

## Supplementary Information for

RiPP enzyme heterocomplex structure-guided discovery of a bacterial borosin  $\alpha$ -*N*-methylated peptide natural product

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**Table S1| All primers, plasmids, and genes used in this manuscript.****a. Primers used in this study**

<b>ID</b>	<b>Description</b>	<b>Sequence (5'-3')</b>
prKKC1035	His-SonA_extBBD_fwd	AAAACGTTAAAAACGTTAAGTGG
prKKC1036	His-SonA_modBBD_rev	GAGCTTTTCCATATCCC
prKKC1037	His-SonA_lessBBD_fwd	AGTGGTGATAGTAGCTATC
prKKC1041	SonA_KTLSdel_for	GGTGATAGTAGCTATCAATC
prKKC1042	SonA_MEKCLKLdel_rev	ATCCCCAGTCATTACAG
prKKC1043	SonA_KTLGDSdel_for	AGCTATCAATCTTACCTTG
prKKC1044	SonA_KTLSext_for	AGTAAAACGTTAAGTGGTGATAGTAGCT
prKKC1045	SonA_MEKCLKLText_for	ATGGAAAAGCTCAAACGTTAAGTGGTG
prKKC1046	SonA_KTLSext_rev	TAACGTTTTGAGCTTTTCCATATCCCC
prKKC1047	SonA_QSYdel_for	CTTGTTATTTACATGGTAATG
prKKC1048	SonA_QSYdel_rev2	ATAGCTACTATCACCACTTAAC
prKKC1005	pET28b_bb_rev_signal	CATGGTATATCTCCTTCTTAAAGTTAAACA
prKKC1003	pET28b_bb_fw_signal	TGAGATCCGGCTGCTAACAAA
prKKC1000	Son_1059_Nsec1_fw	CTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATCG GCGGCCGTCCAAAATCCTTT
prKKC1002	Son_1059_C-histag_rev	ATGGTGATGGTGATGGTGCCGCTGCCATGGGTCATTTT ATTTTTAATTAGTACGC
prKKC1004	insert_gib_Nsec_fw	TGTTTAACTTTAAGAAGGAGATATACCATGAAAAAGATTT GGCTGGCGCTGGCTGGTTT
prKKC1001	insert_gib_Nsec_rev	TTTGTTAGCAGCCGGATCTCAatggtgatggtgatggtgGCC
prTJ1042	SO0867_FGib	tcaCATATGAAAGATGCCCTGTCTATG
prTJ1043	SO0867_RGib	ATTCGGATCCCGtgactggaagtacagtccctcGAACTGCGCGAC AAC
prTJ1044	pET_SO0867_FGib	agtcaCGGGATCCGAATTCGAGCTCCGTC
prTJ1045	pET_SO0867_RGib	GGCATCTTTCATATGtgactggaagtacagtccc
prTJ1054	SonA_Xacore_F	ggaAGGGATAGTAGCTATCAATCTTACC
prTJ1055	SonA_Xacore_R	ctcgatACCACTTAACGTTTTGAGCTT
prFM1144	pBAD_seq_fw	CCTACCTGACGCTTTTTATCGCAA
prFM1145	pBAD_seq_rev	GCGTTCTGATTTAATCTGTATCAGGCT
prFM1150	SonCluster_screen_fw	GTGCGCCAAAGCAATATGGTGAGTT
prFM1151	SonCluster_screen_rev	GCGCTATGACTTCCAAATCGGCAAT
prFM1152	1479US_fw	CCCGGGGGATCCACTAGTGCTACAATAGGGGTAAAG
prFM1155	1479DS_rev	GAACAAAAGCTGGAGCTCAAATCAGTTGATTATAATGCT
prFM1167	pSMV3_bb_rev	ACTAGTGGATCCCCCGG
prFM1168	pSMV3_bb_fw	GAGCTCCAGCTTTTGTTCCC

prFM1180	ΔSO1478_US_seq_rev	GCGAGCATTACCCATAAAGAAC
prFM1181	ΔSO1478_DS_seq_fw	CGTGCGTCTTACTTACGTTTA
prFM1182	ΔSO1479_US_seq_rev	CGGCATGTTCAATATAGCTGC
prFM1183	ΔSO1479_DS_seq_fw	GAGCTGGAGATTAATAGCGTACA
prFM1196	SO1479USnewREV	CTTCAATTAATCACCATTACCATGTGAAATTCCAGACAT GTTTTCTCCTTATTG
prFM1197	SO1479DSnewFW	CAATAAGGAGAAAACATGTCTGGAATTTACATGGTAAT GGTGATTAATTG
prFM1216	pBBAD_gib_rev	CGCTAGCCCCAAAAAACGGG
prFM1217	pBBAD_gib_fw	CCTGCAGGCATGCAAGC
prFM1218	SO1478UF-Spel	TAGAACTAGTACAAAAAGCGCCATTGGC
prFM1219	SO1478UR-XhoI	TAGACTCGAGCACACAGACGAGTGATCCC
prFM1220	SO1478DF-XhoI	TAGACTCGAGATTGAAGTGTGTTATTGAATCATTATTAAC AATAAGG
prFM1221	SO1478DR-SacI	TAGAGAGCTCAGAGAATCTAATAAGTAAGAGATAGCAAG CTG
M13F	Universal primer	GTAAAACGACGGCCAGT
M13R	Universal primer	GGAAACAGCTATGACCATG
prmMRJ_026	sonA_fwd	ATGTCTGGATTATCGGATTTTTTTTACCCAGTTAGGCCAA GATG
prmMRJ_027	sonA_rev	TTAATCACCATTACCATGTGAAATAACAAGGTAAGATTG ATAGCTACTATCACCA
prmMRJ_024	sonM_fwd	TTGGGATCACTCGTCTGTGTGGGCACT
prmMRJ_025	sonM_rev	TTATCCCAAATCTTCGGGACCGATCCCTAAGTTAGC

