The Biosynthesis of Thiol- and Thioether-containing Cofactors and Secondary Metabolites Catalyzed by Radical S-Adenosylmethionine Enzymes

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Sulfur atoms are present as thiol and thioether functional groups in amino acids, coenzymes, cofactors, and various products of secondary metabolic pathways. The biosynthetic pathways for several sulfur-containing biomolecules require the substitution of sulfur for hydrogen at unreactive aliphatic or electron-rich aromatic carbon atoms. Examples discussed in this review include biotin, lipoic acid, methylthioether modifications found in some nucleic acids and proteins, and thioether cross-links found in peptide natural products. Radical SAM (S-adenosyl-l-methionine) enzymes use an iron-sulfur cluster to catalyze the reduction of SAM to methionine and a highly reactive 5′-deoxyadenosyl radical; this radical can abstract hydrogen atoms at unreactive positions, facilitating the introduction of a variety of functional groups. Radical SAM enzymes that catalyze sulfur insertion reactions contain a second iron-sulfur cluster that facilitates the chemistry, either by donating the cluster’s endogenous sulfide or by binding and activating exogenous sulfide or sulfur-containing substrates. The use of radical chemistry involving iron-sulfur clusters is an efficient anaerobic route to the generation of carbon-sulfur bonds in cofactors, secondary metabolites, and other natural products.

Radical SAM Enzymes Can Introduce New Sulfur-containing Functional Groups

A number of cofactors and secondary metabolites contain sulfur that is added to carbon atoms that would not be predicted to accept sulfur nucleophiles in a polar reaction (Fig. 1). For example, the precursor to biotin contains unreactive aliphatic methyl and methylene carbon atoms at the positions where a sulfur atom is added during the last step in the biosynthetic pathway. The site of methylthiolation of aspartate 89 in the ribosomal protein S12 is on the β-carbon, adjacent to the side-chain carboxylate, a site that could potentially be deprotonated to an nucleophilic enolate, but that is difficult to transform into an electrophilic carbon. These sites can, however, be activated through hydrogen atom abstraction in a radical enzyme reaction; the product is a carbon radical that is electron deficient and will readily form new C–S bonds. Radical SAM enzymes carry out hydrogen atom abstraction from unreactive carbon atoms in a variety of substrates by coupling hydrogen atom transfer to reduction of the SAM sulfonium center.

The radical SAM superfamily includes thousands of enzymes that catalyze a diverse array of biochemical reactions (5). The enzymes contain a core (βα)₆ architecture that binds a [4Fe-4S]²⁺ cluster to a conserved CXXXCCXC motif found in a loop between the first β strand and α helix (6). Although three of the iron atoms in the cluster are bound by the cysteine residues in the conserved motif, the fourth position is occupied by the methionyl amine and carboxylate groups of SAM, bringing the sulfonium group of SAM into van der Waals contact with both iron and sulfur atoms in the cluster. Reduction of the cluster to a [4Fe-4S]³⁻ state results in partial occupancy of antibonding orbitals in the sulfonium group (7), triggering rapid cleavage of the sulfonium C5′-S bond to yield a 5′-deoxyadenosyl (5′-dA′) radical. The 5′-dA′ radical can be readily quenched by abstracting a hydrogen atom from nearby atoms in the substrate, generating 5′-deoxyadenosine (5′-dAH) and a high-energy substrate-centered radical that reacts in a variety of ways, depending on the detailed structure of the substrate and the enzyme active site.

Sulfur is present as a relatively low abundance element in all organisms; for example, the human body is 0.2% sulfur by mass (1), whereas rapidly dividing Escherichia coli grown in a sulfate-enriched minimal medium are 0.3% sulfur by mass (2). The vast majority of this sulfur is present as inorganic sulfate, as well as a variety of organic molecules including cysteine, methionine, several related metabolites including homocysteine and S-adenosyl-l-methionine (SAM), and sulfate esters and sulfonamides in polysaccharides and proteins, for example, heparin containing functional groups.

Radical SAM enzymes can introduce new sulfur-containing functional groups involving iron-sulfur clusters in an efficient anaerobic route to the generation of carbon-sulfur bonds in cofactors, secondary metabolites, and other natural products.
**MINIREVIEW: C–S Bond Formation by Radical SAM Enzymes**

SAM FeS cluster binding site is empty (11–14). Reconstitution under mild reducing conditions leads to assembly of a [4Fe-4S]^{2+} cluster at this site, resulting in active enzyme (15).

