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EDITORIAL

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Chemical Mechanisms in Biochemical Reactions

The surprising advances in understanding chemical mechanisms have been eagerly adopted into biological chemistry. In some cases, the novel chemistry from biological reactions provides advances in chemical mechanisms to impact fundamental understandings in chemistry as well as deeper understanding in chemistry and biochemistry. The purpose of this *JACS* Select issue is to provide a cross section of recent articles that have appeared in the *Journal of the American Chemical Society* and report some of these advances. Selection of these articles was a daunting task because of the expanding research focus at the chemical biology interface, leading to an ever-growing number of publications. The articles collected here highlight the diversity of chemical research related to biochemical mechanisms and cannot be fully representative of biological chemistry appearing in the Journal. Likewise, many excellent articles do not appear here because of subject overlap. Nevertheless, the collection gives the reader a feeling for the scope of this expanding field of research and the interesting chemical mechanisms that characterize biology. This Editorial draws on my own impressions of where these efforts are making an impact on future research at the chemistry−biology interface.

Here, I summarize some of the high points of each article and provide some opinion as to where this work will impact the current state of research in the field. With only 24 publications, this selection cannot, in any way, reflect all areas of interest in biochemical mechanisms—they would not be adequately covered even in a selection of hundreds of articles. Since the topics are not intended to be connected by any common mechanism, the articles highlighed below are listed approximately in order of their appearance in *JACS* during 2010 and early 2011. The diversity of research articles in which chemistry and biochemistry overlap will be apparent from this *JACS Select* collection.

Phosphatidylinositol phospholipases are critical elements in cell signaling, catalyzing the release of inositol 1-phosphate. **Bruzik** and co-workers[1] point out that all previously known phospholipase C enzymes catalyze *cis* cyclization to form inositol 1,2-cyclic phosphate as an intermediate. Their present report characterizes a phospholipase C from *Streptomyces antibioticus*, a veritable microbial antibiotic factory. Using short-chain dihexanoyl phosphothioate analogues of phosphatidylinositol, this enzyme is shown to catalyze a *trans* cyclization with 1,6-cyclic phosphate as an intermediate. The enzyme reveals an unprecedented ability to distinguish bridging and nonbridging thio effects. Although the biological function of this enzyme in *Streptomyces* remains to be discovered, it is capable of hydrolyzing structural analogues of phosphatidylinositol known to be present in cell membranes of micro-organisms and plants. Our tendency to categorize chemical mechanisms often leads us to oversimplify in the belief that "if you've seen one phosphatidylinositol phospholipase, you've seen them all." This study provides an example of the evolutionary chemistry where more than one chemical mechanism fulfills the biological imperative to convert reactants to needed products.

Proton transfer in biological systems can occur rapidly when donor−acceptor geometry and distance are optimized. In other cases, the transfer is gated by motions of the protein. Since protein motions take relatively long times, often nanoseconds to milliseconds, the time constants for proton transfer can tell us much about the elements involved in the transfer. **Tonge**, **Meech**, and co-workers[2] have examined the rate of proton transfer in green fluorescent protein (GFP), one of the most useful biological tools for imaging cellular compartments. Light excitation of neutral GFP generates the anionic state with emission of green fluorescence coupled to a three-step proton transfer along amino acids and the chromophore in a proton-transfer chain. Formation of the deprotonated state by the excitation step occurs within the 50 fs time resolution of the instrumentation. As protein conformational motions are considerably slower, photoexcitation changes the pK_a and initiates proton transfer between optimally aligned groups.