#### b. Plasmids used in this study

ID	Description
pMF1181	SonM-gRBS-His-SonA_pET28b
pMF1235	His-SonA_pET28b
pMF1236	His-SonM_pET28b
pMF1230	His-ADE (JW_3640 ASKA collection)
pMF1231	His-SAHN (JW_0155 ASKA collection)
pMF1321	His-SonA_K51-L53dup_pET28b
pMF1322	His-SonA_K51-L53del_pET28b
pMF1397	His-SonA_Q60-Y62del_pET28b
pMF1398	His-SonA_K51-S54del_pET28b
pMF1399	His-SonA_M47-L53del_pET28b
pMF1400	His-SonA_K51-S57del_pET28b
pMF1403	His-SonA_K51-S54dup_pET28b
pMF1404	His-SonA_M47-L53dup_pET28b
pMF1440	SonM-gRBS_His-SonA_Q60-Y62del_pET28b
pMF1488	pET28b-ss-strep-tev-SO0867-tev-His
pMF1246	His-So_1059_ecoli_sig_pET28b
pMF1490	SonNMT-gRBS-His-SonA_XaCORE55-56
p015	pBBad18K
p024	pSMV3
pMF1274	SO1478-79_pBBAD18K
pMF1223	KOso1478_pSMV3

**c. DNA sequences**

UniProt ID	Name	Description
P31441	<i>ade</i>	Adenine deaminase
<p>ATGAATAATTCTATTAACCATAAATTTTCATCACATTAGCCGGGCTGAATACCAGGAATTGTTAGC  CGTTTTCCCGTGGCGACGCTGTTGCCGATTATATTATTGATAATGTCTCTATTCTCGACCTGATCA  ATGGCGGAGAAATTTCCGGCCCAATTGTGATTAAGGACGTTACATTGCCGGTGTGGCGCAG  AATACACTGATGCTCCGGCTTTGCAGCGGATTGATGCTCGCGGCGCAACGGCGGTGCCAGGG  TTTATTGATGCTCACCTGCATATTGAATCCAGCATGATGACGCCGGTCACTTTTCAAACCGCTAC  CCTGCCGCGCGGCCTGACGACCGTTATTTGCGACCCTCATGAAATCGTCAACGTGATGGGCGA  AGCCGGATTGCGCTGGTTTGCCCGCTGTGCCGAACAGGCAAGGCAAACCAGTACTTACAGGT  CAGCTCTTGCGTACCCGCCCTGGAAGGCTGCGATGTTAACGGTGCCAGTTTTACCCTTGAACA  GATGCTCGCCTGGCGGGACCATCCGCAGGTTACCGGCCTTGAGAAATGATGGACTACCCTG  GCGTAATTAGCGGGCAGAATGCGCTGCTCGATAAACTGGATGCATTTGCCACCTGACGCTGG  ACGGTCACTGCCCGGTTTGGTGGTAAAGAAGTAAACGCCTATATTACTGCGGGTATTGAAAA  CTGCCACGAAAGTTATCAGCTGGAAGAAGGACGCCGAAATTACAACCTCGGCATGTCGTTGAT  GATCCGCGAAGGGTCCGCTGCCCGCAATCTCAACGCGCTGGCACCGTTGATCAACGAATTTAA  CAGCCCGCAATGCATGCTCTGTACCGATGACCGTAACCCGTGGGAGATCGCCATGAAGGACA  CATCGATGCCTTAATTCGCCGCTGATCGAACAACACAATGTGCCGCTGCATGTGGCATATCGC  GTCGCCAGCTGGTCGACGGCGCGCCACTTTGGTCTGAATCACCTCGGCTTACTGGCACCCGG  CAAGCAGGCCGATATCGTCCTGTTGAGCGATGCGCGTAAGGTCACGGTGCAGCAGGTACTGGT  GAAAGGCGAGCCGATTGATGCGCAAACCTTACAGGCGGAAGAGTGGCGGAGACTGGCACAAT  CCGCTCCGCCATATGGCAACACCATTGCCCGCCAGCCAGTTTCCGCCAGCGACTTTGCCCTGC  AATTTACGCCCGGAAAACGCTATCGGGTCATTGACGTCATCCATAACGAATTGATTACGCACTC  CCTACTCCAGCGTCTACAGCGAAAATGGTTTTGATCGCGATGATGTGAGCTTTATTGCCGTACTT  GAGCGTTACGGGCAACGGCTGGCTCCGGCTTGTGGTTTGTGTTGGCGGCTTTGGACTGAATGAA  GGTGCCTGGCTGCGACGGTACGCCATGACAGCCATAATATTGTGGTGCATCGGTGCGAGTGCC  GAAGAGATGGCGCTGGCGGTCAATCAGGTGATTGAGGATGGCGGCGGGCTGTGCGTGGTACG  TAACGGCCAGGTACAAAGTCATCTGCCGTTACCCATTGCCGGGCTGATGAGCACCGACACGGC  GCAGTCGCTGGCGGAACAAATTGACGCCTTGAAGCCGCCGCCCGTGAATGCGGTCCGTTAC  CCGATGAGCCGTTTATTGAGATGGCGTTTCTTTCTCTGCCAGTGATCCCCGCGCTAAAATAAC  CAGTCAGGGGCTATTTGATGGCGAGAAGTTTGCTTCACTACGCTGGAAGTCACGGAATAA</p>		
P0AF12	<i>sahn</i>	S-adenosylhomocysteine nucleosidase
<p>ATGAAAATCGGCATCATTGGTGAATGGAAGAAGAAGTTACGCTGCTGCGTGACAAAATCGAAA  ACCGTCAAACATCAGTCTCGGCGGTTGCGAAATCTATACCGGCCAACTGAATGGAACCGAGG  TTGCGCTTCTGAAATCGGGCATCGGTAAGTTCGCTGCGGCGCTGGGTGCCACTTTGCTGTTGG  AACACTGCAAGCATGTGATTATTAACACCGGTTCTGCCGGTGGCCTGGCACCAACGTTGA  AAGTGGCGATATCGTTGTCTCGGACGAAACGACGTTATCACGACGCGGATGTGACGGCATTG  GTTATGAATACGGTCAGTTACAGGCTGTCCGGCAGGCTTTAAAGCTGACGATAAACTGATCGC  TGCCGCTGAGGCCTGCATTGCCGAACGAACTTAAACGCTGTACGTGGCCTGATTGTTAGCGG  CGACGCTTTCATCAACGGTCTGTTGGTCTGGCGAAAATCCGCCACAACCTTCCACAGGCCATT  GCTGTAGAGATGGAAGCGACGGCAATCGCCATGTCTGCCACAATTTCAACGTCCCGTTTGTG  GTCGTACGCGCCATCTCCGACGTGGCCGATCAACAGTCTCATCTTAGCTTCGATGAGTTCTG  GCTGTTGCCGCTAACAGTCCAGCCTGATGGTTGAGTCACTGGTGCAGAACTTGCACATGGC  TAA</p>		
Q8EGW3	<i>sonM (so1478)</i>	Borosin methyltransferase
<p>ATGGGATCACTCGTCTGTGTGGGCACTGGGTTACAGCTCGCGGGGCAAATTAGCGTATTAAGC  CGCAGCTATATTGAACATGCCGATATTGATTTTCACTCTTACCTGACGGTTTCTCGCAGCGTTG  GTTGACGAAGCTCAACCCCAATGTCATCAATTTGCAGCAGTTTTATGCGCAAAATGGTGAAGTT  AAAAATCGCCGAGACACCTACGAGCAAATGGTCAATGCCATTCTAGATGCGGTGAGAGCGGGT  AAAAAAACCGTGTGTGCACTCTACGGTCATCCGGGGGATTTGCCTGTGTATCCCATATGGCGA  TAACTCGGGCGAAGGCCGAAGGGTTTTCGGCAAAGATGGAGCCGGGGATTTCCGGCCGAAGCT  TGCCGTGTTGGGCGACTTAGGGATTGACCCCGGCAACTCGGGGCATCAAAGTTTTGAAGCTAGC  CAGTTTATGTTTTTCAACCATGTGCCCGATCCCACTACCCACTTATTACTCTGGCAAATCGCCAT</p>		