When reconstituted biotin synthase is mixed with dethiobi- 
tin, SAM, and an enzymatic reducing system, the enzyme 
undergoes ~1 turnover per dimer in 15 min and then turns over 
more slowly, reaching ~4 turnovers per dimer after 4 h (16). 
Enzyme turnover is accompanied by reduction and destruction 
of one [2Fe-2S]^{2+} cluster per dimer (12, 15). Because there is 
no other sulfur source added to the enzyme, this strongly suggests 
that the [2Fe-2S]^{2+} cluster is the source of sulfur for biotin 
formation. When the enzyme reaction is run with excess 
[34S]sulfide in both the [4Fe-4S]^{2+} cluster and the buffer, but 
has natural abundance sulfide in the [2Fe-2S]^{2+} cluster, biotin 
formed in the first turnover contains only natural abundance 
sulfur (16). Additional slower turnovers incorporate [34S]sul-
fide, possibly suggesting that the [2Fe-2S]^{2+} cluster is sponta-
neously reassembled using sulfide from the buffer.

Because the enzyme reaction requires 2 eq of SAM and gen-
erates 2 eq of 5′-dAH and methionine, but each monomer only 
has one binding site for SAM, and then the enzyme must gen-
erate a dethiobiobiotin-derived intermediate that remains bound 
while 5′-dAH and methionine dissociate and a second equiva-
 lent of SAM binds. The formation of an intermediate had been 
suggested by earlier studies, but the identity could not be con-
firmed (17). Using two methods, this intermediate was shown 
to be 9-mercaptodethiobiobiotin (9-MDTB). First, the compound 
co-eluted on LC-MS with a synthetic sample of 9-MDTB 
and had the correct mass (18). Second, when 9-[methyl-
2H_3]dethiobiobiotin was used as a substrate, the 9-MDTB formed 
retained only two deuterium atoms, indicating that one deute-
rion had been transferred to 5′-dAH(D) during formation of 
the intermediate and that the newly added thiol group was at C9 
(18).

Formation of 9-MDTB from a dethiobiobiotin carbon radical 
and sulfide requires that an electron must be removed from 
sulfide during catalysis, which would likely end up in one of the 
FeS clusters. When biotin synthase underwent a reaction with 
only ~1 eq of SAM, a new EPR signal was formed in approxi-
mately stoichiometric concentration with 9-MDTB, consisting 
of two overlapping rhombic signals in an approximate 2:1 ratio 
with g = 1.880, 1.955, and 2.010 and g = 1.845, 1.940, and 2.000 
(19). Prior observation of these EPR signals and subsequent Mössbauer analysis suggested that the signals were attributable 
to the generation of a [2Fe-2S]^{2+} cluster (12). However, the pres-
ence of 9-MDTB suggested that one of the sulfides had been 
incorporated into this intermediate, and thus more likely, the 
cluster was formally a [2Fe-3S(RS)]^{2+} cluster.

The structure of this intermediate was further investigated 
using the pulsed EPR technique hyperfine sublevel correlation 
(HYSCORE) spectroscopy, using 9-[methyl-^{13}C]dethiobiobiotin 
and [guanidino-^{15}N]arginine-labeled enzyme to probe the 
chemical environment of the paramagnetic FeS cluster (20). 
The formation of 9-[methylene-^{13}C]9-mercaptodethiobiobiotin 
in the presence of the paramagnetic cluster resulted in the observation 
of a strong cross-peak (at 2.9 and 4.7 MHz), which is 
simulated with an isotropic hyperfine tensor A_{iso} = 2.7 MHz. 
This strong hyperfine interaction, together with the presence of 

Once a substrate-centered carbon radical has been gener-
ated, the introduction of a sulfur atom or a sulfur-containing 
functional group would seem straightforward. Carbon radicals 
are rapidly quenched by reaction with disulfides and polysul-
fides, often generating a mixture of products that includes new 
C–S bonds. An enzyme that catalyzes this reaction in a con-
trolled manner must deal with two chemical problems. First, 
any stable sulfur-containing substrate will have an even number 
of electrons, and the enzyme must remove an electron prior to 
or at the same time as C–S bond formation. Second, at neutral 
pH, a reduced sulfide or thiol will usually have S–H bonds, and 
the substrate radical can be quenched more rapidly by abstract-
ing a hydrogen atom from an S–H bond, rather than generating 
a new C–S bond. These problems are solved by using a second 
“auxiliary” FeS cluster to provide the sulfur or to bind the sul-
fur-containing substrate.

