SUPPORTING INFORMATION

Supporting Tables and Figures

Table S1. Primers used for all cloning described in the Experimental Procedures. The bold and underlined nucleotides indicate the codon mutated to alanine.

Primer Name	Sequence
C104A	Forward 5′-gcctgaacgtcgcccacgac**gct**aatctgcgctgcaaatattgttttgc-3′
	Reverse 5′-gcaaaacaatatttgcagcgcagatt**agc**gtcgtgggcgacgttcaggc-3′
C104/108/111A	Forward 5′-cccacgac**gct**aatctgcgc**gcc**aaaatat**gct**ttgccagcaccggtaatttcaagg-3′
	Reverse 5′-ccttgaaattaccggtgctggcaaa**agc**atattt**ggc**gcgcagatt**agc**gtcgtggg-3′
C400A	Forward 5′-cgacatctataaacgtgaagaa**gcc**gcaaattgttgggctcgc-3′
	Reverse 5′-gcgagcccaacaatttgc**ggc**ttcttcacgtttatagatgtcg-3′
C409A	Forward 5′-gcaaattgttgggctcgcttttat**gcc**tctggcggttgtttcgc-3′
	Reverse 5′-gcgaaacaaccgccaga**ggc**ataaaagcgagcccaacaatttgc-3′
C400/403/409A	Forward 5′-cgtgaagaa**gcc**gcaaat**gct**tgggctcgcttttat**gcc**-3′
	Reverse 5′-**ggc**ataaaagcgagccca**agc**atttgc**ggc**ttcttcacg-3′
C344A	Forward 5′-gcatcaaaaagcgcctgcagggc**gct**ggtgcaggcttcgaatatattgc-3′
	Reverse 5′-gcaatatattcgaagcctgcacc**agc**gccctgcaggcgctttttgatgc-3′
C362A	Forward 5′-cggatgaagaaatctacccg**gcc**caccaatttgttggtattgaagaattcaaactgg-3′
	Reverse 5′-ccagtttgaattcttcaataccaacaaattggtg**ggc**cgggtagatttcttcatccg-3′
C413A	Forward 5′-cgcttttattgctctggcggt**gct**ttcgcaaacaactacaacatcaacgg-3′
	Reverse 5′-ccgttgatgttgtagttgtttgcgaa**agc**accgccagagcaataaaagcg-3′
C409/413A	Forward 5′-cgcttttat**gcc**tctggcggt**gct**ttcgcaaacaactacaacatcaacgg-3′
	Reverse 5′-ccgttgatgttgtagttgtttgcgaa**agc**accgccaga**ggc**ataaaagcg-3′

Table S2. Monoisotopic masses corresponding to the peaks of the various charge states with the associated ppm error from the mass spectrum of unmodified Tte1186a (**Fig. 4A, black**) and modified Tte1186a (**Fig. 4A, red**) isolated from the reaction not treated with iodoacetamide. The masses observed in the spectrum of unmodified Tte1186a were consistent with the peptide containing six reduced cysteine residues, while the observed masses in the spectrum of modified Tte1186a were consistent with the loss of two hydrogen atoms.

	Unmodified Tte1186a (black spectrum)			**Modified Tte1186a (red spectrum)**		
Charge State	**Observed $[M+nH]^{n+}$**	**Calculated $[M+nH]^{n+}$**	**Error (ppm)**	**Observed $[M+nH]^{n+}$**	**Calculated $[M+nH]^{n+}$**	**Error (ppm)**
4	1777.8408	1777.8419	0.62	1777.3321	1777.3380	3.32
5	1422.4745	1422.4750	0.35	1422.0676	1422.0719	3.02
6	1185.5631	1185.5637	0.51	1185.2243	1185.2278	2.95
7	1016.3408	1016.3414	0.59	1016.0506	1016.0534	2.76
8	889.4245	889.4246	0.11	889.1708	889.1726	2.02
9	790.7117	790.7116	0.13	790.4855	790.4876	2.66
10	711.7401	711.7411	1.41	711.5355	711.5396	5.76

Table S3. Monoisotopic masses corresponding to the peaks of the various charge states with the associated ppm error from the mass spectrum of unmodified Tte1186a (**Fig. 4B, black)** or modified Tte1186a (**Fig. 4B, red)** isolated from reactions that were subsequently treated with iodoacetamide. The masses of the peaks in the spectrum of unmodified Tte1186a were consistent with Tte1186a containing six carbamidomethylated cysteine residues, while the masses in the spectrum of modified Tte1186a were consistent with peptide missing two hydrogen atoms and containing only five carbamidomethylated cysteine residues.

