

SPASM and Twitch Domains in *S*-Adenosylmethionine (SAM) Radical Enzymes*

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ABSTRACT

***S*-adenosylmethionine (SAM, also known as AdoMet) radical enzymes use SAM and a [4Fe-4S] cluster to catalyze a diverse array of reactions. They adopt a partial (TIM) barrel fold with N- and C-terminal extensions that tailor the structure of the enzyme to its specific function. One extension, termed a SPASM domain, binds two auxiliary [4Fe-4S] clusters and is present within peptide-modifying enzymes. The first structure of a SPASM-containing enzyme, anaerobic sulfatase maturing enzyme (anSME), revealed unexpected similarities to two non-SPASM proteins, butirosin biosynthetic enzyme (BtrN) and molybdenum cofactor biosynthetic enzyme (MoaA). The latter two enzymes bind one auxiliary cluster and exhibit a partial SPASM motif, coined a Twitch domain. Here we review the structure and function of auxiliary cluster domains within the SAM radical enzyme superfamily.**

cysteine ligands to the iron atoms of the cluster, with the fourth ligand coming from the bidentate coordination of SAM to the unique iron (2,3). Direct ligation of SAM to the cluster facilitates reductive cleavage of the C-S bond through an inner sphere electron transfer event, forming methionine and a 5'-deoxyadenosyl radical (5'dAdo•) (Fig. 1A) (4). The abstraction of an H-atom from the substrate by 5'dAdo•, producing a substrate radical, ends the mechanistic similarity between enzymes of this superfamily; each enzyme utilizes a different mechanism to generate product. Structures of the first seven members of the SAM radical superfamily were used to define a core fold for binding SAM and for the generation of 5'dAdo• species. This core consists of a partial (β/α)₆ triose-phosphate isomerase (TIM) barrel (5). Outside of the core fold, the structure can vary greatly, with N- and C-terminal extensions that are functionalized for binding other cofactors or substrates. The SPASM subfamily is an example of a functionalized C-terminal extension for the binding of two auxiliary clusters.

Members of the *S*-adenosylmethionine (SAM) radical superfamily catalyze a wide variety of radical-mediated reactions, including complex chemical transformations and rearrangements; modifications of peptides; DNA, and RNA; dehydrogenations; and sulfur insertions (1). Despite this diversity, there are unifying structural and mechanistic themes. For instance, SAM radical enzymes typically bind a [4Fe-4S] cluster using a conserved CX₃CX ϕ C motif (where ϕ is an aromatic residue). This motif provides three

Haft and Basu recognized that enzymes with this C-terminal extension appear to be involved in the modification of ribosomally translated peptides (6,7). This subclass is referred to as SPASM after the biochemically characterized members, AlbA, PqqE, anSMEs, and MftC, which are involved in subtilosin A, pyrroloquinoline quinone, anaerobic sulfatase and mycofactocin maturation, respectively. The SPASM subfamily, accession TIGR04085, is composed of 281 sequences. However, recent similarity network analysis by

Babbitt and co-workers using the Structure Function Linkage Database (8) identified additionally related sequences, expanding the number to 1,380 (Fig. 1B).

Enzymes in this subfamily were expected to use a seven-cysteine (9) motif to bind two auxiliary [4Fe-4S] clusters, leaving a unique iron for substrate binding (7,10). However, the first and thus far only structure of a SPASM protein, the anaerobic sulfatase maturing enzyme (anSME) from *Clostridium perfringens*, showed full cysteine ligation to both [4Fe-4S] clusters (11). anSME was also the first structure of a SAM radical enzyme with dehydrogenase activity, and it, along with the structures of butirosin biosynthetic enzyme BtrN from *Bacillus circulans*, and molybdenum cofactor biosynthetic enzyme MoaA (12) (Fig. 2), revealed unexpected structural homology between SPASM and non-SPASM enzymes (13). In particular, the comparison of anSME, BtrN and MoaA led to the identification of a truncated SPASM domain used for binding a single auxiliary [4Fe-4S] cluster, which has been termed a Twitch domain (Fig. 1B)(11,13). This review discusses the function and architecture of the newly defined SPASM/Twitch subclass and explores the roles of auxiliary clusters in radical SAM chemistry with particular focus on the auxiliary clusters of this subclass. We suggest that SPASM and Twitch domains will constitute a larger subfamily than anticipated.

