMed: 5891407]

Biographies

David Christianson received the AB, AM, and PhD from Harvard University before moving to the University of Pennsylvania, where he has been a member of the faculty for 28 years. His current research focuses on metal-dependent arginases, deacetylases, and enzymes of terpenoid biosynthesis. His accomplishments have been recognized by numerous awards, including the Pfizer Award in Enzyme Chemistry and the Repligen Award in Chemistry of Biological Processes from the American Chemical Society, and Fellowships from the John

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