	Unmodified Tte1186a (black spectrum)			**Modified Tte1186a (red spectrum)**		
Charge State	**Observed $[M+nH]^{n+}$**	**Calculated $[M+nH]^{n+}$**	**Error (ppm)**	**Observed $[M+nH]^{n+}$**	**Calculated $[M+nH]^{n+}$**	**Error (ppm)**
4	1863.3894	1863.3741	8.21	1848.6252	1848.6148	5.63
5	1490.9087	1490.9007	5.37	1479.0985	1479.0933	3.52
6	1242.5912	1242.5852	4.83	1232.7507	1232.7456	4.14
7	1065.2221	1065.2169	4.88	1056.7865	1056.783	3.31
8	932.1949	932.1907	4.51	924.8140	924.8110	3.24
9	828.7314	828.7259	6.64	822.1693	822.1662	3.77
10	745.9575	745.9540	4.69	740.0540	740.0503	5.00

Table S4. Comparison of the observed and calculated monoisotopic masses of the various charge states of each *b*- or *y*-ion and the associated ppm error from the MS/MS fragmentation of the + 8 charge state of unmodified Tte1186a (**Fig. 5A**) and modified Tte1186a (**Fig. 5B**). The fragments corresponding to *b*51 through *b*54 and *y*10 through *y*13 were not detected (n.d.) in the CID fragmentation mass spectrum of the modified peptide. The (*) indicates the calculated *m/z* corresponds to the theoretical mass of the Tte1186a peptide minus two hydrogens and one carbamidomethyl group relative to the unmodified peptide.

		unmodified Tte1186a (black)			**modified Tte1186a (red)**		
Ion	**Charge**	**Observed *m/z***	**Calculated *m/z***	**Error (ppm)**	**Observed *m/z***	**Calculated *m/z***	**Error (ppm)**
*b*5	1				506.2117	506.2106	2.2
*b*5 -H_2O	1				488.2012	488.2001	2.3
*b*6	1				643.2711	643.2695	2.5
*b*7	1				780.3304	780.3284	2.5
*b*7 -H_2O	1				762.3197	762.3179	2.5
*b*8	2				459.1984	459.1973	2.3
*b*9	2				527.7280	527.7268	2.3
*b*10 -H_2O	2				562.2388	562.2375	2.4
*b*21	3				773.7043	773.7022	2.6
*b*36	5				817.2419	817.2399	2.5
*b*42	6				785.9136	785.9118	2.4
*b*44	6				833.9286	833.9266	2.4
*b*45	6	845.7674	845.7661	1.5	845.7682	845.7661	2.4
*b*46	6				860.2737	860.2715	2.6
*b*46 -H_2O	6				857.2719	857.2697	2.6
*b*47	6				886.9455	886.9433	2.5
*b*48	7				778.6771	778.6751	2.6
*b*48	6	908.2870	908.2864	0.7			
*b*49	7	791.1088	791.1082	0.8	791.1100	791.1082	2.3
*b*49 -H_2O	7				788.5375	788.5353	2.8
*b*50	7	801.2584	801.2564	2.5	801.2581	801.2564	2.2
*b*50	6	934.6336	934.6312	2.6			
*b*50	5				1121.3591	1121.3560	2.8
*b*50 -H_2O	7	798.6805	798.6834	3.6	798.6854	798.6834	2.4
*b*51	7	824.1167	824.1179	1.5	n.d.	815.8280*	
*b*52	7	842.4186	842.4172	1.7	n.d.	834.1273*	
*b*52 -NH_3	7	839.9870	839.9848	2.6	n.d.	831.6949*	
*b*53	7	856.8540	856.8526	1.6	n.d.	848.5627*	
*b*53 -H_2O	7	854.2805	854.2796	1.1	n.d.	845.9897*	
*b*54	7	869.2875	869.2857	2.1	n.d.	860.9958*	
*b*54 -H_2O	7	866.7145	866.7128	2.0	n.d.	858.4229*	
*b*55	7	892.1492	892.1472	2.2	883.7172*	883.7133*	4.4