Sugar-transfer reactions often occur with inversion of chemistry at the anomeric carbon, a stereochemical signature for an S_N2 nucleophilic displacement. In other cases they occur with retention of configuration, indicating a covalent intermediate in a double-displacement mechanism. In the past decade, kinetic isotope effect measurements on several sugar transferases have revealed that, regardless of the inversion or retention of stereochemistry at the reaction center, the transition states are distinctly dissociative, with S_N1 character. The original findings were made with *N*-ribosyltransferases, where a combination of transition-state analysis and structural studies revealed migration of the anomeric carbon in the reaction coordinate. The mechanism was called "nucleophilic displacement by electrophile migration", and a similar mechanism was later shown to characterize the lysozyme reaction. Now, **Davies** and co-workers[3] demonstrate a similar chemical strategy for a third class of enzymes, a stereochemistry-retaining *O*-GlcNAc hydrolase. A bicyclic oxazoline intermediate is observed crystallographically using a glucopyranosyl fluoride to chemically stabilize the intermediate. These novel results define the geometric reaction coordinate for a reaction with a stabilized oxazoline intermediate. With multiple classes of sugar transferases now shown to catalyze their reactions by electrophile migration, a common mechanistic theme is emerging.

Vitamin synthesis always involves chemistry that is unique from the synthetic pathways of the host. Riboflavin synthesis involves a remarkable transformation where two molecules of 6,7-dimethyl-8-ribityllumazine fuse to form an intermediate that fragments to riboflavin and a substituted pyrimidinedione. Amazingly, this reaction, though enzyme-catalyzed in cells, occurs spontaneously at elevated temperatures under anoxic aqueous conditions. Previous mechanisms proposed involvement of a nucleophilic amino acid side chain, but the solution chemistry and the lack of a crystallographically convenient nucleophile led to growing doubt about such a mechanism. Now, **Fischer**, **Bacher**, and colleagues[4] have proposed a new mechanism for the

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riboflavin synthase from *Escherichia coli* where the reaction begins with transfer of a hydride ion from one substituted lumazine to the second, followed by a 4 + 2 cycloaddition. The pentacyclic intermediate is experimentally documented. This mechanism has eluded researchers since the first report of this remarkable reaction by Plaut in 1960.[5] The new mechanism is consistent with earlier literature, including previously unexplained isotope effects.

Methyltransferases using *S*-adenosylmethionine (AdoMet) as the methyl donor are gaining increased interest as their role in epigenetic control becomes better understood. Thus, DNA methyltransferases determine patterns of gene expression in development, and protein methyltransferases control chromatin structure and function by methylating histone tails. New activities are still being uncovered. Chemical biology tools to capture enzymes or other proteins that interact with AdoMet would be of great utility. **Zhou** and co-workers[6] have described the single-step synthesis of keto-AdoMet from *S*-adenosylhomocysteine and bromoacetone to form an analogue of AdoMet with acetone replacing the methyl group. The activity of cellular methyltransferases transfers the ketone from keto-AdoMet to provide a reactive group for subsequent derivitation with chromphores containing hydroxylamines or hydrazines. The resulting stable oximes and hydrazones permit identification of previously unidentified targets for methylation by AdoMet.

Although DNA damage gets the headlines for gamma-radiation of nucleic acids, damage to polymers of RNA was originally characterized to initiate understanding of the chemical process leading to strand scission. The hydroxyl radical ($HO^{\bullet}$) generated by radiation interacts with π-bonds to generate nucleobase radicals, eventually leading to strand breaks. As strand breaks involve ribosyl phosphodiester bonds, nucleobase radicals are implicated in forming sugar radicals to initiate strand scission. **Greenberg** and colleagues[7] generated a specific C5 radical of uridine by Norrish type-I photocleavage of a nucleoside precursor. Computational models indicated a mechanism of strand cleavage under anaerobic conditions with C2′ H-atom abstraction by the C5 radical of the adjacent 5′-nucleotide. Thiol competition demonstrated that strand scission directly from the pyrimidine base radical is slow relative to the efficient strand break accomplished by radical transfer via C2′-H abstraction.