**Biotin Synthase Uses an Auxiliary [2Fe-2S]^{2+} Cluster to 
Provide Sulfur for Thioether Formation**

Biotin (Fig. 1) is a cofactor in a number of enzymes that cat-
alize carboxyl transfer reactions, where carbon dioxide reacts 
with the N5′ position in the ureido ring (8). The thioether ring 
is sterically bulky and provides protection against hydrolysis, 
thereby prolonging the lifetime of this unstable carboxybiotin 
intermediate. The thioether functional group is introduced 
in the last step of the biosynthetic pathway. The precursor dethio-
biotin contains a methyl group at C9 derived from l-alanine and 
a methylene group at C6 derived from pimelic acid (9). Biotin 
synthase inserts a sulfur atom between these two positions. The 
enzyme uses 2 eq of SAM as a net oxidant and generates 2 eq of 
5′-dAH with hydrogen atoms derived from both C9 and C6 
(10). Early studies established that the enzyme is purified with a 
[2Fe-2S]^{2+} cluster that is bound to Cys-97, Cys-121, Cys-188, 
and Arg-260 (E. coli numbering), whereas the canonical radical 

![Biotin](https://example.com/biotin.png)

![Lipoyl H-Protein](https://example.com/lipoyl.png)

![N6-isopentenyl-2-methylthioadenosine](https://example.com/n6-isopentenyl.png)

![3-methylthioaspartyl-S12 protein](https://example.com/3-methylthio.png)

![Cysteine to α-carbon thioether](https://example.com/cysteine.png)

**FIGURE 1. Biomolecules that contain sulfur atoms where C–S bond formation is catalyzed by radical SAM enzymes.** The new bonds formed and atoms added by radical SAM enzyme reactions are indicated in red.
MINIREVIEW: C–S Bond Formation by Radical SAM Enzymes

The crystal structure of biotin synthase has been solved in the presence of FeS clusters, dethiobiotin, and SAM (Fig. 2A) (21). Most notable in this structure is a closed enzyme active site generated by two additional β strands that complete a full (βα)₈ “TIM” barrel structure. This barrel encapsulates an active site in which dethiobiotin and the [2Fe-2S]²⁺ cluster are buried within the barrel, with the C9 position of dethiobiotin in between the C5’ position of SAM (3.9 Å C–C distance) and the μ-sulfide of the [2Fe-2S]²⁺ cluster (4.7 Å C–S distance) (Fig. 2B). The approximately linear relationship of the reactive atoms suggests a straightforward mechanism.

In the proposed mechanism (Fig. 3A), reductive cleavage of SAM generates a transient 5′-dAH radical that abstracts a hydrogen atom from the C9 position of dethiobiotin, generating 5′-dAH and a dethiobiotinyl C9 radical. This radical intermediate moves ~2.9 Å through the active site using a hinge motion secured by hydrogen bonds to the ureido ring and is quenched by a reaction with the μ-sulfide of the cluster. The sulfide is concurrently oxidized by transferring one electron into the cluster, generating 9-MDTB tightly bound as a ligand to a [2Fe-S(RS)]²⁺ cluster. After dissociation of 5′-dAH and methionine and association of a second equivalent of SAM, a similar reaction sequence directed at the C6 position closes the thioether ring and fully reduces the original [2Fe-2S]²⁺ cluster to two Fe²⁺ ions that dissociate from the enzyme. Further turnover requires reassembly of this cluster, which in vitro is slowly spontaneous, and in vivo is most likely carried out by the Isc or Suf FeS cluster assembly systems (22).

**Lipoyl Synthase Uses an Auxiliary [4Fe-4S]²⁺ Cluster to Provide Sulfur for Thiol Formation**

Lipoic acid is biosynthesized by the action of the radical SAM enzyme LipA on octanoylated carrier proteins, including the E2-protein from the pyruvate dehydrogenase complex (23) and the H-protein from the glycine cleavage system (24), generating the functional lipoyl E2-protein and lipoyl H-protein (Fig. 1). The reaction with the octanoyl H-protein has been more thoroughly characterized, and requires 2 eq of SAM and generates 2 eq of 5′-dAH for the formation of each equivalent of lipoyl H-protein (24).