*b*55	6	1040.6715	1040.6705	1.0			
*b*55 -H_2O	7	889.5768	889.5743	2.8			
*b*56	7	906.5846	906.5826	2.2	898.1514*	898.1487*	3
*b*56	6	1057.5139	1057.5118	2.0	1047.6749*	1047.6723*	2.5
*b*56 -H_2O	7	904.0114	904.0097	1.9	895.5783*	895.5758*	2.8
*b*56 -H_2O	6	1054.5123	1054.5101	2.1	1044.6731*	1044.6705*	2.4
*b*57	7	920.7371	920.7352	2.1	912.3034*	912.3014*	2.3
*b*57	6	1074.0251	1074.0232	1.8	1064.1862*	1064.1837*	2.3
*b*57 -H_2O	7	918.1632	918.1623	1.0	909.7306*	909.7284*	2.4
*b*57 -NH_3	6				1061.3516*	1061.3459*	5.3
*b*57 -H_2O	6	1071.0231	1071.0215	1.5			
*b*58	6	1083.5285	1083.5268	1.6	1073.6898*	1073.6873*	2.3
*b*59	7	945.1748	945.1730	1.9	936.7413*	936.7391*	2.3
*b*59	6	1102.5367	1102.5340	2.4	1092.6972*	1092.6944*	2.6
*b*59 -NH_3	7	942.7431	942.7406	2.7			
*b*60	8				835.7823*	835.7800*	2.8
*b*60	7	963.4687	963.4671	1.7	955.0355*	955.0332*	2.4
*b*60	6	1123.8801	1123.8771	2.7	1114.0402*	1114.0375*	2.4
*b*60 -H_2O	6				1111.0382*	1111.0358*	2.2
*b*60 -NH_3	7	961.0365	961.0347	1.9	952.6056*	952.6008*	5
*b*61	8				851.9124*	851.9103*	2.5
*b*61	7	981.9034	981.9017	1.7	973.4703*	973.4679*	2.5
*b*61	6	1145.3859	1145.3842	1.5	1135.5473*	1135.5446*	2.4
*b*61 -NH_3	7	979.4731	979.4694	3.8			
*b*61 -H_2O	7				970.8971*	970.8949*	2.2
*b*61 -NH_3	8				849.7847*	849.7820*	3.3
*b*61 -NH_3	6				1132.7105*	1132.7069*	3.2
*b*62	7				996.3316*	996.3294*	2.2
*b*62 -NH_3	8				869.7894*	869.7858*	4.1
*b*62 -NH_3	7	1002.3337	1002.3309	2.8	993.9017*	993.8970*	4.7
*b*63	8	895.3010	895.3011	0.1	887.9237*	887.9214*	2.5
*b*63	7	1023.0596	1023.0573	2.2	1014.6258*	1014.6235*	2.3
*b*63	6				1183.5623*	1183.5595*	2.4
*b*63 -NH_3	8				885.7976*	885.7931*	5
*b*63 -NH_3	7	1020.6293	1020.6250	4.2	1012.1959*	1012.1911*	4.8
*b*64	8	915.6858	915.6840	2.0	908.3070*	908.3044*	2.9
*b*64	7				1037.9208*	1037.9182*	2.5
*b*64 -H_2O	8	913.4333	913.4327	0.7	906.0557*	906.0530*	2.9
*b*64 -NH_3	7				1035.4890*	1035.4859*	3.1
*y*2	1	296.1246	296.1241	1.7	296.1248	296.1241	2.5
*y*2 -NH_3	1				279.0982	279.0975	2.4
*y*3	1	424.1835	424.1827	1.9	424.1837	424.1827	2.4
*y*3 -H_2O	1	406.1729	406.1721	2.0			

*y*4	1	584.2144	584.2133	1.9	584.2147	584.2133	2.4
*y*4 -H_2O	1	566.2036	566.2028	1.4			
*y*4 -NH_3	1	567.1878	567.1868	1.8	567.1880	567.1868	2.1
*y*5	1	713.2574	713.2559	2.1	713.2577	713.2559	2.5
*y*5 -H_2O	1	695.2467	695.2454	1.9	695.2470	695.2454	2.3
*y*5 -NH_3	1				696.2311	696.2294	2.5
*y*6	1	841.3160	841.3145	1.8	841.3165	841.3145	2.4
*y*6 -H_2O	1	823.3059	823.3039	2.4	823.3061	823.3039	2.6
*y*6 -NH_3	1				824.2901	824.2879	2.6
*y*7	1	955.3593	955.3574	2.0	955.3600	955.3574	2.7
*y*8	1	1012.3810	1012.3789	2.1	1012.3814	1012.3789	2.5
*y*8 -H_2O	1				994.3708	994.3683	2.5
*y*8 -NH_3	1	995.3537	995.3523	1.4	995.3549	995.3523	2.6
*y*9	1	1111.4498	1111.4473	2.2	1111.4502	1111.4473	2.6
*y*10	1	1212.4981	1212.4950	2.6	n.d.	1211.4872*	
*y*11	1	1372.5291	1372.5256	2.6	n.d.	1371.5178*	
*y*12	1	1459.5605	1459.5577	1.9	n.d.	1458.5498*	
*y*13	1	1560.6111	1560.6053	3.7	n.d.	1559.5975*	
*y*15	2				895.3530*	895.3506*	2.8
*y*19	2				1118.4328*	1118.4297*	2.8
*y*20	2				1161.9490*	1161.9458*	2.8
*y*53 -H_2O	7	879.1475	879.1405	8.0			
*y*61 -H_2O	8				876.5478*	876.5417*	6.9
*y*61 -H_2O	7	1010.0576	1010.0519	5.6	1001.6247*	1001.6180*	6.7

Table S5. *g*-values of the EPR spectra determined by the data simulations.