Bacterial antibiotic resistance can result from methylation of an adenosine nucleotide within the peptidyltransferase catalytic site of the ribosome. Gene products RlmN and Cfr have recently been shown to be AdoMet (SAM) transferases catalyzing methyl transfer to the purine ring of adenosine in 23S rRNA to generate 2,8-dimethyladenosine. Sequence analysis indentified the proteins as members of the radical SAM family containing 4Fe-4S clusters. AdoMet plays two roles in these antibiotic resistance-conferring enzymes: forming a 5′-adenosyl radical to activate the purine base, and then donating a methyl group. **Mankin**, **Fujimori**, and co-workers make two major contributions with this work, as it reveals the first known methyl transfer catalyzed by a member of the radical SAM superfamily, and it demonstrates an important mechanism for the acquisition of antibiotic resistance.[8] In subsequent work reported in *Science*[9] and *Proceedings of the National Academy of Science*,[10] these investigators have demonstrated intermediate transfer of the methyl group to a protein Cys prior to reaction with activated carbon of the adenine ring. This work, initiated with the *JACS* article, has been the subject of a *Science* editorial by JoAnne Stubbe entitled "The Two Faces of SAM".[11] These reports have benchmarked a new class of chemical methyl-transfer reactions and has initiated a surprising new catalytic mechanism for a class of enzymes already known for over 30 years.

The chemical diversity of natural product chemistry appears infinite, and finding patterns to explain recurring chemical motifs is an essential first step to organize, classify, and understand the catalytic machinery for natural product synthesis. An excellent example of this approach comes from **Walsh** and co-workers[12] in their discovery of a three-enzyme pathway capable of making the 2-amino-3-hydroxycyclopent-2-enone five-membered ring (the C_5N ring) that appears to participate in amide linkage to polyketide-derived scaffolds. Three tandem open reading frames from a biosynthetic gene cluster in *Streptomyces aizunenesis* provide all the enzyme activities needed to form the C_5N ring. The first enzyme condenses succinyl-CoA and glycine to yield 5-aminolevulinate (ALA) in a pyridoxyl phosphate-dependent aminolevulinate synthetase. ALA is converted to ALA-CoA via an ALA-AMP intermediate with the second enzyme, an acyl-CoA ligase, forming the chemically unstable ALA-CoA, but also cyclizing it to the C_5N ring, a substrate for the third enzyme. This ATP-dependent amide synthetase completes the reaction. Examples of natural products containing this chemical building block include manumycin A, asukamycin, limocrocin, and reductiomycin. Unraveling these mechanisms demonstrates the power of combining genetics, biochemistry, and chemistry to bestow order on the nearly unimaginable scope of natural product chemistry.

Protein dynamic reorganization occurring along the reaction coordinate has long been assumed to contribute to distinct catalytic steps, most importantly in the conversion of enzyme-bound reactant to enzyme-stabilized transition state. **Childs** and **Boxer**[13] report a light-sensitive probe to examine this question in the well-studied Δ^5-3-ketosteroid isomerase (KSI). Is there a conformational response at the active site of KSI following an electrostatic change of bound reactants that simulates the catalytic reaction coordinate? A photoacid bound to the active-site oxyanion hole resembles the enolate intermediate known to exist on the reaction coordinate. Photoactivation generates an excited state resembling the starting material. Thus, the state change resembles two important steps on the reaction coordinate, and the question is if the catalytic site is dynamically perturbed as determined by the use of fluorescence probes. The results indicate the active site does not reorganize in response to this electrostatic change resembling distinct steps in the catalytic cycle. A conclusion for this enzyme is that the catalytic site is electrostatically preorganized, and reorganization upon conversion of bound reactants (from intermediate-like to substrate-like) is not observed. Time scales interrogated in these experiments are limited to 100 ps, and the authors argue that these are relevant time scales for protein motion linked to catalysis. Contribution to catalysis from fast thermal motions (breathing modes) of 0.1−10 ps could not be excluded. There is literature to support coupling of "promotional vibration modes" that occur on the femtosecond to low picosecond time scale to reaction coordinate barrier crossing.[14] Protein dynamic reorganization remains an active area of research with time scales difficult to access experimentally, and the present work is a bold step in investigating time-linked enzymatic reorganization.