Function of SPASM/Twitch Subfamily

A chief function of this subfamily appears to be in the post-translational modification of peptides. Post-translational modifications expand the chemical repertoire of enzymes by generating modified amino acids that are well suited to perform specific reactions, priming peptides for cofactor biosynthesis, or rigidifying the protein or peptide (Fig. 2).

anSME catalyzes the co- or post-translational modification of an arylsulfatase active site cysteine or serine residue to a catalytically essential formylglycine (FGly) moiety in anaerobic organisms (10,14-17). The active site FGly allows sulfatases to perform their hydrolysis function, removing sulfate groups from a wide array of substrates (e.g. sulfated polysaccharides, sulfolipids, and steroid sulfates) (18-20). Sulfatase activity is important in humans

and bacteria with a lack of activity leading to disease in humans (21) and an inability to colonize the mucosal layer of the host's gut upon inhibition in bacteria (22). anSMEs are known to use SAM radical chemistry, in addition to two auxiliary [4Fe-4S] clusters, to perform the dehydrogenation of a serine or cysteine residue to the FGly moiety (Fig. 2). Until recently, very little was known about the structure and mechanism of anSME in comparison to the related formylglycine generating enzymes found in eukaryotes and aerobically-living prokaryotes. With the structural and mechanistic information on anSME from *C. perfringens* (11,17), our understanding of the anaerobic sulfatase maturing enzyme family is on the rise.

AlbA is involved in the post-translational modification of a linear peptide into the cyclic peptide natural product, subtilisin A. In particular, AlbA is responsible for forming three thioether bonds between the sulfur atoms of three cysteine residues and the α carbons of two phenylalanines and one threonine, yielding a rigidified peptide (Fig. 2) (23). Mutation of the corresponding gene, *alba*, indicates that AlbA is essential for subtilisin A biosynthesis and shows that thioether bond formation is a critical aspect of subtilisin A maturation (24). Peptide-derived natural products that contain thioether bonds, such as subtilisin A, are collectively known as sactipeptides. In terms of the medical relevance of this class of compounds, subtilisin A, which is produced by the soil bacterium *Bacillus subtilis*, shows antimicrobial activity against both Gram-negative and Gram-positive bacteria, as well as some human pathogens (25). Another sactipeptide produced by *B. subtilis*, sporulation killing factor, is excreted under nutrient limitation to lyse neighboring cells in what can only be described as cannibalistic behavior (26-28). A different SAM radical enzyme, SkfB, is responsible for the formation of the thioether bond of this sactipeptide (29).

PqqE is one of several enzymes that participate in the maturation of the bacterial redox cofactor pyrroloquinoline quinone (PQQ) from a peptide precursor (Fig. 2). In particular, PQQ is formed post-translationally from a skeleton peptide PqqA through complex rearrangements initiated by the cross-linking of glutamyl and tyrosyl sidechains (30,31). PQQ is then excised

from PqqA and attached to its target enzyme. PqqE is known to contain two [4Fe-4S] clusters and to reductively cleave SAM (32), and although it believed to be involved in an early step in PQQ biogenesis, the exact reaction catalyzed has not been identified (33).

BtrN, a 2-deoxy-*scyllo*-inosamine dehydrogenase, is a member of the Twitch structural subclass of SAM radical enzymes, and is involved in the biosynthesis of the aminoglycoside antibiotic butirosin B. BtrN is also a member of a recently described functional subclass of the SAM radical enzymes, SAM radical dehydrogenases, with the sulfatase maturases anSME and AtsB being the only other biochemically characterized members (10,17,34). BtrN contains a Twitch domain (13), which houses one auxiliary [4Fe-4S] cluster. Using SAM radical chemistry, BtrN catalyzes the oxidation of the C3 hydroxyl group of 2-deoxy-*scyllo*-inosamine by a H-atom abstraction, deprotonation and one-electron oxidation to produce the ketone group in 3- amino-2,3-dideoxy-*scyllo*-inosose (amino-DOI) (Fig. 2) (35,36).

The Twitch subclass enzyme MoaA, is involved in the biosynthesis of molybdopterin (Moco). MoaA catalyzes the first step in Moco biosynthesis, the complex rearrangement of guanosine triphosphate to 3'-8-*c*H₂GTP, which is then converted to Moco in subsequent steps (Fig. 2) (37,38). In humans, defects in molybdenum cofactor biogenesis leads to death shortly after birth, and these patients show neurological abnormalities including untreatable seizures and attenuated brain growth (39).