LipA contains two [4Fe-4S]²⁺ clusters per protein polypeptide; in addition to a [4Fe-4S]²⁺ cluster bound to the canonical radical SAM motif, there is a second [4Fe-4S]²⁺ cluster bound to Cys-68, Cys-73, and Cys-79 (E. coli numbering). Although the wild-type enzyme contained ~7 eq of iron and sulfur per polypeptide, a variant in which these three cysteine residues were changed to alanine contained only ~3 eq of iron and sulfur per polypeptide and was inactive both for H-protein lipoylation and for conversion of SAM to 5′-dAH (25). Informed by the role of the auxiliary FeS cluster in biotin synthase, this second [4Fe-4S]²⁺ cluster was proposed to be a sulfur donor for the conversion of the octanoyl group to the lipoyl group. When enzyme turnover was carried out with a mixture of ³²S-labeled enzyme and natural abundance ³⁵S-enzyme, the lipoyl H-protein generated was a mixture of products with either two ³⁴S atoms or two ³²S atoms incorporated; only ~9% of the product contained both ³⁴S and ³²S in the same lipoyl group (26). This result demonstrated either that the thiol addition reaction was highly concerted or that it was a stepwise reaction in which a monothiolated intermediate remained tightly bound to LipA during dissociation of 5′-dAH and methionine and binding of a second equivalent of SAM.

Up to this point, studies of LipA were hampered by the difficulty of obtaining large quantities of substrate and characterizing a large lipoylated protein product. Roach and co-workers (27) discovered that LipA from the thermophilic organism Sulfolobus solfataricus would accept an octanoylated tetrapeptide as a substrate, allowing chemical synthesis of the substrate and direct characterization of intermediates and products by NMR and LC-MS analysis. When the reaction mixture was quenched in acid, they were able to detect formation of a monothiolated...
intermediate that was identified as a 6-thiooctanoyl peptide by NMR analysis (28).

Using full-length octanoyl H-protein and LipA from E. coli, Booker and co-workers (29) were able to detect a species in which the H-protein and LipA co-purified on an ion exchange column, suggesting the presence of a covalent cross-link. The cross-link was rapidly destroyed by treatment with acid, consistent with ligation of the monothiolated H-protein to an FeS cluster on LipA. Using Mössbauer spectroscopy, they were able to establish that this cross-linked protein also contained a modified FeS cluster. Although the initial enzyme contains two [4Fe-4S]2+ clusters, the isolated cross-linked species contains a paramagnetic [3Fe-4S]0 or [3Fe-3S(RS)]2+ cluster. Switching to an octanoylated 8-residue peptide and using LipA from Thermus thermophilus, they were able to measure kinetically competent formation ($k_1 = 1.1 \text{ min}^{-1}$) and decay ($k_2 = 0.017 \text{ min}^{-1}$) of the 6-thiooctanoyl peptide intermediate using LC-MS. They also observed formation of the paramagnetic cluster in parallel with this intermediate, which then decayed to predominantly free Fe2+ during conversion of 6-thiooctanoyl peptide to lipoyl peptide.

Together these data are consistent with a mechanism (Fig. 3B) in which reductive cleavage of SAM generates a 5′-dA radical that abstracts a hydrogen atom from the carbon of another residue in the peptide generates a radical that is quenched by capture of the cysteine sulfur, generating a stable thioether peptide cross-link.

**FIGURE 3.** The proposed mechanisms for radical SAM enzymes that introduce sulfur atoms as thioether or thiol functional groups. A, the proposed mechanism for thioether bond formation in biotin catalyzed by biotin synthase. In the first half-reaction, a C9 dethiobiotinyl radical is quenched by a reaction with the [2Fe-2S]2+ cluster, leading to formation of 9-MDBTB, which remains as a ligand to a [2Fe-5(SR)]2+ cluster. The second half-reaction is similar, with radical SAM chemistry directed at the C6 position, closing the thioether ring. B, the proposed mechanism for the stepwise introduction of two thiol groups into octanoyl proteins catalyzed by LipA. In the first half-reaction, a C6 octanoyl radical is quenched by the auxiliary [4Fe-4S]2+ cluster, leading to formation of a 6-thiooctanoyl protein, which is proposed to remain as a tightly bound ligand to a [3Fe-3S(RS)]2+ cluster. The second half-reaction leads to introduction of another sulfur from this intermediate cluster at the C8 position, generating lipoylated protein and leading to dissociation of the remnant FeS cluster. C, the proposed mechanism for introduction of the methylthiol functional group into aspartate 89 of the ribosomal S12 protein catalyzed by the radical SAM enzyme RimO. In the first half-reaction, a sulfide (or possibly persulfide) ion bound at the auxiliary [4Fe-4S]2+ cluster carries out a nucleophilic attack on the methyl group of SAM, generating a tightly bound methanethiol intermediate. In the second half-reaction, reductive cleavage of SAM generates a 5′-dA′ radical that abstracts a hydrogen atom from Asp-89. The aspartyl C3 radical is quenched by the transfer of the methanethiol group, resulting in formation of the methylthiolated protein residue with concomitant reduction of the FeS cluster. D, the formation of a thioether peptide cross-link by the enzyme AlbA. The peptide substrate is proposed to bind as a ligand to the auxiliary [4Fe-4S]2+ cluster. Abstraction of a hydrogen atom from the c-carbon of another residue in the peptide generates a radical that is quenched by capture of the cysteine sulfur, generating a stable thioether peptide cross-link.
cess, is instead unstable and ejects one Fe\(^{2+}\) ion to generate a [3Fe-3S(RS)]\(^{2+}\) cluster. This intermediate complex is stable while 5'-dAH and methionine dissociate and a second equivalent of SAM binds to the enzyme. A second reaction sequence focused on the C8 position generates a C8 radical that is quenched by a reaction with an adjacent sulfide in the FeS cluster, generating the reduced lipoylated product and destabilizing the remnant FeS cluster such that it dissociates from the enzyme. As with biotin synthase, further in vivo turnover likely requires regeneration of the [4Fe-4S]\(^{2+}\) cluster by the Isc or Suf FeS cluster assembly systems.