Variant	SAM	g_1	g_2	g_3	σ1	σ2	σ3
RC	–	2.0405	1.9190	1.9055	0.0350	0.0324	0.0579
RC	+	1.9925	1.8950	1.8400	0.0190	0.0280	0.0350
AC1a	+/–	2.0631	1.9322	1.8799	0.0712	0.0270	0.1145
AC1b	+/–	2.0221	1.9509	1.900	0.0274	0.0166	0.0354
AC2a	+/–	2.0500	1.9255	1.8865	0.0470	0.0367	0.0556
AC2b	+/–	2.0850	1.9397	1.8672	0.0330	0.0152	0.1197

Figure S1. *Caldanaerobacter subterraneus* subsp. *tengcongensis* MB4 *tte1186* (**a**) and *tte1186a* (**c**) nucleotide sequences optimized for recombinant expression in *E. coli*. The corresponding protein sequences for Tte1186 and Tte1186a are shown in (**b**) and (**d**) respectively. The nucleotide sequences in bold represent the *Nde*I and *Xho*I restriction endonuclease sites that were used to clone the gene into pET28a. The start and stop codons are shown in italic. The underlined sequence corresponds to the His_6 purification tag.

a) *ATG*GGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGC**CATATG**TCGGC
TACCATGCATAAGTTTAAGCGTCTGGGCCTGAATATCGTTGTTGATCCGGTTTCGGGCGCAATCCACG
TCGTTGATGACGTGGTTTACGATGTCCTGGACTATTACGAAAACCATAGTCGTGAAGAAATCGTTAAT
CTGCTGAAGGATAAGTACAAAGAAGAAGACATCCTGGAAGCGATCTCCGAAGTGGATGAACTGAAGGG
CAACGGTCTGCTGTTTACCGAAGATATTTACAAAGACATTGCGATCAGTCGTGCCGATTCCGTTATCA
AAGCGATGTGCCTGAACGTCGCCCACGACTGTAATCTGCGCTGCAAATATTGTTTTGCCAGCACCGGT
AATTTCAAGGGCGGTCGTAAACTGATGGATTTTGAAACGGGTCGCAAAGCGATTGACTTCCTGATCAA
GAGCTCTGGCAAACGTCGCAACATTGAAATCGATTTCTTTGGCGGTGAACCGCTGCTGAATTTTGAAG
TCGTGAAACAGCTGGTTGAATACGGCAAGCAAAAAGCGAAGGAAAACAAAAAGAATATCAAGTTCACC
ATCACCACGAACGCCGTTCTGCTGGATGACGAAAAGATCGAATACTTCAACGAAAACTTCTCAAACGT
TGTCCTGTCGCTGGATGGCCGCAAAGAAGTCAATGACCAGATGCGTGTGCGTGCAGATGGTAGCGGCA
CGTACGACGTCATTGTGCCGAAAATCCAAAAGTTTGTGAAAGCGCGTGGTAAAAAGGAATATTACGTT
CGCGGCACCTTTACGGCGAAAAATCTGGATTTCGTTGAAGACGTCCTGCATATTGCCGATCTGGGTGT
GTATGAAATCTCTGTTGAACCGGTGGTTGAAAAGGATGACAAAGATTACACCCTGAAAGAAGAACACC
TGGATCGTATTTTCGAAGAATATGACCGCCTGGCAGAAGAATATATCCGTCGCTACGAAGAAGGCCGT
CCGTTCGCTTTCTACCATTTCAAGATCGATCTGAAAGGCGGTCCGTGCATCAAAAAGCGCCTGCAGGG
CTGTGGTGCAGGCTTCGAATATATTGCTGTGACGCCGGATGAAGAAATCTACCCGTGCCACCAATTTG
TTGGTATTGAAGAATTCAAACTGGGTACCCTGGATGAAGGCATTACGAACATCGAACTGCAGCGTAAG
TTTATGGAAAGCGACATCTATAAACGTGAAGAATGCGCAAATTGTTGGGCTCGCTTTTATTGCTCTGG
CGGTTGTTTCGCAAACAACTACAACATCAACGGCGATATCAACAAGCCGTATAAACTGGCTTGTGAAA
TGCAGAAACGTCGCATTGAAAATGCAATTGCTATCAAGGCATACCTGACCCTGCGTGGTGAAAAAGGC
GATTATCAGCGTGTGCAACGCGACAAAGCGGCCAACCGC*TAA***CTCGAG**