The SAM Radical Core Fold

SAM radical enzymes adopt a core fold, with exceptions described in a recent review (40) and below. This core is responsible for SAM binding and radical generation, and has been defined as a partial TIM barrel (β/α)₆ with six α -helices making up the outside of the partial barrel and the six parallel β -sheets forming the inner face of the barrel (Fig. 3A) (5). The active site is located within the lateral opening of the partial TIM barrel, and includes a [4Fe-4S] cluster, SAM and substrate binding sites. Although some SAM radical enzymes are composed of a complete TIM barrel (41,42), most have the partial barrel

architecture. Recently, the structural diversity of this enzyme superfamily was expanded when a significant deviation was reported for the queuosine biosynthetic enzyme 7-carboxy-7-deazaguanine synthase (QueE). QueE was found to have a hypermodified barrel fold in which three of the six α -helices are replaced by two loops and one 3₁₀ helix (43).

The [4Fe-4S] cluster motif, more popularly known as the CX₃CX ϕ C motif, binds the [4Fe-4S] cluster responsible for initiating radical chemistry. The three cysteines of this motif reside on the loop linking β 1 to α 1, termed the cluster-binding loop (Fig. 3A). This motif, which is largely but not absolutely conserved (42,43), allows for binding of SAM to the cluster through the open coordination site on the unique iron atom of the cluster, and through hydrophobic interactions from the aromatic residue (ϕ).

With respect to cluster binding, the largest deviations observed through crystallographic studies thus far are found in the SAM radical enzymes QueE, mentioned above, and ThiC, which is involved in the biosynthesis of thiamin pyrimidine moiety. In the former case, the cysteine motif has an eleven amino acid insertion between the first two cysteines (CX₁₄CX ϕ C) (43), and in the latter, the cluster-binding region is not part of the SAM radical core, but is instead found in a separate domain (42). For QueE, the 11-residue insert does not alter the way in which SAM binds, and is instead believed to be important for binding of the physiological reductase (43). For ThiC, no structure is available of the fully reconstituted protein and thus the exact mode of cluster and SAM binding remains to be determined.

SAM binding is facilitated by four different motifs, some of which are more highly conserved than others. The "GGE motif", named after residues in pyruvate formate lyase activating enzyme and MoaA, resides at the C-terminal end of β 2 and interacts with the amino group of SAM, helping to orient the methyl group of the SAM. Interactions between residues from β 4, called the "ribose motif", and the hydroxyls of the ribose moiety appear to play an important role in positioning of 5'dAdo• with respect to substrate for H-atom abstraction (44). The "GXIXGXXE motif", which is only partially conserved in terms of sequence, is located on β 5 and provides

hydrophobic interactions with the adenine ring of SAM. Finally, the $\beta 6$ motif is responsible for hydrogen bonding via backbone atoms to the adenine ring. Interestingly, the structure of BtrN shows that residues at the end of $\beta 5$ can replace the function of the $\beta 6$ motif. BtrN thus contains both the “GXIXGXE” motif and the $\beta 6$ motif on $\beta 5$ (13). We suggest that the $\beta 6$ motif be renamed the “adenine binding motif.” Taken together, all of these motifs, which span across the SAM radical core, work together to ensure that SAM is positioned in the correct orientation for H-atom abstraction from the substrate after homolytic cleavage.

SPASM/Twitch Domain Fold

In addition to the core fold, SAM radical enzymes contain N- or C-terminal extensions. The SPASM domain at the C-terminal end of the core fold binds two auxiliary [4Fe-4S] clusters using a conserved seven cysteine motif, CX_9 .₁₅ $GX_4X_nCX_2CX_5CX_3CX_nC$ (9). Based on the reactions performed by the biochemically characterized SPASM members and the presence of only seven cysteine residues in the conserved cysteine motif, an open coordination site on one auxiliary [4Fe-4S] cluster has been proposed to ligate the peptide substrate (7,10). Through the work of Goldman *et al.* (11), we now have a snapshot of one of these enzymes, anSME, both with and without substrate bound. This structure shows that both clusters of anSME are fully ligated by cysteine residues, with auxiliary cluster I (Aux I) 16.9 Å from the SAM radical cluster and 12.9 Å from the second auxiliary cluster (Aux II) (Fig. 3B,E). Aux I is ligated by four cysteine residues: one before the predicted SPASM domain seven cysteine motif, two within the motif, and one downstream cysteine. A conserved β -hairpin motif lies between the second and third coordination sites of Aux I, with an alpha helix following ($\alpha 2'$). This helix leads to the second auxiliary cluster (Aux II) (Fig. 3B). Aux II is coordinated by four cysteine residues of the seven cysteine motif, but not in the same order as the primary sequence. Unexpectedly, the protein chain travels back and forth between the two auxiliary clusters such that cysteines 4, 5, 6, and 8 coordinate Aux II whereas cysteine 7 is the downstream cysteine ligand to Aux I (Fig. 3B).