Isomerization reactions exemplify the simplest class of enzymes, with their one substrate and one product. Yet, surprises are possible. Most members of the isomerase superfamily contain two approximately symmetric domains, each providing a catalytic-site cysteine to act in concert as an acid/base pair for stereospecific proton transfer in the racemization reaction. Interest in the

transformation of maleate to fumarate by maleate isomerase (MI) arose from the observation that the catalytic sites of Asp/Glu racemases are similar to that of MI from *Nocardia farcinica* IFM. Kinetic, mutational, and crystallographic studies by **Grogan** and colleagues support an unprecedented mechanism for *cis*−*trans* isomerizations by showing a covalent succinylcysteine covalent intermediate to Cys76.[15] Replacement of Cys76 with Ser reduces catalysis by 3 orders of magnitude, and replacement with Ala renders the enzyme inactive. In two possible mechanisms, the first step is addition of the Cys thioate in a Michael nucleophilic attack to form the covalent intermediate, followed by bond rotation and stereospecific deprotonation with elimination of the covalent Cys76. This newly uncovered mechanism establishes that the racemase superfamily is more diverse than proposed originally.

Base-catalyzed solvolysis of RNA to yield strand cleavage depends on the well-known mechanism of 2′-hydroxyl ionization followed by attack of the hydroxyl ion to displace the adjacent 5′-nucleoside and form a cyclic 2′,3′-phosphodiester on the initiating ribosyl group. Hydrolysis of the cyclic phosphodiester occurs from a solvent nucleophile. Despite the long-standing knowledge of this mechanism, understanding of the transition state is incomplete and has been based on indirect and substituent studies. Kinetic isotope effects provide direct access to detailed information about bond cleavage at the transition states. **Harris, Piccirilli, Anderson,** and co-workers[16] measured the primary ^{18}O and solvent $^{2}H_2O$ isotope effects to reveal the mechanism of base-catalyzed 2′-*O*-transphosphorylation of 5′-UpG-3′. A combination of these isotope effects and their pH dependence implicated a concerted mechanism with advanced bond formation to the attacking nucleophile and bond cleavage from the leaving group in a product-like transition state. The synthetic steps and analytical chemistry to measure these isotope effects provide an experimental approach for all related phosphotransferase reactions.

Another example of the power of kinetic isotope effects to provide information about transition states in complex reactions comes from the **Sauve** laboratory and the study of the ADP-ribosylation of acetyllysine catalyzed by Sir2 from a bacterial source.[17] The sirtuin family of enzymes catalyze the transfer of an acetyl group from acetyllysines of histones and regulatory proteins to nicotinamide adenine dinucleotide (NAD) to form free nicotinamide and 2′-*O*-acetyl-ADP-ribose. This reaction breaks and forms almost a dozen covalent bonds on conversion of reactants to solution-equilibrated products. In addition to the chemical complexity of this catalysis, the physiological function of the sirtuins has been implicated in regulation of processes as diverse as DNA repair, insulin secretion, and mammalian lifespan. A hotly disputed early step in the chemical reaction is loss of the nicotinamide and its replacement by the acetyl carbonyl oxygen from acetyllysine.[18] Of dispute is the nature of this transition state. At one extreme, the attacking carbonyl oxygen can act in a nucleophilic displacement to form an S_N2 transition state, and at the other extreme, nicotinamide could depart in an S_N1 mechanism to form the highly reactive ribocation, which would then capture the carbonyl oxygen of the acetyllysine in a subsequent step. Numerous studies from chemical reactivity series and crystallographic studies have promoted both mechanisms; however, all such indirect studies are speculative. Only kinetic isotope studies provide definitive information about transition states. Sauve solved this mechanism for the Sir2 of *Archaeoglobus fulgidus* by measuring a full family of kinetic isotope effects and using computational quantum chemical approaches to find a transition state consistent with the intrinsic isotope effects. The results establish a highly dissociated ribocation transition state with very low bond orders to the departing nicotinamide and the carbonyl oxygen of the acetyllysine. This solution to the hotly debated issue is chemically satisfying since the carbonyl oxygen of acetyllysine is a poor nucleophile and the nicotinamide of NAD is a highly activated leaving group. The sirtuins also must deal with the problem of distance, getting the nucleophilic oxygen within striking distance of the anomeric carbon of NAD, which is not likely to be easy when the acetyllysine is attached to one of a large family of target proteins. Other ribosyltransferases form a ribocation at the transition state, followed by migration of the C1′-ribocation by a ribosyl ring pucker mechanism through space to move it several angstroms to the incipient nucleophile.[19] Since ribocations are highly reactive, they react at diffusion rates, even with insipid nucleophiles like the carbonyl oxygen of acetyllysine, which cannot resist the approaching ribocation. Thus, generation of the ribocation transition state solves problems of both chemistry and geometry. It is anticipated that this chemically satisfying transition-state structure will permeate the extended family of sirtuins. But, as demonstrated in other enzyme reactions, digging into the true solution of biochemical problems often leads to surprises.