b) MGSSHHHHHHSSGLVPRGSHMSATMHKFKRLGLNIVVDPVSGAIHVVDDVVYDVLDYYENHSREEIVN
LLKDKYKEEDILEAISEVDELKGNGLLFTEDIYKDIAISRADSVIKAMCLNVAHDCNLRCKYCFASTG
NFKGGRKLMDFETGRKAIDFLIKSSGKRRNIEIDFFGGEPLLNFEVVKQLVEYGKQKAKENKKNIKFT
ITTNAVLLDDEKIEYFNENFSNVVLSLDGRKEVNDQMRVRADGSGTYDVIVPKIQKFVKARGKKEYYV
RGTFTAKNLDFVEDVLHIADLGVYEISVEPVVEKDDKDYTLKEEHLDRIFEEYDRLAEEYIRRYEEGR
PFAFYHFKIDLKGGPCIKKRLQGCGAGFEYIAVTPDEEIYPCHQFVGIEEFKLGTLDEGITNIELQRK
FMESDIYKREECANCWARFYCSGGCFANNYNINGDINKPYKLACEMQKRRIENAIAIKAYLTLRGEKG
DYQRVQRDKAANR

c) *ATG*GGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGC**CATATG**CGTCG
CATTATCACCATTAACAAACCGACGCTGCGTGAAAGTCTGAAAAAGCCGGGCTGCGGTGAATGTCAGG
CGAGCTGCCAATCTGCCTGTAAGACCAGCTGCACGGTGGGCAACCAGGAATGTCAATATAAT*TAA***CTC**
GAG

d) MGSSHHHHHHSSGLVPRGSHMRRIITINKPTLRESLKKPGCGECQASCQSACKTSCTVGNQECQYN

Figure S2. Purification of Tte1186a and Tte1186. **A**. Tte1186 maturase (lane 2) and Tte1186a peptide (lane 3) were purified by affinity chromatography to 95% homogeneity as determined by SDS-PAGE analysis. Lane 1 is the molecular weight marker. **B**. UV-visible spectra of as-isolated Tte1186 (red), reconstituted Tte1186 (black), and reconstituted Tte1186 after size exclusion chromatography (blue). **C**. Mass spectrum of purified Tte1186a peptide. The various charge states of the peptide are indicated above the respective peaks. **D**. Deconvoluted and deisotoped mass spectrum of Tte1186a generated from **C** using Xtract software (Thermo Fisher). **E**. Zoomed in view of the deconvoluted and deisotoped spectrum in **D**. The peak with *m/z* = 7108.3298 corresponds to the monoisotopic $[M+H]^+$ of Tte1186a. The other peaks correspond to oxidized peptide (+O) and/or potassium (+K) adducts, as indicated above the respective peaks.