Based on sequence homology, it is predicted that this cluster-binding architecture will be a common feature of SPASM domain-containing enzymes (7,11,13), with the caveat that some SPASM proteins may not display full cysteine ligation.

The protein topology around Aux I of anSME shows structural similarity to the auxiliary cluster domain of the non-SPASM SAM radical enzyme MoaA. MoaA also displays a ~ 16 Å distance between its SAM radical cluster and its auxiliary cluster and adopts an abridged SPASM domain architecture (Fig. 3C,F). This abridged SPASM fold provides three cysteine ligands to bind one [4Fe-4S] cluster, and contains the β -hairpin motif and $\alpha 2'$, all of which are found in the first half of the SPASM domain. Based on the similarity between anSME and MoaA, this abridged SPASM domain was subsequently coined the Twitch domain (11,13). MoaA contains no cysteine ligands following $\alpha 2'$, and the resulting open coordination site on the [4Fe-4S] cluster is used for substrate binding (45) as was originally proposed for the SPASM enzymes.

BtrN also contains a Twitch domain that binds one [4Fe-4S] cluster similar to the auxiliary cluster-binding domain of MoaA. However, the auxiliary cluster in BtrN is fully ligated (13), showing the versatility of the Twitch domain. Like anSME and MoaA, the auxiliary cluster of BtrN is ~ 16 Å from the SAM radical cluster (Fig. 3D,G) and has a domain architecture made up of a β -hairpin followed by $\alpha 2'$, with cysteine ligands flanking both ends of the β -hairpin. Similar to anSME, cysteine residues following $\alpha 2'$ ligate an auxiliary cluster. Unlike anSME and MoaA, BtrN only has one cysteine ligand before the β -hairpin (Fig. 3D). Thus, both the Aux I of anSME and the Aux cluster of BtrN have full ligation by cysteine residues, although the cysteine positions in the primary sequence are different.

Overall, these structural snapshots show two different coordination modes of [4Fe-4S] clusters in Twitch domains, one in which the [4Fe-4S] cluster can bind substrate directly to an open coordination site (MoaA) and one in which it cannot (BtrN). They also reveal two different varieties of SAM radical dehydrogenases, one with one auxiliary [4Fe-4S] cluster (BtrN) and one with two auxiliary clusters (anSME).

Given the structural homology between anSME, BtrN and MoaA, we propose that the entire area of sequence space shown in Fig. 1B be considered its own SAM radical enzyme subclass, encompassing ~15% of the enzymes currently identified as belonging to the SAM radical superfamily in the Structure Linkage Database (8). The three available structures of SPASM/Twitch proteins represent the three edges of sequence space and thus exemplify the structural diversity within this subclass.

Roles of Auxiliary Clusters in the SPASM/Twitch Subclass

Recent structural data has served to clarify the function(s) of auxiliary clusters in SPASM/Twitch enzymes (11,13). For anSME and BtrN, crystal structures show that auxiliary clusters are fully ligated both in the presence and absence of substrates, and that substrates are bound through protein-mediated hydrogen bonding interactions at distances of 9-10 Å away from their auxiliary clusters (Fig. 4A, B). These long distances also suggest that auxiliary clusters do not function in substrate deprotonation. For anSME, both crystallographic and mutagenesis data support Asp277 as being responsible for the deprotonation step (11). In particular, Asp277 is adjacent to the substrate and its mutation to Asn results in a protein with only 0.8% of wild-type activity and increased uncoupling of SAM cleavage from product formation (11). For BtrN, Arg152 has been proposed to play a role in substrate deprotonation based solely on its location in the active site (13). Instead of binding or deprotonating substrate, the observed 9-10 Å distances between cluster and substrate are consistent with a role for the auxiliary clusters as electron acceptors during substrate oxidation. There is diversity in the number of auxiliary clusters needed for substrate oxidation. anSME has two auxiliary clusters (12.9 Å apart) to provide a route for the electron from the buried active site to the protein surface, whereas BtrN only needs one auxiliary cluster for the electron to reach the protein surface, where it would be accessible to an external electron acceptor.

The extent to which SPASM/Twitch enzymes will display full ligation of their auxiliary clusters is not known at this time. Current sequence data suggests that variations will occur.

AlbA, for example, does not contain an eighth cysteine in its C-terminal auxiliary cluster domain. As more structures become available, it will be very interesting to discover how many of these enzymes, if any, i) do indeed have an open iron coordination site for substrate ligation, ii) use a non-cysteine residue for ligation of an auxiliary cluster, as in biotin synthase (BioB), which has an arginine ligand (41), and/or iii) use a cysteine that is distal in primary sequence from its SPASM motif to provide the last auxiliary cluster ligand.