TGCAGGCGAACATACCTTAACCCAATTTTCATACCTCGAGTGATAGGTTGCAGATCCTCGTGGAG  
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 TCCAAGCCCCGCGTATCGAGCGTTTACCTTTAGCGAATTTACCCCAAGCACACTTAATGCCGAT  
 TAGTACGTTGTTAATTCCGCCAGCAAAAAAGCTGGAGTACAACATGCTATTTTGGCTAAGTTAG  
 GGATCGGTCCCGAAGATTTGGGATAA

Q8EGW2	<i>sonA (so1479)</i>	Borosin RiPP precursor
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ATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGCGCAGTTAATGGAAGACTATAA  
 ACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAAACAAATTAACGCTGTAATG  
 ACTGGGGATATGGAAGAGCTCAAACGTTAAGTGGTATAGTAGCTATCAATCTTACCTGTTAT  
 TTCACATGGTAATGGTGATTAA

Q8EHZ5	<i>so1059</i>	Aminopeptidase
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GTGAATTTGTTTAAAGCTAGCCTAGTCGCCCTCACCTGCTCAGCATTAAATGTCGTGCGCCGTCC  
 AAAATCCTTTAAATACAGGACCAAGGGATACGAGTCCCTATATCAGCCAGTATCAGGCAAGTTT  
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 TTCAGTGCGACAACGAAAGTGAACCTTCAATTTAAGCGAAGTGCCCAAGCAACTGAGCTTAGATC  
 TCAACAAAGCACAAATTAAGCGCTTTTTGATCAACGGCACCGCCGTTTATCCTAATTACAATGGC  
 GCCTATATCAGCCTCAACACTCGTTACTGACTTCAGGGGAAACACAATTGAAGTGCAATTCA  
 CCCGAGCCACAGTACCAATGGTGAAGTTTTGCACCGGTTCCAAGACCCAGTTGATGGCAAGG  
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 CCCGTGAAACGACCGTCACCCAGCTGGCGCCTTAAATCGTTGGGAGTTTCTGAAACGCCTA  
 AGTTAAGCCCTTATAACTTCTCCATGCATGCTGGCCCATATTATATGTGGCAGGATAATTCTGGC  
 CGTTATCCAATGCGCTTATTCGCACGGCAATCCGTTGCCAGCCAAGTGAATCCACAGGACTGGT  
 TTACCTACACCAACAAGGGTTAACCTTCTTTGACGCCTACTTTGGTATTCCTTACCCCTTTAAA  
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 CCTTCGCCGAAGATCACTTCTCCACAAGCGGAAATGACCGCCGAGCAAAAACAAAACCTTAGC  
 GGGCGTAATTATGCACGAAATGGCCACCAATGGTTTGGCGATCTGGTGACCATGAAGTGGTG  
 GAACAGCCTCTGGTTAAACGAAAGTTTTGCTTCTTTATGGGTACACTTGCAACCCAAGAAGCC  
 ACTGAATTTACCAATGCATGGCGCAGTTTTTATGCTCAAGGTAACAACGTGCCTATGAGCAAG  
 ACAGCTTAGTGACCACTCATCCATTGAAGTACCCGTGGCAACAACCCAAAATGCCTTCGATAA  
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 GATGTGGTGTTCGCGCGGGCGTGAGCAACTACCTCAAACAATATAGTTATCAAAATGCCGAGC  
 TAGATGACTTCATCAATAGCCTCGCCAAAGCCTCGAATCGTGACTTAAGCGCATGGACGCAGGA  
 ATGGCTTTACAGCGCAGGGGTAACACGATCAAGGCAGAGTACCGCTGCGACGGTAACCGCAT  
 CAGCGAATTTAGCCTACTGCAATATCCTGCCAGCAGCGAACTGCCAACACTTAGGGAGCAAAAA  
 GTCCAAATCGCGCTCTTCAAAAAGGACGTTTCGATTTACGACATGAGACCACAGTGCCAGTCA  
 CCTACAAAGGTGAGCGCACGGAAGTGAAGCAATTAGTCGGTGAGCGTTGCCAGATCTGGTTT  
 ACCCTAACTACGATGACTGGGGCTATGTAAAGGTACAACCTCGATGACAAATCCTTTGATACCGC  
 CAAGCAACAGTAAAGCAAGGTGACAGACCCGCTTCTGCGTTCAATGTTATGGCAAAGCCTGTG  
 GGACAGTGTGCGTGAGGGCAACCTGAGTTTGGACCAATACCTGAGCACAGTGTTTATTAATGCA  
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 ACCTTGCAGCAATCGACCAATCCAGCAAAACTATGCTGAAAATGCAGTAAAAGGCTTAGCACA  
 AATGAGCCTTCGAAAACCATGGAGAGTAGAGGCAACAATGACTTCCAGCGCCGCTGGTTTAA  
 GCCTATATTCAATTAGCGGGCGATCGCGACAGTTTACGCCACTTAGCACAATTAAGTATGGCA  
 CAACCAAAATCAAAGGGTTAACCTTAGATCAAGATCTGCGCTGGGCTATCATAACTCAGCTCAA  
 TCGCTACGACTTCCCAATGCGCAGCAACGTATTACACGGGAAAGCGCACGCGATAACTCAGA  
 TTCAGGGGAAAAGTCTGCATTAGCTGCACAGGTTATCCGCCAGAAGCCGCGGCGAAGCGTGC  
 TTGGTTAAGTAAGGTGCATACCGATGCACTGCCCTTTGCAAAACAGCGCATTATCATGGAAAAC  
 CTTTATCCTGCTGAGCAAAAGCTCTTAAGTGCGGCCACCGCCGAAGAGCGCCTCGAAACTTA  
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 ATTGTAACACTACAACAGTATTGCGGCCCTTAGATAAAGTATTAGACAGCCAACAGGCTTATCTAAT  
 CTTAGCCGTGCAACCTTATTAGAAACGCGCCAAGCCGAGCAGCGCTGCGTACTAATTAATAA  
 AAATGACCCATTGA