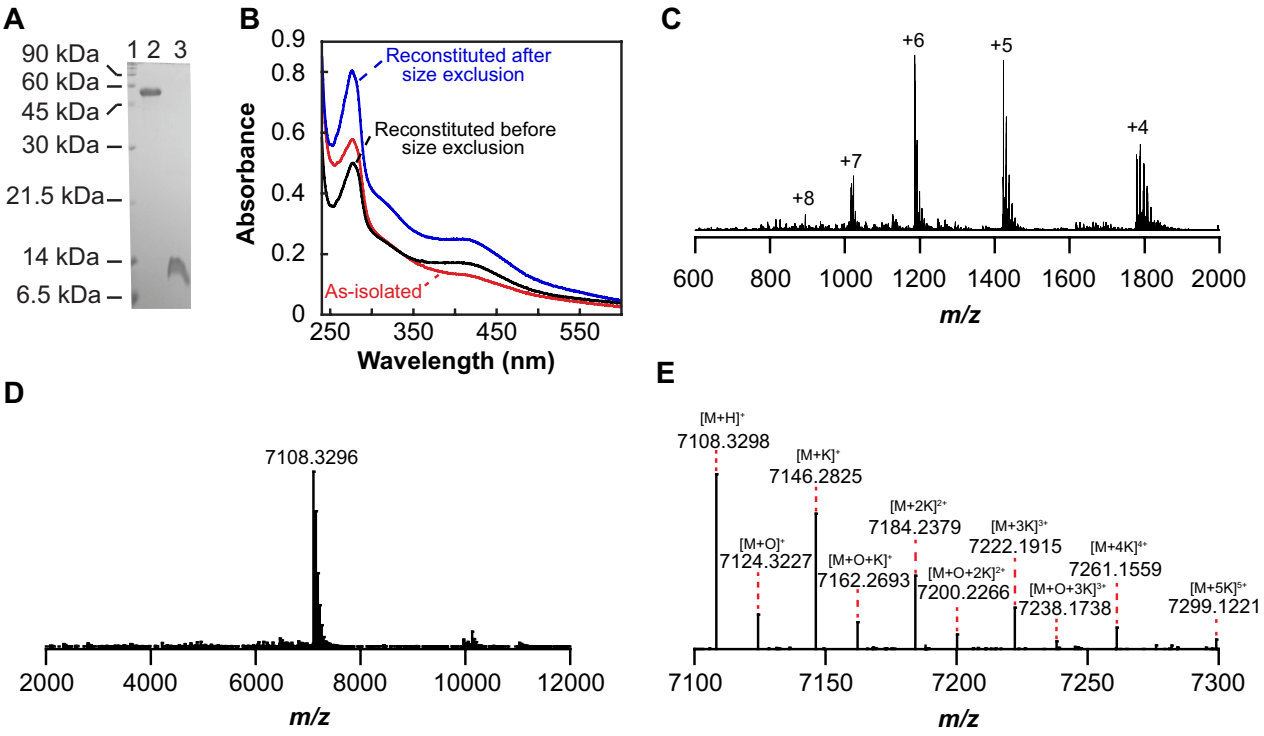

Figure S3. Clustal Omega sequence alignments between anSME from *Clostridium perfringens* ATCC 13124 (gi 122959045), QhpD from *Paracoccus denitrificans* PD1222 (gi 119374208), Tte1186 from *C. subterraneus* subsp. *tengcongensis* MB4 (gi 20516186), and AlbA from *Bacillus subtilis* subsp. *subtilis* str 168 (gi 27734208). The CxxxCxxC RS signature sequence is highlighted by the blue box and the seven conserved Cys resides in the C-terminal SPASM domain are highlighted by the red box. Each of the seven conserved Cys residues in anSME were observed binding either AC1 or AC2 in the crystal structure.[1]

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AlbA      MFIEQMFPFINESVRVHQLPEGGVLEIDYLRDNVSISDFEYLDLNKTAYELCMRMDGQKT   60
anSME     ------------------------------------------------------------   0
Tte1186   -----------MSATMHKFKR---LGLNIVVDPVSGAIHVVDDVVYDVLDYYENHSREEI   46
QhpD      -----MGALTLIRHNAHRVDV---DGHAMLMHVPTTSLFELDGVARDVYDLFRRAPAVD-   51


AlbA      AEQILAEQCAVYDESPEDHKDWY--YDMLNMLQNKQVIQLGNRASRHTITTSGSNEFPMP   118
anSME     ------------------------------------------------------MPPL   4
Tte1186   VNLLKDK-Y-----KEEDI---LEAISEVDELKGNGLLFTEDI-YKD---IAISRADSVI   93
QhpD      PDLMRAE-LG-PRHGPDTLSECLQSFLALDILRNAE---AADI-PR----PVVKVEEIPL   101


AlbA      LHATFELTHCNLKCAHCYLESSPEALGTVSI--EQFKKTADMLFD------NGVLTCEI   170
anSME     SLLIKPASSCNLKCTYCFYHSLSDNRNVKSYGIMRDEV-LESMVKRVLNEANGHCSFAF   63
Tte1186   KAMCLNVAHICNLRCKYCFASTGNFKGGRKLMDFETGRKAIDFLIKS--SGKRRNIEIDF   151
QhpD      STIILNVNTCCNLACTYCYKEDLTTPAKGQKMGFETAKASFELLLKQ--AHARDRVNVVF   159
                    *** * :*:                  .   : :..     .     :


AlbA      TGGEIFV--HPNANEILDYVCKK------FKKVAVLTNGTLMRKESLELLKTYKQKIIVG   222
anSME     QGGEPTLAGLEFFEKLMELQRKHNYK-NLKIYNSLQTNGTLIDESWAKFLSEN--KFLVG   120
Tte1186   FGGEPLL-NFEVVKQLVEYGKQKAKENKKNIKFTITTNAVLLDDEKIEYFNEN--FSNVV   208
QhpD      FGGEPLS-NMPLIRELVAYARPRAAELGKAVDFSLTTNATLLTPELVDWFDAH--RFALT   216
           ***         .:::     :          :: **..*:  .  . :.       :


AlbA      ISLDSVNSEVHDSFRGR---KGSFAQTCKTIKLLSD----HGIFVRVAM----------S   265
anSME     LSMDGP-KEIHNLNRKDCCGLDTFSKVERAAELFKKYKVEFNILCVVTSNTARHVNKVYK   179
Tte1186   LSLDGR-KEVNDQMRVRADGSGTYDVIVPKIQKFVKARGKKEYYVRG-------------   254
QhpD      VSMDGP-KALHDANRKTVGGKGTYDLVARNVRMLLSRYRSRPVGGRV-------------   262
          :*:*.  . :.:  *       ::       . : .