Roles of Auxiliary Clusters in Other SAM Radical Enzymes

A number of auxiliary cluster-containing non-SPASM/non-Twitch SAM radical enzymes have been identified and the roles of their auxiliary clusters are under investigation. BioB, for example, has been proposed to use its auxiliary [2Fe-2S] in a sacrificial manner, donating one of its sulfur atoms to perform a sulfur insertion reaction during biotin formation (41,46-48). Similarly, lipoyl synthase (LipA) contains an auxiliary [4Fe-4S] cluster that is proposed to be the source of the two sulfur atoms needed to form lipoic acid (49-51). Methylthioltransferases (MTTases), as their name suggests, transfer a methylthiol group to macromolecular substrates including the S12 ribosomal protein (catalyzed by RimO) and tRNA-A37 (catalyzed by MiaB). Methylthioltransferases also have auxiliary clusters (52,53), which, unlike for BioB and LipA, are not currently believed to serve as the source of sulfur for generation of the methylthiol group (54). Instead, a recent crystal structure of RimO has led to the proposal that its auxiliary cluster is responsible for binding a polysulfide moiety that serves as the sulfur source (55). set of enzymes is still in its infancy although hydrogenase cofactor maturation is an active area of research.

Predictions

The structures of the SPASM protein anSME and of the Twitch proteins BtrN and MoaA allow us to make predictions about other enzymes in the SPASM/Twitch subclass for which there are no structures. As mentioned above, AlbA and SkfB catalyze the internal peptide linkage of a cysteine sidechain sulfur to a C α position in the maturation of two natural products generated from ribosomally translated peptide scaffolds, subtilosin

A and sporulation killing factor (Fig. 2). Following H-atom abstraction from the C α position, formation of a covalent bond with cysteine requires a one electron oxidation and deprotonation of its sulfur. The parallels in this system to the dehydrogenases (deprotonation and one electron oxidation) again made the direct ligation of substrate to an auxiliary cluster an attractive hypothesis (23,29). AlbA contains seven cysteines in a SPASM motif, whereas SkfB contains five cysteine residues in a C-terminal domain. Using anSME as a model for SPASM proteins and BtrN for Twitch proteins, we predict that AlbA's structure will parallel that of anSME, and SkfB will contain a Twitch domain similar to MoaA and BtrN. If these predictions are correct, then both SAM radical dehydrogenases and thioether-bond forming enzymes will use both the SPASM and Twitch enzyme architectures to catalyze analogous reactions.

We can also use the recent structural data on this enzyme subclass to consider whether AlbA and SkfB will use an auxiliary cluster to bind their substrate. Because the distances between the SAM cluster and the Aux cluster (~16 Å) as well as the SAM binding motifs appear to be conserved in this subclass, we can predict for AlbA and SkfB that the distance between clusters will be ~16 Å, and that SAM will bind in a similar fashion as observed previously (5). The structures of anSME, BtrN, and MoaA also reveal similar distances (9.3 Å on average) between the H-atom abstraction site on the substrate and the Aux cluster (9.6, 8.6, and 9.6 Å in BtrN, anSME, and MoaA, respectively) (Fig. 4A-C). For BtrN and anSME, these long distances were used in part to make the argument that the Aux cluster was unlikely to be involved in the substrate deprotonation step that follows the H-atom abstraction (11,13). In MoaA, where a molecule of GTP is both the H-atom abstraction site (3' hydroxyl) (37) and the auxiliary cluster ligand (N1 of guanosine base) (56), this separation (7.4 Å) is both intramolecular and distal. Based on these numbers, if AlbA or SkfB peptidyl-substrate binds such that one substrate residue (glycine in Fig. 4D) is close enough to SAM for H-atom abstraction and a substrate cysteine is close enough to the auxiliary cluster for ligation, the distance between the glycine C α and cysteine S would be at least 6 Å, which is too long for

covalent bond formation. Although structures of AlbA and SkfB will be essential to resolve this question, our current structural data on anSME and BtrN predict that the auxiliary cluster of AlbA and SkfB will play a role in oxidation of substrate and not in its binding. Intriguingly, the peptidyl-substrate of anSME binds in a tight turn (Fig. 4B), perhaps foreshadowing how AlbA and SkfB bind their peptidyl substrate to afford covalent linkage between two residues of the same peptide.