Q8EIH2	<i>so0867</i>	Collagenolytic serine protease
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ATGTGATAAAACTCTCACCGTTGGCGTTAGCAATTACAGGAGCTATGACGTTGTGACCCGTAC  
 CGGCTATGGCAAAGATGCCCTGTCTATGAAAAGATGCCATTCTCGTGGTGTATAAAGACAA  
 TGCAACTAAAGCAGAGCGCTCGGCGGCTCAGCGTTAATTCGTGGAACATTAACCGATGTCAAT

GCCGATGGTGTGCGACGATAAATTTCCGCATTTACTTAAACGGCAAGCTGGCCCGTTTAGCATTGC  
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 TGGGAAATCACCACAGGTAGCTCTGATGTGGTATTGGCGTTATCGATACCGGTGTGGATTACA  
 ACCATCCCGATTTGCAAGCCAATATGTGGTCAATGCGGGCGAAATTGCGGGCAATGGCATCG  
 ATGATGACGCAAACGGTGTATCGACGATATTCATGGTTACAGTGCCGTGAATAATAACGGAAA  
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 ATTGCCAGTACAACTCGCAATGACCGTATGTCAGACTTCTACAATGGGGTTTAAACCAGTGTTG  
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 CTGGTACCTCGATGGCAACTCCCACGTGACGGGCGCCGCGCTCTCGTTTGGGCATTAACC  
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 AACCGGTAACAGGTTGCGGGTACTCGCCTCAATGTGGCTAATGCGCTGGAGCAGGCAAACCC  
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 GTGTCACCTGCACTTGAAGGGGTTTCAATTATCAAGTTCAACCGTTTCTGCGGGTGGTTCATTTA  
 CCTTAAACGTGGCGACAACCGCGCAAACGGTTTGGGGTATTACAGCATTACGGTGACGGGCA  
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 TTGAGTTTCCGTTGAAGGATTAGGCGTAGCCTTACCAACACCAGTACAGATGCAAATGATGA  
 TATCGTCAGTTACAGCTGGGACTTTGGTGTGATGGTACAACTCTACTGAGATGAACCAAGCCAT  
 GTTTATACCTCTGCGGGTACTTATACTGTGCTTTGACTGTGACCGATTCAATGGATCACAGCAA  
 CACTGTGACGATGCCTGTACAAGTCTATGAGCATAATATTAATGCTGCTGTTAGCCGCGCATT  
 GTATCGCGCCGTGGCAGTGCAATGGTAGATCTCACTTGGGATAGTGCTTTAGGTGAAAACGTT  
 GCCCTTACCAGGATGGTGAAGTGGTGGTAACGACTGAGAACGATGGTAGCTATCGTGATCGC  
 TTTACGACCACCGCCTCAAGTGTGACTTATCAAGTGTGTGAAACGACAGGAACATTATGTTCTG  
 CGCCAGTTGTCGCGCAGTTCTAA