AlbA      VFEK----NMWEIHDMAQKVRDLGAKAFSYNWVDDFGRGRDIVHPTKDAEQ---------   312
anSME     YFKEKDFKFLQFINCLDPLYEEKGKYNYSLKPKD-----------------YTKF---LK   219
Tte1186   TF---TAKNLDFVEDV-LHIADLGVYEISVEPVVE----KDDKDYTLKEEHLDRIFEEYD   306
QhpD      TL---TRGVTDVIGIHDHLKNELGFHEVGFGPATS----GPIAVFNLDAEALKRAFEDMK   315
           :          :        : *    .


AlbA      ------HRKFMEYEQHVIDEFKDLIPIIPYERKRAANCG---AGWKSIVISPFGEVRPCA   363
anSME     NLFDFWYEDFLNGNRVSIRYFDGLLETILLGKS--SSCGMNGTCTCQFVVESDGSVYPCD   277
Tte1186   RLAEEYIRRYEEGRPFAFYHFKIDLKGGPCIKKRLQGCG---AGFEYIAVTPDEEIYPCH   363
QhpD      TLGRRYVEAACRGENIGFSNMHQLLTDIAQGTKKAVFCG---AGLGMLAVDKDGELHLCH   372
                 .   . .   :  :.  :       .    **   :    :.:    .:  *


AlbA      LFP--KEFSLGNIFHDSYESIFNSPLVHKLWQAQAPRFSEFCMKDKCPFSGYC-GGCYLK   420
anSME     FYV-LDKWRLGNIQDMTMKELFETNKNHEFIKLSF-KVHEFC--KKCKWFRLCKGGCRRC   333
Tte1186   QFVGIEEFKLGTLDEGITN----IELQRKFMESDI-YKREFC--ANCWARFYCSGGCFAN   416
QhpD      RFVGSNQPTYGNVAKGIDI----PKLAGFIETAQD-RSAYGC--KTCRIRSICAGGCYHE   425
           :   .:   *.: .             :   .       *    .*    * * ***


AlbA      GLNSNKYHRK---NICS----WAKNE-QLEDVVQLI------------------------   448
anSME     RDSK-EDSALELNYYCQSYKEFFEYAFPR-----LINVANNIK-----------------   370
Tte1186   NYNINGDINKPYKLACEMQKRRIENAIAIKAYLTLRGEKGDYQRVQRDKAANR-------   469
QhpD      SYARQGDPFAPVWHYCDLMRDWVDFG--IESYVRIMQANPSFFRSQLEPRIARSGTAREV   483
                      *.       .          :