We can contrast these predictions with auxiliary cluster-containing SAM radical enzymes in different subfamilies where auxiliary clusters are believed to be involved in more than accepting an electron. For example, in the sulfur insertion enzyme BioB, the auxiliary [2Fe-2S] cluster is thought to play a direct role in the conversion of dethiobiotin (DTB) to biotin by donating a sulfur atom and accepting an electron from the resulting mercapto intermediate (57). This cluster is highly unusual in that it is positioned in the middle of a TIM barrel, ligated by three cysteines residing from β 2, β 3, and β 5 strands of the SAM radical core fold, and an unprecedented arginine residue contributed by the C-terminal region (41). The distance between this Aux cluster and the SAM radical cluster in BioB is 12 Å, ~4 Å closer than for BtrN, anSME and MoaA. Also, the distance between the H-atom abstraction site at the C9 position of DTB and the Aux cluster is 4.6 Å, ~5 Å closer than for the SPASM/Twitch proteins (Fig. 4E). The structure of the methylthioltransferase RimO was also recently solved (55). As mentioned above, this structure has led to the hypothesis that the Aux cluster is not the direct sulfur source but may bind the sulfur source (55). Interestingly, the cluster-to-cluster distance in RimO is 8.4 Å, which is even closer than in BioB (12.0 Å), and twice as close as in the SPASM/Twitch architecture (Fig. 4F). Thus, the structures of the Aux-cluster subset of SAM radical enzymes that are emerging show variation in cluster-to-cluster distance, in function (sulfur source (BioB), substrate binding (MoaA), and substrate oxidation (anSME, BtrN)), and in fold (both SPASM/Twitch and non-SPASM/Twitch). We have just scratched the surface of the structural exploration of this amazing superfamily, and more surprises likely await us.

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FOOTNOTES

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²The abbreviations used are: SAM, S-adenosylmethionine; 5′dAdo•, 5′-deoxyadenosyl radical; TIM, triose-phosphate isomerase; anSME, anaerobic sulfatase maturing enzyme; FGly, formylglycine; PQQ, pyrroloquinoline quinone; DOIA, 2-deoxy-*scyllo*-inosamine; BtrN, 2-deoxy-*scyllo*-inosamine dehydrogenase; MoaA, molybdenum cofactor biosynthetic enzyme; MPT, molybdopterin; 3′-8-*c*H₂GTP, (8*S*)-3′,8-*cyclo*-7,8-dihydroguanosine 5′-triphosphate; QueE, 7-carboxy-7-deazaguanine synthase; MTTases, methylthioltransferases; DTB, dethiobiotin; BioB, biotin synthase; LipA, lipopyl synthase.

FIGURE LEGENDS

Figure 1: The SAM radical SPASM/Twitch subfamily similarity network. **A.** General mechanism for reductive cleavage of SAM by inner sphere electron transfer from the [4Fe-4S] to the C-S bond, forming a 5′-deoxyadenosyl radical and methionine. **B.** Protein Sequence Similarity Network (58) of the SPASM/Twitch domain containing proteins of the SAM radical superfamily. The nodes represent protein sequences with 40% sequence identity at an E-value (Expectation or Expect value) of 1×10^{-22} . Proteins with SPASM domains map to 153 nodes (green dots). This figure was generated using Cytoscape (59).

Figure 2: SPASM/Twitch subfamily reactions. The involvement of SPASM/Twitch domain containing enzymes, anSME, MoaA, BtrN and AlbA/SkfB in the biosynthesis of their respective products. The specific reaction catalyzed by SPASM enzyme PqqE is not indicated as its substrate is not known. For AlbA, only one of the three thioether bond formations is shown. H-atoms known or proposed to be abstracted by the 5′-deoxyadenosyl radical are shown in blue. DOIA, 2-deoxy-*scyllo*-inosamine; amino-DOI, 3-amino-2,3-dideoxy-*scyllo*-inosose

Figure 3: Folds of SPASM/Twitch subfamily members. **A.** Topology of SAM (β/α)₆ core fold (magenta). Cluster cysteine ligands are indicated as yellow spheres. **B.** General topology of a SPASM domain-containing SAM radical enzyme. The SPASM domain (green), at the C-terminus of the SAM radical core, binds two [4Fe-4S] clusters. **C.** Topology of a Twitch domain-containing SAM radical enzyme with an open iron coordination site for substrate (yellow circle). **D.** Topology of a Twitch domain-containing SAM radical enzyme with a fully ligated auxiliary cluster. **E-G.** Ribbon representations of the crystal structures of anSME, MoaA, and BtrN, respectively. The SAM core is indicated in magenta followed by the SPASM or Twitch domains in green. The SAM to Aux I cluster distances for these enzymes, as well as the Aux I to Aux II distance for anSME are shown as black dashed lines.