Antibiotic resistance in Gram-negative bacteria is a rapidly expanding health problem, driven by the presence of more than 870 unique, naturally occurring β-lactamases. These enzymes inactivate β-lactams like penicillin and related antibiotics. These antibiotics kill bacteria by interfering with cell wall synthesis. The pharmaceutical response to the emergence of β-lactamases has been synthesis of β-lactamase inhibitors. Four classes of β-lactamases are known, three of which use an active-site serine as the nucleophile and the fourth which uses zinc atoms in their familiar biochemical role of water activation or to interact with reactants as Lewis acids. Of these, the class D serine-based β-lactamases are the most problematic. Called oxacillinases, they hydrolyze penicillins, cephalosporins, and carbapenems and are resistant to inhibitors of other β-lactamases, including clavulanate, sulbactam, and tazobactam. **Bonomo**, **Romero, Buynak**, and co-workers[20] report the synthesis and mechanism of action of five novel 6-alkylidene-2′-substituted penicillanic acid sulfones with effective IC_{50} values in the nanomolar range. Mass spectrometry and X-ray crystallography demonstrate covalent adducts consistent with formation of an acyl-enzyme intermediate. The inhibitor is not fragmented, and the adduct shows the carbonyl oxygen deep in the oxyanion hole. As class D β-lactamases are the most problematic drug targets, the new results provide an important and significant advance in the ever-evolving cat-and-mouse game between drugs and mutation of drug targets.

In a remarkable biochemical conversion—the one essential for the biological nitrogen cycle—atmospheric nitrogen gas is converted to two molecules of ammonia by the transfer of six electrons. The nitrogenase responsible for this reaction uses two proteins, a Fe protein and a MoFe protein, named for their metal contents. Energy from the hydrolysis of multiple ATP molecules by the Fe protein (more accurately called a [4Fe-4S] protein) is used to deposit electrons one at a time to the MoFe protein until nitrogen reduction can occur. The Fe protein is the only documented functional reductant for the MoFe protein. **Seefeldt** and co-workers[21] now report a remarkable mutant of the MoFe protein to permit uncoupling of the Fe protein role from the nitrogen reductant role of the MoFe protein. By replacing Tyr98 with a His in the MoFe protein, the Fe-protein in an ATP-dependent reductant role can be replaced directly by a

low-potential reductant, in this case polyaminocarboxylate-ligated Eu^{II}. This discovery uncouples the ATP requirement for the reduction of atmospheric nitrogen to ammonia. Enzymes are already used in a growing number of industrial applications for large-scale chemistry, and this report provides an early step toward realizing the concept of biological nitrogen fixation.