**Methylthioltransferases Catalyze Both Traditional Methyl Transfer and Sulfur Insertion Reactions**

The addition of a methylthiol group has been discovered as a common post-transcriptional modification on certain tRNAs, where the methylthiol group is found on adenosine 37 (Fig. 1) next to the anticodon, and as a post-translational modification on protein S12 in the ribosome 23S subunit, where it is found on aspartate 89 (Fig. 1), a residue located near the peptidyltransferase site in the intact ribosome. The methylthiol group on tRNAs is introduced at C2 of 6-isopentenyladenosine by the enzyme MiaB (30, 31), and at C2 of 6-threonyladenosine by the homologous enzyme MtaB (32). The methylthiol group on the ribosomal S12 protein is introduced at the C3 position of Asp-89 by the homologous enzyme RimO (33); it is as yet unclear whether this occurs prior to or after assembly of the 23S subunit. Because MiaB and RimO are homologous enzymes that catalyze chemically identical reactions, they are believed to utilize the same fundamental mechanism, and they will be discussed interchangeably in this review.

Both MiaB and RimO from *Thermotoga maritima* contain two [4Fe-4S]\(^{2+}\) clusters (34, 35). In *T. maritima* MiaB, the canonical radical SAM [4Fe-4S]\(^{2+}\) cluster binds to Cys-150, Cys-154, and Cys-157, whereas a second [4Fe-4S]\(^{2+}\) cluster binds to Cys-10, Cys-46, and Cys-79 (34). In *T. maritima* RimO, a similar auxiliary [4Fe-4S]\(^{2+}\) cluster is bound to Cys-17, Cys-53, and Cys-82 (35). These cysteine residues account for coordination to three iron atoms within the cluster, suggesting that the fourth ligation position is available to coordinate to the substrate or to participate in catalysis. When MiaB is assayed with selenide (Se\(^{2-}\)) substituted for sulfide in the assay buffer, the tRNA is modified with methylselenide, suggesting that the *in vitro* sulfur source is sulfide, persulfide, or polysulfides, which could be binding to the available coordination position of the auxiliary FeS cluster (30). The source of the methyl group was identified as SAM using [methyl-\(^{14}\)C]SAM, which is produced from the production of \(^{3}\)H-labeled N\(^{6}\)-isopentenyl-2-methylthioadenosine (30). The transfer of a methyl group from SAM should produce S-adenosylhomocysteine (SAH), which was detected in 3-fold excess as compared with the amount of methylselenilated RNA (36, 37).

The incorporation of selenide from the buffer and a methyl group from SAM suggests two plausible reaction sequences. One possibility is that the substrate is thiolated in a reaction similar to that catalyzed by BioB and LipA, and the resulting thiolated intermediate is subsequently capped with a methyl group from SAM in a traditional nucleophilic methyltrans-

ferase reaction. Alternatively, sulfide (or selenide) could bind to the auxiliary FeS cluster at the open coordination position and become capped with a methyl group to generate a methanethiolate (or methylselenolate) intermediate, which is then transferred to the substrate in a radical reaction. To explore these possibilities, the MiaB reaction was run in the presence of exogenous methylselenide, and the methylselenilated tRNA product contained ~9:1 selenium to sulfur in the modified base. Further, HYSCORE spectroscopy was carried out using \([^{77}\text{Se}]\)methylselenide and MiaB in which the radical SAM cluster had been eliminated by mutagenesis and the auxiliary FeS cluster had been reduced to a paramagnetic [4Fe-4S]\(^{2+}\) oxidation state using dithionite. The observation of a strong cross-coupling peak with a hyperfine interaction tensor of 3.8 MHz indicates that methylselenide is binding directly to the auxiliary cluster (37).