Figure 4: Auxiliary clusters positions in SPASM/Twitch enzymes. Distances shown as follows: SAM cluster to Aux cluster (green dashed lines); H-atom abstraction position on substrate to Aux cluster (black dashed lines); and H-atom abstraction position to C5′ of SAM (purple dashed lines). **A.** BtrN, DOIA, 2-deoxy-*scyllo*-inosamine. **B.** anSME with peptide bound (green). **C.** MoaA. **D.** Hypothetical AlbA model estimating distances between the site of H-atom abstraction on a peptide glycine to the site of thioether

bond formation on the sulfur of a peptide cysteine (red dashed line) and to the Aux cluster (blue dashed line). E. BioB with dethiobiotin (DTB) bound, and F. RimO.

FIGURES

Figure 1.

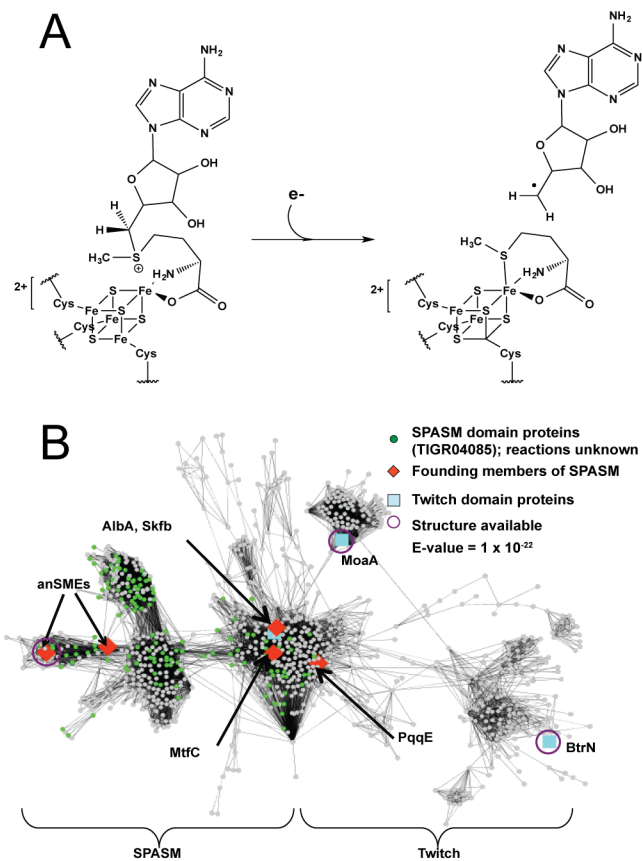


Figure 2.