Despite generations of intense public health measures, immunization programs, and development of antibiotics with action against tuberculosis, this infection remains one of the most devastating worldwide health problems. Now the spread and intensity of the disease are complicated by the advent of "MDX", or multiple-drug-resistant tuberculosis. **Johnsson** and co-workers[22] report a class of benzothiazinone prodrugs with action against virulent *Mycobacterium tuberculosis* that is 20 times more active than isoniazid, a frontline tuberculosis drug. The target for the benzothiazinones is known to be an epimerase required for the conversion of decaprenylphosphoryl-β-D-ribose to decaprenylphosphoryl-β-D-arabinose, a reaction essential for cell wall synthesis in the bacterium, but the mechanism of action had not been established. Now it is shown that a nitro group essential for the biological action of the benzothiazinones is the target of a biological reduction to form a nitroso group. The increased chemical reactivity targets an essential Cys amino acid side chain of the epimerase to form a covalent semimercaptal adduct of the activated benzothiazinone. This new family of antimycobacterial prodrugs has a unique mechanism of action, which gives them potential utility for sole-agent use or for use in drug combinations with existing antimycobacterial drugs.

Chemistry students know that molecular associations are described by the familiar components of Gibbs free energy ($\Delta G = \Delta H - T\Delta S$). In complex biochemical systems of ligand−protein, protein−protein, or other macromolecular interactions, we are most familiar with the enthapic components of hydrogen, ionic, and van der Waals contacts. These interactions are generally stabilizing for both partners of the binding interaction, causing increased organization or decreased freedom of motion in all of the binding partners and causing an entropic penalty that may be difficult to avoid. Furthermore, entropic energies can equal or exceed those of enthalpy, the most common focus of ligand design. Entropic penalties are almost inevitable in structure-based design with step-by-step building of a ligand to contact as many interactions as possible; however, if both enthalpy and entropy are favorable, ligands with exceptional affinity might be found. Transition-state analogues provide exceptional examples of this concept, where rigid inhibitors with the largest favorable enthalpic contribution to binding are not the most tightly bound because of the entropic penalty.[23] By building increased flexibility into these analogues, the dynamic motion of the protein can be matched to dynamic motion of the inhibitor, leading to more favorable entropic contributions and causing increased binding affinity (ΔG).[24] **Akke** and co-workers explore these principles by examining the conformational entropy of carbohydrate-based ligands that bind to galectin-3, a protein involved in cell surface effects influencing cell growth and differentiation.[25] Here, conformational entropy modulates the affinity of the interaction between the protein and the carbohydrates. Remarkably, NMR studies reveal that the target protein structure responds to ligand binding by increased dynamic fluctuations, and these changes are prominent in the hydrophobic core. Here the conformational entropy is similar in overall energetic contribution to the enthalpic interactions of binding. These findings support the incorporation of entropic thermodynamic components in ligand design approaches for carbohydrate-binding proteins where hydrophobic effects are limited.

DNA synthesis in all organisms requires a balanced source of deoxynucleotide triphosphates. Ribonucleotide reductases (RNRs) are responsible for reduction to the nucleotides, and a single enzyme acts on all of the precursors needed for DNA synthesis. Mammals and *Escherichia coli* have similar RNRs with an $\alpha 2/\beta 2$ subunit structure. Ribonucleotide reduction and regulatory interactions occur in the α subunits; the β subunits contain Fe and are the sites of the di-Fe-tyrosyl radical essential for reduction occurring in the α subunits. Remarkably, the site of tyrosyl radical formation (Tyr122β) and the catalytic site where the radical is used are separated by over 35 Å. Long-range proton-coupled electron transfer (PCET) is proposed to occur in the *E. coli* enzyme through a long path of amino acids including Tyr122β, the site of radical formation, and reversible tranfers through four additional amino acids in both subunits to generate a thiyl radical at the catalytic site. The rate of chemical conversion of reactants to products is limited by a rate-limiting conformational gating of some sort in the PCET process. Until the present results, in elegant studies from the **Stubbe** laboratory,[26] the rates of the individual complex conversions were unknown. Specific insertion of a 3-NO_2-Tyr122β, a "hot oxidant", into functional protein provides a probe for investigations by stopped-flow spectroscopy, freeze quench EPR, and rapid chemical quench. The results reveal the new radical to be predominantly localized on Tyr356, one of the intermediate transfer elements. This example is a unique experimental approach to analyze conformational gating distinct from chemistry.