Further studies have subsequently been carried out with RimO, which accepts a 12-residue peptide substrate corresponding to Arg-83 to Tyr-95 of the ribosomal S12 protein. In the presence of SAM, but in the absence of the peptide substrate and a reductant, the enzyme produces 1 eq of SAH (36). When the assay is repeated with [methyl-\(^{14}\)C]SAM, 1 eq of radiolabel becomes associated with the enzyme in a manner that can be readily released by treatment with hydroxide or urea. GC-MS analysis was used to confirm the identity of the intermediate as methanethiol, which is formed in a 1:1 stoichiometry with SAH. When the reaction was supplemented with exogenous methanethiol together with [methyl-\(^{2}\)H\(_3\)]SAM, both unlabeled (from methanethiol) and \(^{2}\)H-labeled (from SAM) methylthiolated peptides were generated in an ~1:1 ratio, suggesting that exogenous methanethiol can compete with enzyme-generated methanethiol. Perhaps most informative, in contrast to the production of SAH, which occurs in the presence or absence of a reductant, the generation of 5'-dAH and the methylthiolated product both required the presence of a reductant.

The structure of *T. maritima* RimO has been solved (37) with two [4Fe-4S]\(^{2+}\) clusters but in the absence of SAM or the peptide or protein substrate (Fig. 2C). The overall structure contains three major domains. The radical SAM domain contains a [4Fe-4S]\(^{2+}\) cluster bound at the C-terminal end of a partial (\(\beta\)\(_{\alpha}\)) barrel, similar to the structural topology observed in the radical SAM enzymes HemN and MoaA. A second [4Fe-4S]\(^{2+}\) cluster is bound at the N-terminal end of a five-stranded \(\beta\) sheet in the UPF0004 domain. Finally, a small domain containing five \(\beta\) strands, which has homology with MiaB, has been termed the TRAM domain and is proposed to be involved with recognizing with the RNA or protein substrate. The two [4Fe-4S]\(^{2+}\) clusters are ~8 Å apart, and a chain of electron density is observed between these clusters that is modeled as a pentasulfide chain. This chain occupies space that is most likely occupied by SAM in the active enzyme, but it may show the likely binding site of sulfide or persulfide, as well as the methanethiol intermediate (Fig. 2D).

Together these data suggest the following reaction sequence (Fig. 3C). Sulfide or persulfide is bound at the unique iron of the auxiliary [4Fe-4S]\(^{2+}\) cluster. *In vivo* this sulfur may be deposited by the action of IscS or a similar cysteine desulfurase. SAM
MINIREVIEW: C–S Bond Formation by Radical SAM Enzymes

Several radical SAM enzymes that catalyze C–S bond formation have now been sufficiently characterized that a general theme has emerged. All of these enzymes have a second auxiliary FeS cluster that is crucial for the C–S bond formation reaction. In those enzymes where only a sulfur atom is introduced, this sulfur atom can be scavenged from the FeS cluster itself, but at the cost of low turnover rates due to the need for cluster repair following each catalytic cycle. In those enzymes that can generate or bind a substrate that already contains a sulfur atom, this sulfur atom can be activated through coordination to one iron site within a catalytic auxiliary FeS cluster.

The use of an auxiliary FeS cluster to help catalyze C–S bond formation serves two purposes. First, coordination of a thiol group such as cysteine to the FeS cluster eliminates the thiol proton, which could otherwise quench the substrate radical in a wasteful side reaction. Second, the FeS cluster allows rapid inner sphere electron transfer out of the sulfur-containing substrate or intermediate, which is essential to generate a stable electron-paired C–S bond using organic radical chemistry. Undoubtedly, there are likely to be many more radical SAM enzymes that catalyze C–S bond formation.
enzymes that generate C–S bonds, particularly in both ribosomal and nonribosomal peptide natural product pathways, but the general principles elucidated through the studies described above can serve as a roadmap that guides future work on other systems.

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