n/a	<i>his6-sonM</i>	Hexahistidine-tagged borosin methyltransferase for heterologous expression
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ATGCATCATCATCATCACAGCAGCATGGGATCACTCGTCTGTGTGGGCACTGGGTTACAGC  
 TCGCGGGGCAAATTAGCGTATTAAGCCGAGCTATATTGAACATGCCGATATTGATTTTCACTC  
 TTACCTGACGGTTTCTCGCAGCGTTGGTTGACGAAGCTCAACCCCAATGTCATCAATTTGCAGC  
 AGTTTTATGCGCAAATGGTGAAGTTAAAAATCGCCGAGACACCTACGAGCAAATGGTCAATGC  
 CATTCTAGATGCGGTGAGAGCGGGTAAAAAACCGTGTGTGCACTCTACGGTCATCCGGGGGT  
 ATTTGCCTGTGTATCCCATATGGCGATAACTCGGGCGAAGGCCGAAGGGTTTTCGGCAAAGAT  
 GGAGCCGGGATTTCCGGCCGAAGCTTGCTGTGGGCGGACTTAGGGATTGACCCCGGCAACT  
 CGGGGCATCAAAGTTTTGAAGCTAGCCAGTTTATGTTTTTCAACCATGTGCCGATCCCCTAC  
 CCACTTACTCTGGCAAATCGCCATTGCAGGCGAACATACCTTAACCAATTTCATACCTCGA  
 GTGATAGGTTGCAGATCCTCGTGGAGCAGTTGAATCAATGGTATCCCCTCGACCATGAGGTGG  
 TCATATACGAAGCGGCCAATTTGCCAATCCAAGCCCCGCGTATCGAGCGTTTACCTTTAGCGAA  
 TTTACCCCAAGCACACTTAATGCCGATTAGTACGTTGTTAATTCGCCAGCAAAAAAGCTGGAG  
 TACAATATGCTATTTTGGCTAAGTTAGGGATCGGTCCCGAAGATTTGGGATAA

n/a	<i>his6-sonA</i>	Hexahistidine-tagged borosin precursor for heterologous expression
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ATGCATCATCATCATCATCATGCTGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC  
 GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTAT  
 GAACAAATTAACGCTGTAATGACTGGGGATATGAAAAGCTCAAACGTTAAGTGGTGTAGTA  
 GCTATCAATCTTACCTGTTATTTACATGGTAATGGTGATTAA

n/a	<i>his6-sonA_Q60-Y62del</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATATGGAAAAGCTCAAACGTTAAGTGGTGATAGTA GCTATCTTGTATTTCACATGGTAATGGTGATTAA		
n/a	<i>his6-sonA_K51-S54del</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATATGGAAAAGCTCGGTGATAGTAGCTATCAATCTT ACCTTGTTATTTCACATGGTAATGGTGATTAA		
n/a	<i>his6-sonA_K51-L53del</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATATGGAAAAGCTCAGTGGTGATAGTAGCTATCAAT CTTACCTTGTTATTTCACATGGTAATGGTGATTAA		
n/a	<i>his6-sonA_M47-L53del</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATAGTGGTGATAGTAGCTATCAATCTTACCTTGTTAT TTCACATGGTAATGGTGATTAA		
n/a	<i>his6-sonA_K51-S57del</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATATGGAAAAGCTCAGCTATCAATCTTACCTTGTTAT TTCACATGGTAATGGTGATTAA		
n/a	<i>his6-sonA_M47-L53dup</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATATGGAAAAGCTCAAACGTTAATGGAAAAGCTCA AAACGTTAAGTGGTGATAGTAGCTATCAATCTTACCTTGTTATTTCACATGGTAATGGTGATTAA		
n/a	<i>his6-sonA_K51-S54dup</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATATGGAAAAGCTCAAACGTTAAGTAAAACGTTAA GTGGTGATAGTAGCTATCAATCTTACCTTGTTATTTCACATGGTAATGGTGATTAA		
n/a	<i>his6-sonA_K51-L53dup</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATATGGAAAAGCTCAAACGTTAAAACGTTAAGTG GTGATAGTAGCTATCAATCTTACCTTGTTATTTCACATGGTAATGGTGATTAA		
n/a	<i>his6-sonA_XaCORE55-56</i>	Hexahistidine-tagged borosin precursor variant for heterologous expression
ATGCATCATCATCATCACATGTCTGGATTATCGGATTTTTTTACCCAGTTAGGCCAAGATGC GCAGTTAATGGAAGACTATAAACAGAATCCTGAGGCGGTGATGCGTGCCACGGATTAAGTATGAT GAACAAATTAACGCTGTAATGACTGGGGATATGGAAAAGCTCAAACGTTAAGTGGTatcgagggg AGGGATAGTAGCTATCAATCTTACCTTGTTATTTCACATGGTAATGGTGATTAA		