Specific activation of enzymes by altering the chemical reactivity of groups at the catalytic site has been a long-sought goal at the interface of catalysis and chemistry. **Toney, Larsen,** and co-workers[27] have taken a step in this direction by illuminating the pyridoxal phosphate cofactor involved in the reaction catalyzed by aspartate aminotransferase. In this reaction, isotope effects have indicated a rate-limiting step to be proton loss from the aspartate external aldimine, a species with an absorption band at 430 nm. Excitation of this band increases the rate of the enzyme reaction considerably, attributed to photoenhancement and consistent with solution studies of Schiff base complexes. By placing the pyridoxal-aspartate Schiff base in different environments, including catalytic site mutants of the enzyme and in water, it was shown that the extent of enhancement corresponds to the intrinsic reactivity of the environment, with the least reactive Schiff base environments benefiting the most from the photodynamic stimulation. Time-resolved spectroscopy established a triplet-state photoactivation pathway as a consequence of lowering the pK_a of the Ca−H bond as much as 11−19 units for the Schiff base in water. These remarkable results suggest a method to obtain a population of synchronized catalytic states in enzymes with light-sensitive chromophores.

Xanthine oxidoreductase (xanthine oxidase) catalyzes two regiochemically distinct reactions by oxidizing both C2 and C8 of hypoxanthine. The enzyme is of biomedical significance, as it is required for production of uric acid, the causative agent of gout. Xanthine oxidase is the target of pharmacological agents, most commonly allopurinol, a purine analogue that is also activated by the action of this enzyme to form a tight-binding inhibitor. Newer agents, including febuxostat, have recently been approved by the U.S. FDA for use as xanthine oxidase inhibitors. Xanthine oxidase is a molybdenum-containing oxidoreductase, and in

order for reaction to occur, the susceptible site of oxidation must be brought near the Mo cofactor in the catalytic site. Several mechanistic proposals have been made, but there has been no definitive resolution. In their recent work, **Nishino** and co-workers[28] have solved two high-resolution crystal structures with rat and bovine enzymes in complex with uric acid at the catalytic sites. The bovine enzyme was a reduced species of the enzyme with a chemically trapped reaction intermediate at the catalytic site. The results are consistent with only one of the previous proposals for the catalytic action of this protein. Hydrogen bonds from Glu802, Glu1261, and Arg880 hold the purine in close proximity to the Mo cofactor, and Glu1261 and a thiol ligand to the Mo also serve as acid−base centers for the reaction.

Proteins are subject to a host of post-translational modifications that serve to regulate their function. A recent discovery is the nitration of protein tyrosine residues, a possibly damaging change associated with human diseases of metabolism and aging. Chemical logic implicates reactive nitrogen compounds including peroxynitrite anion ($ONOO^-$) and nitrogen dioxide ($^{\bullet}NO_2$). **Groves** and colleagues[29] have now shown that generated peroxynitrite reacts primarily at the catalytic site Tyr34 of the model enzyme, *Escherichia coli* manganese superoxide dismutase. Surprisingly, the efficiency of nitration was found to depend on CO_2, implicating carbonate radical ($^{\bullet}CO_3^-$) in peroxynitrite-dependent nitration. The observed lack of manganese superoxide dismutase activity under disease conditions is likely due to nitration of Tyr34 by a peroxynitrite + CO_2-dependent inactivation mechanism.

The actions of fatty acid amide hydrolases (FAAHs) are linked to signaling pathways leading to pain and inflammation. Finding inhibitors of FAAH and determining their mechanism of action has been a priority in the development of drug leads for this new target related to major health needs. **Stevens**, **Boger**, and colleagues[30] report two crystal structures of an exceptionally potent α-ketoheterocycle inhibitor (20 pM) in complex with a humanized version of rat FAAH. The inhibitor binds as its ketone rather than its gem-diol form, and a solution fluoride ion binds the oxyanion hole of the enzyme prior to inhibitor binding. Noncovalent and covalent forms are captured in these crystal structures, leading to identification of key anchoring interactions before and after covalent inactivation of the FAAH.