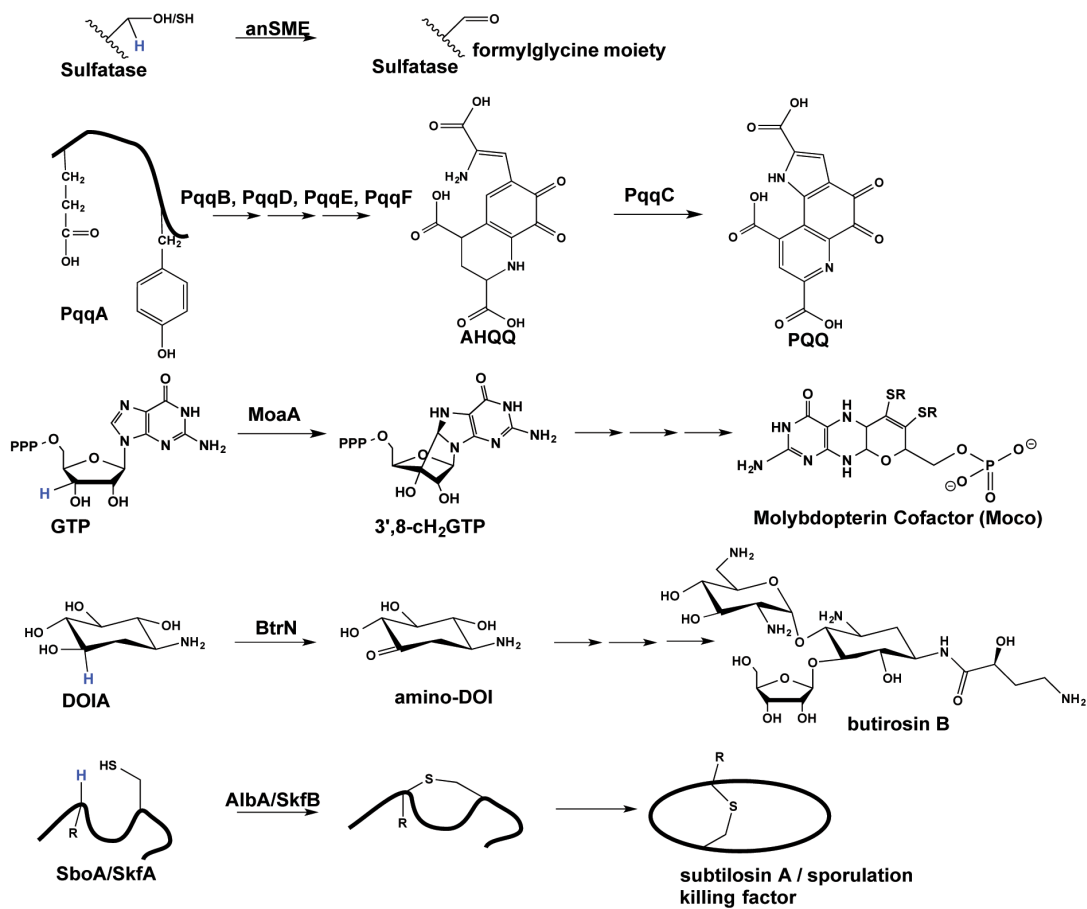


Figure 3.

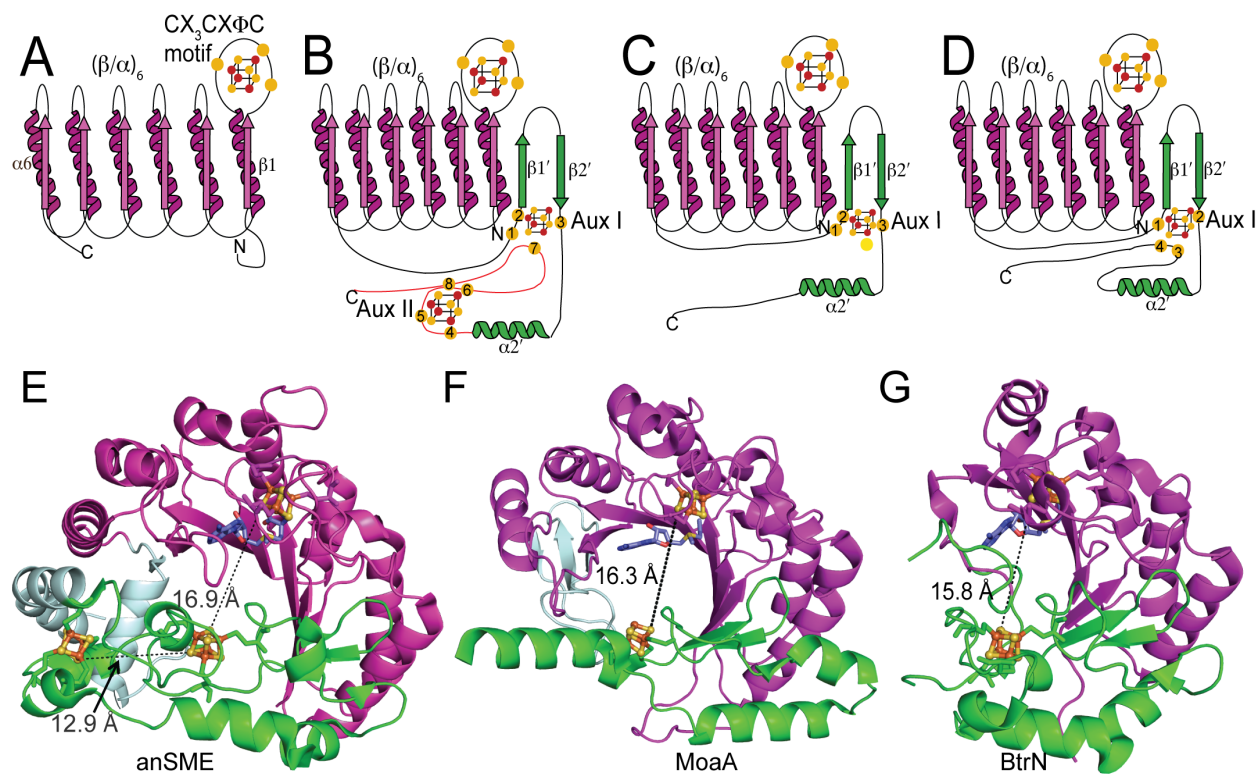


Figure 4.