n/a	<i>pET28b-ss-strep-tev-so0867-tev-his</i>	<i>S. oneidensis</i> protease with N-terminal <i>E. coli</i> periplasmic signal sequence, strep tag, TEV cleavage site. C-terminal TEV cleavage site and his-tag.
<p>ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATCGGCGTGAGC  CACCCGCAGTTCGAAAAGAGCGCAGAGGGACTGTACTTCCAGTCACATATGAAAGATGCCCT  GTCTATGAAAAGATGCCATTCTCGTGGTGTATAAAGACAATGCAACTAAAGCAGAGCGCTCGG  CGGCTCAGCGTTTAATTCGTGGAACATTAACCGATGTCAATGCCGATGGTGTGACGATAAATT  TCCGCATTTACTTAACGGCAAGCTGGCCCGTTTAGCATTGCGTAAAGGGCGCCAATATTGAAGAT  GCCATTAAGGTGATCAGCCGTCATCCTGCGGTGAAATACGCTGAGCCTAACTATATTATCAAAG  CGATAGGTACACCTGATGACCCAAGTTTTGCCAGCCTATGGGGAATGAATAACACTGGCCAAAG  CGGTGGCACTGCCGATGCGGATATCGATGCACCTGAAGCTTGGGAAATCACCACAGGTAGCTC  TGATGTGGTGAATTGGCGTTATCGATACCGGTGTGGATTACAACCATCCCGATTTGCAAGCCAAT  ATGTGGGTCAATGCGGGCGAAATTGCGGGCAATGGCATCGATGATGACGCAAACGGTGTATC  GACGATATTCATGTTACAGTGCCGTGAATAATAACGGAAATCCAATGGACGGAAACGGCCAC  GGTACCCATGTTTCTGGCACGATTGGCGCTAAAGGTAATAACGGTGTCCGGTGTAGTAGGCGTT  AACTGGGACGTTAAAATTGCCGGATGTCAGTTCCTCGATACTGACGGTTATGGCTCTACCGCGG  GCGCTATTGCGTGTATCGATTATTTACCAACCTTAAGGTTAATCATGGGGTTGATATTAAGCGG  ACCAACAACCTTTGGGGCGGTGGCGGCTTTAGTCAAGCATTAAAAGATGCAATTGAAGCGGGT  GGTGAAGCAGGGATTTTATTTGTTGCCGCAGCAGGAAACGATGCCGTCGATAATGATGCGAGC  CCACACTATCCTTCAAGCTATAACTCTGATGTGGTGTCTCCATTGCCAGTACAACCTCGCAATGA  CCGATGTGCAACTTCTACAATGGGGTTAACCAGTGTGATATGGGCGCGCCAGGCTCGGC  GATTTTATCAACTGTCCGTGGCGGTGGTTACGCGACTTACTCTGGTACCTCGATGGCAACTCCC  CACGTGACGGGCGCCGCGCTCTCGTTTGGGCATTAAACCCTGACTTAACACCTGTTGAAATG  AAAGAACTACTGATGGCCTCAGGTGATGCGAATGCGGATTAACCGGTAAAACGGTTGCGGGT  ACTCGCCTCAATGTGGCTAATGCGCTGGAGCAGGCAAACCCATCACCTAGCTATAAGTTTACC  GTATCACCGGCTTACAATCTGTGCAAGCAGGCGCTGCAAGCTATAACTTCAAGTGTGGCA  GTGTGGCAGGTTGGGATGGTGTGTCGCATTGACTGTGACGCGTGTACCTGCACCTGAAAGGGG  TTTCATTATCAAGTTCAACCGTTTCTGCGGGTGGTTCATTTACCTTAAACGTGGCGACAACCGC  GCAAACGGTTTGGGGTATTACAGCATTACGGTGACGGGCAATGATGGTGCAGTTGAAAAGTC  TAAAGTCGTGTCGCTTAATGTCTTCCACAAGGATTAACGACTTTACCTATGGTAATGAGA  ACTCTGTAGCCATTCCGGATAACAACCCTAATGGCGTTATTAGCACAATAGAAGTCGCCGACGATGT  ACAGATTTTTGGTGTGCTCGCTGACGTGAATATCAGTCACACTTGGATTGGTACTTACGCGTT  GTGCTAACCTCACCAGCGGGTACTCAAGTGGTGTACATAACCGCGATGGTGGCAGTGCTGAT  AATATCGTTAAGAGCTGGGATCTAAGCGCATTGATGGTGA  AAAATGTACGTGGTACTTGGTCAT  TATCTGTCGATGACAATGTGGGTTGAGATACCGGAACGCTTAATAACTGGGGCTTAGTCATCAG  CGGCTTAGGTGAGGCTTCCCCAGCTGCGCCTGTTGCAGTTTTGAGTTTCCGTTGAAGGATT  AGGCGTAGCCTTACCAACACCAGTACAGATGCAAATGATGATATCGTCAGTTACAGCTGGGAC  TTTGGTGTGTTACAAACTCTACTGAGATGAACCCAAGCCATGTTTATACCTCTGCGGGTACTTA  TACTGTGCTTTGACTGTGACCGATTCAATGGATCACAGCAACACTGTGACGATGCCTGTACAA  GTCTATGAGCATAATTAATGCTGCTGTTAGCCGCGCATTAGTATCGCGCCGTGGCAGTGCAA  TGGTAGATCTCACTTGGGATAGTGCTTTAGGTGAAAACGTTGCCCTTACC  CGGAATGGTGAAGTGT  GGTGGTAACGACTGAGAACGATGGTAGCTATCGTGATCGCTTACGACCACCGCCTCAAGTGT  GACTTATCAAGTGTGTGAAACGACAGGAACATTATGTTCTGCGCCAGTTGTGCGCGCAGTTGCGAG  GGACTGTACTTCCAGTCACGGGATCCGAATTCGAGCTCCGTGCAAGCTTGGCGCCGCACTC  GAGCACCACCACCACCAC</p>		
n/a	<i>His-so_1059_ecoli_sig_p ET28b</i>	<i>S. oneidensis</i> protease with N-terminal <i>E. coli</i> periplasmic signal sequence and C-terminal hexahistidine tag for heterologous expression
<p>ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATCGGCGGCCGTC  CAAAATCCTTTAAATACAGGACCAAGGGATACGAGTCCCTATATCAGCCAGTATCAGGCAAGTT  TGCGTTCACAGGTGATATCGAATGTACATTATGAGTTGGATTTTCAACTGACTGGCGATACCGA  ATTCAGTGCGACAACGAAAGTGAACCTCAATTTAAGCGAAGTGCCCAAGCAACTGAGCTTAGAT  CTCAACAAAGCACAATTAAGCGCTTTTTGATCAACGGCACCGCGTTTATCCTAATTACAATGG  CGCCTATATCAGCCTCAACACTCGCTTACTGACTTCAGGGGAAAACACAATTGAAGTGAATTC  ACCCGAGCCCACAGTACCAATGGTGAAGGTTTGCACCGGTTCCAAGACCCAGTTGATGGCAAG  GTTTACCTTTACTCCATTTTGAACCTGCCGCCGCCAACAATGTTTGGCGTATTTGATCAACC  CGACTTGAAGGCCAATTATAAAATCAGCGTAACTGCACCTAGGGATTGGCAAGTTATCAGTACG  ACCCGTGAAACGACCGTCACCCAGCTGGCGCCTTTAATCGTTGGGAGTTTCTGAAACGCCT  AAGTTAAGCCCTTATAACTTCTCCATGCATGCTGGCCCATATTATATGTGGCAGGATAATTCTGG</p>		