AlbA      --   448
anSME     --   370
Tte1186   --   469
QhpD      LQ   485
```

Fig S4. Continuous wave X-band EPR Component analysis. **A)** EPR of the FeS clusters present in the ΔRC/ΔAC2 variant in the absence of SAM (black) can be well simulated with two components (summed simulation in red), AC1a (fuchsia, 81%) and AC1b (blue, 19%). **B)** The ΔRC/ΔAC1 variant in the absence of SAM (black) can also be well simulated (summed simulation in red) with two components, AC2a (teal, 63%) and AC2b (green, 37%). Simulations were carried out using EasySpin, using a least squares fit varying the three principle *g*-values, *g*-strain (σ), and the percent composition for each component. X-band CW spectra were recorded at T= 10 K, 9.4 GHz microwave frequency, 10G modulation amplitude, 100 μW microwave power.

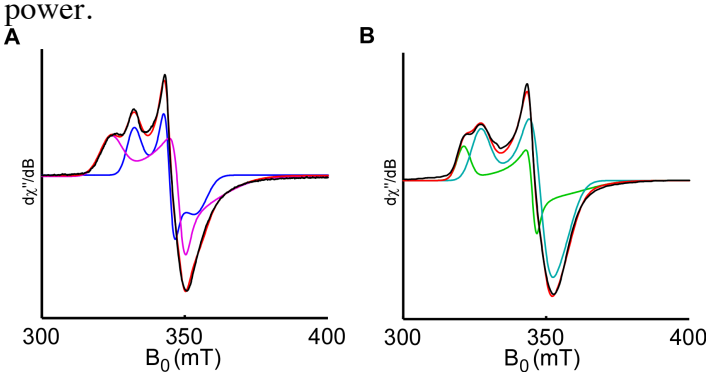
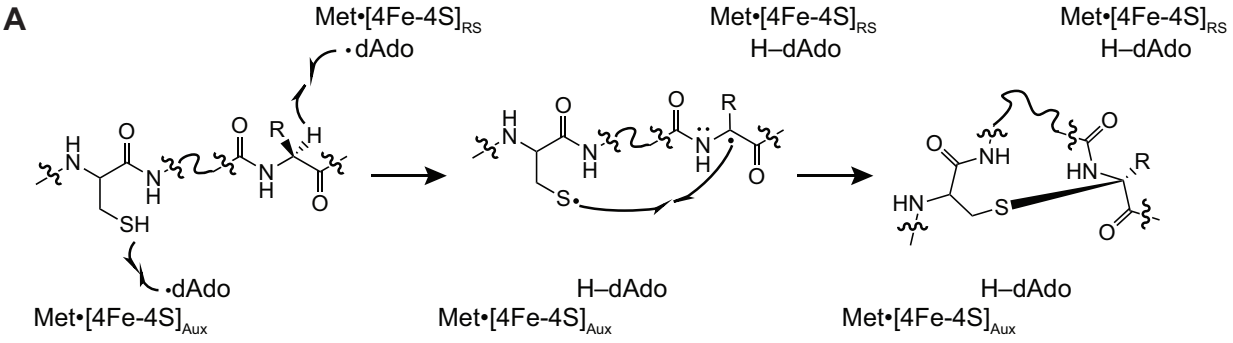
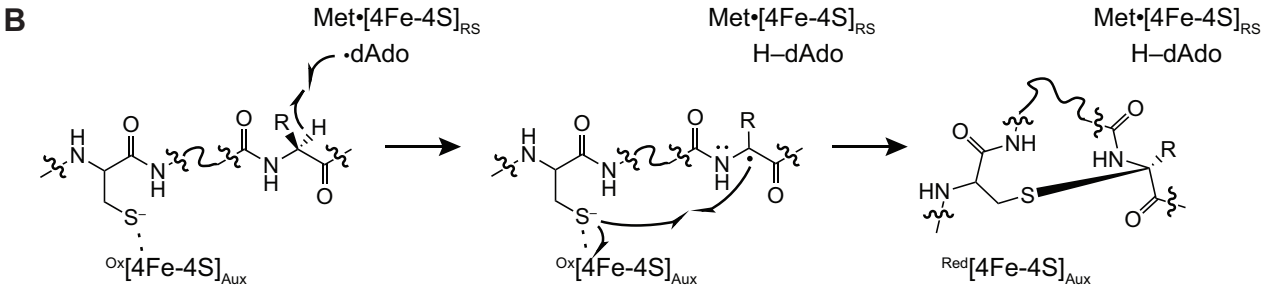
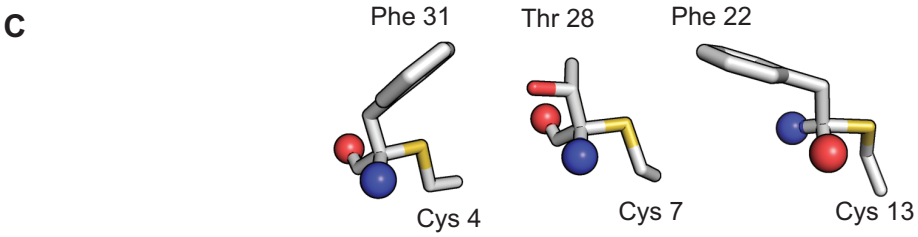


Figure S5. Proposed mechanisms for thioether bond formation. Mechanism (**A**) postulates simultaneous H-atom abstraction from two positions followed by bond formation. Such a di-radical mechanism is unlikely as highly unstable intermediates are being generated simultaneously, and requires the ability of the enzyme to generate two dAdo•. Mechanism (**B**) is more commonly discussed and requests that the peptide-based radical combine with the Cys side-chain, followed by electron transfer to an as yet identified cluster (either RS or Aux). While this is more plausible, the fact that the regiochemistry of the reaction can be either *si-* or *re-* in the same substrate, such as in SboA (**C**), makes this highly unlikely. For example, the RS enzyme AlbA introduces three crosslinks into its peptide substrate (SboA). As (**C**) shows, comparison of the NMR structure of the substrate reveals that the enzyme introduces two regiochemically distinct crosslinks using the same active site. The amide nitrogen (blue) and the carbonyl oxygen (red) of the peptide bond are shown as spheres to help visualize the change in stereochemistry. (**C**) was generated using PyMol [2] using the PDB coordinates 1PXQ [3].







Supplemental References

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