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Preface to Methods in Enzymology, Volume 606

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As early as five decades ago, the notion that radicals would be involved in enzymology was a heretical thought, and was rarely invoked, even if a reasonable polar mechanism could not be drawn. However, elucidation of the role of vitamin B₁₂ in the reaction catalyzed by adenosylcobalamin dependent enzymes revealed that enzymatic transformations could involve free radicals as discrete intermediates. The intermediacy of the highly reactive oxidant, 5'-deoxyadenosyl radical (5'-dAdo•), which was postulated in the context of these transformations, revealed how unactivated substrates could be poised for a myriad of complex radical mediated transformations initiated by H-atom abstraction.

The pioneering studies of Frey and colleagues on lysine 2,3-aminomutase (LAM) revealed H-atom abstraction events in an enzyme that instead of a cobalamin, appeared to use S-adenosyl-L-methionine (SAM) and a 4Fe-4S cluster to catalyze similar transformations. LAM catalyzes reductive cleavage of SAM to generate 5'-dAdo•, which subsequently activates lysine via H-atom abstraction to initiate the reaction. Direct observation of many of the proposed reaction intermediates with both native substrate and substrates analogs cemented the paradigm that 4Fe-4S/SAM enzymes catalyze radical-mediated transformations. But, had it not been for bioinformatics, the story may have ended there and this volume of *Methods in Enzymology* would not exist.

In 2001, using the sequence of LAM as a guide, Sofia and colleagues proposed the presence of so-called radical SAM superfamily, which is distributed across all domains of life. The key insight was the realization that a CxxxCxxC motif is almost always present in these proteins. Subsequent studies have revealed that the motif binds the 4Fe-4S cluster, which in turn binds SAM. With decreasing costs and the widespread use of genome sequencing over the last decade, the community has come to realize that the radical SAM superfamily is, in fact, widespread and includes well over 100,000 examples across various genomes.

Despite their ubiquity, biochemical studies of the radical SAM superfamily have been slow because of numerous challenges. Most radical SAM enzymes are oxygen sensitive and all manipulations must be carried out under anoxic conditions. In most cases, the substrates are not known. Furthermore, obtaining these proteins in cofactor replete form can be an additional impediment. Methods for *In vitro* reconstitution of the cluster(s) are often unique to each enzyme and are usually discovered through trial and error. It is fair to say that *in vitro* reconstitution is still an art! Finally, the discovery that some members require additional auxiliary clusters, accessory proteins, or other cofactors, such as B₁₂, further highlight the challenges of working with this superfamily.

Fortunately, these challenges have not limited the field, but instead, have spurred many labs to search for creative solutions. Indeed, in the last decade, numerous radical SAM enzymes have been characterized; many of which are involved in biological transformations, which among others, include metabolic reactions, RNA/DNA modification, and biosynthesis of natural products.

In this *Methods in Enzymology* volume I have tried to highlight the challenges and solutions in the field by inviting chapters that detail various aspects of radical SAM enzymology. Many of the contributions focus on specific methods for a particular enzyme, while others focus on general methods necessary for the discovery and characterization of new members. However, the common goal is to provide a starting point for characterization of unknown radical SAM enzymes.

The increasing pace of discovery over the last decade in the radical SAM field foreshadows an exciting future, though there are still many challenges. The sheer numbers of radical SAM enzymes of unknown function underscores how little we know about this class of enzymes. However, as functional annotations begins to catch up with bioinformatics, predicting an enzyme's biological role may become possible. Unfortunately, obtaining recombinant proteins from a heterologous host in cofactor replete form is likely to remain a challenge for the foreseeable future; but, the solution is likely to come from the nexus of this field and the iron-sulfur cluster biogenesis community. If the last decade is any indication of things to come, we are in for a wild and exciting future. I hope this snapshot of current methods in the field helps accelerate discoveries.