CCGTTATCCAATGCGCTTATTTCGCACGGCAATCCGTTGCCAGCCAAGTGAATCCACAGGACTG  
GTTTACCTACACCAAACAAGGGTTAACCTTCTTTGACGCCTACTTTGGTATTCCCTTACCCCTTTA  
AAAAATATGATCAGATCCTCGTGCCTGATTTTCTCTACGGCGCGATGGAAAACGCAGGAGCAGT  
GACCTTCGCCGAAGATCACTTCCTCCACAAAAGCGGAAATGACCGCCGAGCAAAAACAAAACCTTA  
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GTGATGTGGTGTTCGCGCGGCGTGAGCAACTACCTCAAACAATATAGTTATCAAAAATGCCGA  
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ATGGCACAACCAAATCAAAGGGTTAACCTTAGATCAAGATCTGCGCTGGGCTATCATAACTCA  
GCTCAATCGCTACGACTTCCCAATGCGCAGCAACGTATTACACGGGAAAGCGCACGCGATAA  
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CAACCGATTGTAACATAACAGTATTGCGGCCTTAGATAAAGTATTAGACAGCCAAACAGGCTT  
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AAAAATAAAATGACCCATGGCAGCGGCCACCATCACCATCACCATTTGA

**Table S2| Crystallographic data and statistics.**

<b>Data collection statistics</b>		
	SonM—SonA_Q60-Y62del— SAM	SonM—SonA_Q60-Y62del— SAH
PDB ID	8T1T	8T1S
Resolution (Å)	1.55	2.00
Diffraction source	APS Argonne 23ID-D	
Wavelength (Å)	1.0332	
Detector	Dectris Pilatus3-6M	
Rotation range per image (°)	1	
Total rotation range (°)	300	
Space group	P1	P2 <sub>1</sub>
Unit-cell parameters (Å)	a = 52.5, b = 59.3, c = 60.1; α = 95.9 γ = 96.5, β = 112.6	a = 52.3, b = 108.7, c = 59.1; α = γ = 90.0, β = 93.7
Resolution range (Å)	1.55 (1.65-1.55)	2.00 (2.10-2.00)
N° of reflections (last bin)	246150 (43667)	240759 (34051)
N° of unique reflections (last bin)	87728 (15117)	44465 (6044)
Completeness (%) (last bin)	92.1 (92.8)	99.7 (100.0)
Redundancy	2.80 (2.89)	5.41 (5.63)
$\langle I/\sigma(I) \rangle$	12.53 (2.00)	10.89 (4.50)
$R_{\text{meas}}$ (%)	5.4 (71.5)	11.1 (58.2)
CC <sub>1/2</sub>	99.8 (66.7)	99.7 (92.6)
<b>Refinement statistics</b>		
N° of total model atoms	5608	5630
$R_{\text{free}}/R_{\text{work}}$	20.87/17.31	23.81/20.27
Ramachandran favored (%)	97.4	98.9
Ramachandran allowed (%)	2.2	1.1
Ramachandran outliers (%)	0.3	0
<i>Rmsd from ideal</i>		
Bond lengths (Å)	0.002	0.002
Bond angles (°)	0.739	0.553
All-atoms clashscore	11	10.1

**Table S3| Bismethylated SonA core peptide masses searched for (a) and identified (b) in the LC-MS/MS data from 24 hour cultures of wt *S. oneidensis*. Only verified masses are highlighted in yellow.**

**a. Peptides searched for and the corresponding monoisotopic mass for the bismethylated species**

Sequence	Charge		Sequence	Charge	
	2	1		2	1
TLSGDSSYQSYLVISHGNGD	1064.496	2127.983	SYQSYLVISHG	641.3279	1281.648
LSGDSSYQSYLVISHGNGD	1013.972	2026.936	YQSYLVISHG	597.8119	1194.616
SGDSSYQSYLVISHGNGD	957.4298	1913.852	QSYLVISHG	516.2802	1031.553
GDSSYQSYLVISHGNGD	913.9138	1826.82	SYLVISHG	452.2509	903.494
<b>DSSYQSYLVISHGNGD</b>	<b>885.403</b>	1769.798	YLVISHG	408.7349	816.462
SSYQSYLVISHGNGD	827.8896	1654.771	LVISHG	327.2032	653.3986
SYQSYLVISHGNGD	784.3735	1567.739	TLSGDSSYQSYLVISH	892.9392	1784.871
YQSYLVISHGNGD	740.8575	1480.707	LSGDSSYQSYLVISH	842.4154	1683.823
QSYLVISHGNGD	659.3259	1317.644	SGDSSYQSYLVISH	785.8734	1570.739
SYLVISHGNGD	595.2966	1189.585	GDSSYQSYLVISH	742.3574	1483.707
YLVISHGNGD	551.7806	1102.553	DSSYQSYLVISH	713.8466	1426.685
LVISHGNGD	470.2489	939.49	SSYQSYLVISH	656.3332	1311.658
TLSGDSSYQSYLVISHGNG	1006.982	2012.957	SYQSYLVISH	612.8171	1224.626
LSGDSSYQSYLVISHGNG	956.4583	1911.909	YQSYLVISH	569.3011	1137.594
SGDSSYQSYLVISHGNG	899.9163	1798.825	QSYLVISH	487.7695	974.5311
GDSSYQSYLVISHGNG	856.4003	1711.793	SYLVISH	423.7402	846.4725
DSSYQSYLVISHGNG	827.8896	1654.771	YLVISH	380.2242	759.4405
SSYQSYLVISHGNG	770.3761	1539.744	LVISH	298.6925	596.3772
SYQSYLVISHGNG	726.8601	1452.712	TLSGDSSYQSYLVIS	824.4098	1647.812
YQSYLVISHGNG	683.3441	1365.68	LSGDSSYQSYLVIS	773.886	1546.764
QSYLVISHGNG	601.8124	1202.617	SGDSSYQSYLVIS	717.3439	1433.68
SYLVISHGNG	537.7831	1074.558	GDSSYQSYLVIS	673.8279	1346.648
YLVISHGNG	494.2671	987.5264	DSSYQSYLVIS	645.3172	1289.627
LVISHGNG	412.7354	824.463	SSYQSYLVIS	587.8037	1174.6
TLSGDSSYQSYLVISHGN	978.4714	1955.935	SYQSYLVIS	544.2877	1087.568
LSGDSSYQSYLVISHGN	927.9476	1854.887	YQSYLVIS	500.7717	1000.536
SGDSSYQSYLVISHGN	871.4056	1741.803	QSYLVIS	419.24	837.4722
GDSSYQSYLVISHGN	827.8896	1654.771	SYLVIS	355.2107	709.4136
DSSYQSYLVISHGN	799.3788	1597.75	YLVIS	311.6947	622.3816
SSYQSYLVISHGN	741.8654	1482.723	LVIS	230.163	459.3183
SYQSYLVISHGN	698.3493	1395.691	TLSGDSSYQSYLVI	780.8938	1560.78
YQSYLVISHGN	654.8333	1308.659	LSGDSSYQSYLVI	730.3699	1459.732
QSYLVISHGN	573.3017	1145.595	SGDSSYQSYLVI	673.8279	1346.648
SYLVISHGN	509.2724	1017.537	GDSSYQSYLVI	630.3119	1259.616
YLVISHGN	465.7564	930.5049	DSSYQSYLVI	601.8012	1202.594
LVISHGN	384.2247	767.4416	SSYQSYLVI	544.2877	1087.568
TLSGDSSYQSYLVISHG	921.45	1841.892	SYQSYLVI	500.7717	1000.536