Regulation of nitric oxide in human metabolism is important for control of blood pressure and is a target for drug design. One of the important enzymes linked to the proposed pathways is dimethylarginine dimethylaminohydrolase (DDAH). Fragment-based ligand screening for this target led to analysis of 4-halopyridines, which show time-dependent inactivation of DDAH. **Fast** and colleagues[31] have selected one member of this family, 4-bromo-2-methylpyridine, and have characterized its mechanism of inactivation. The inhibitor reacts in a selective, irreversible mechanism with Cys249 at the catalytic site of DDAH but not other Cys-dependent enzymes such as papain. Activation of this halopyridine at the catalytic site appears to occur by the DDAH site-specific activation of this ligand to form a more reactive pyridinium species at the catalytic site, causing site-directed activation. Halopyridines had not been previously identified as protein modification agents, and this work introduces a new class of protein modification tools as well as describing a specific inactivation for a potentially important drug target. In an even more recent study,[32] this group has further explored the mechanism of inactivation by X-ray crystallographic studies.

Prenyl groups are a common precursor for secondary metabolism, and prenyltransferases provide the catalytic machinery to facilitate the reactions. Well-known prenyltransferases, including the polyprenyl synthases and the cyclizing terpene synthases, are all α-helical fold structures. The recent discovery of a prenyltransferase from archea with a triosephosphate isomerase (TIM) barrel fold led to a genomic search for bacterial homologues. **Walker** and colleagues[33] identified two prenyltransferases predicted to be TIM barrel folds in the gene cluster for synthesis of the antibiotic moenomycin. Among the novel surprises found with these enzymes are the use of glycerol-1-phosphate as an acceptor and a reaction that involves a combination of isomerization and prenyl transfer by the same protein. Finally, the use of the well-characterized TIM barrel fold for chemistry previously unknown to this class of enzyme teaches us that we are still in the learning phase for understanding biological catalysts.

Superoxide reductases are responsible for the conversion of superoxide to hydrogen peroxide in biological systems. Oxidative damage from superoxide is one of the penalties aerobes pay in exchange for the metabolic efficiency of oxidative pathways. The reaction mechanism for these enzymes is proposed to proceed via an Fe(III)-OOH intermediate. However, these short-lived species are difficult to characterize. Biomimetic chemistry is sometimes possible for enzymes with metal or vitamin cofactor reaction centers and is profoundly useful for the study of chemical mechanisms. **Kovacs** and co-workers[34] have used a thiolate-liganded Fe(II) biomimetic of superoxide reductase, which also contains a cysteinate-ligated non-heme iron. A second way to examine these systems uses nitric oxide (NO), a chemical mimic of superoxide, as a chemically active spectroscopic probe. Remarkably, NO reacts with the Fe(II) biomimetic, leading to oxidative addition and a Fe(III)-NO^- complex with properties independent of the *cis* or *trans* position of the thiolate mimic of the enzymatic Cys group. The reactivity and intermediate characterization provide insight to N−O and therefore O−O bond activation in this important biomimetic of superoxide reductase.

This highly select summary of recent biochemical mechanisms published in the *Journal of the American Chemical Society* provides a cross-section of novel, interesting, and often surprising approaches used by nature to accomplish useful chemical conversions. A collection of these advances provides a window on many areas of active research at the interface of chemistry and biology. Although this selection has focused on mechanisms, similar collections could be made from the recent annals of *JACS* to highlight logical chemical approaches to inhibitor design or to highlight chemical biological methods to label, select, sense, or track biomolecules. Even a cursory read of the articles gathered here and in other *JACS* Select issues demonstrates the merger of chemical science disciplines. The need to understand chemical mechanisms applies equally to a new step in stereoselective chemical synthesis and to understanding how an enzyme is capable of converting reactants to products at rates trillions of times faster than without catalyst. We hope that readers will benefit from this concentration on chemical mechanisms applied to biochemical problems. Students of both the chemical and biochemical sciences and researchers may benefit from this broad view of mechanistic biochemistry.

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