<b>LSGDSSYQSYLVISHG</b>	870.9261	1740.844	<b>YQSYLVI</b>	457.2557	913.5035
<b>SGDSSYQSYLVISHG</b>	814.3841	1627.76	<b>QSYLVI</b>	375.724	750.4402
<b>GDSSYQSYLVISHG</b>	770.8681	1540.728	<b>SYLVI</b>	311.6947	622.3816
<b>DSSYQSYLVISHG</b>	742.3574	1483.707	<b>YLVI</b>	268.1787	535.3496
<b>SSYQSYLVISHG</b>	684.8439	1368.68	<b>LVI</b>	186.647	372.2862

**b. Monoisotopic masses identified in the MS1 and MS2 data**

Sequence	Parent mass	MS1	Retention time (min)	MS2 data supporting sequence
<b>DSSYQSYLVISHGNGD</b>	885.4041	✓	11.44	✓
<b>SYLVISHGNGD</b>	595.2997	✓	11.50	✗

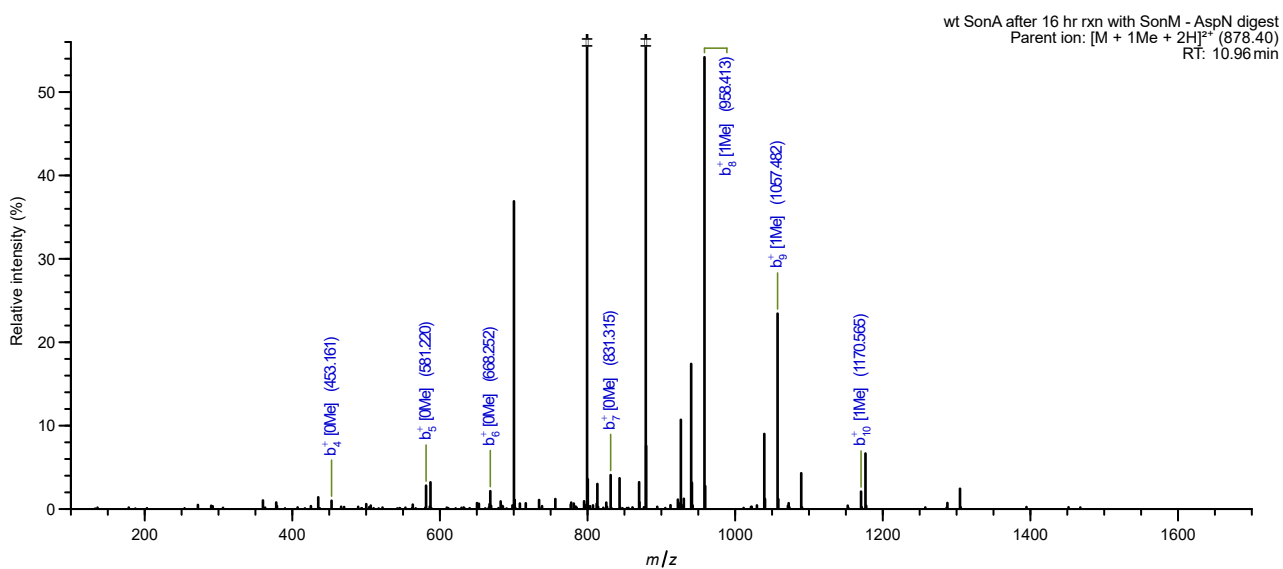
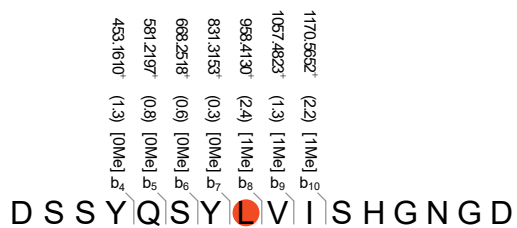
**Figure S1| Mass spectrometric analysis of SonA variants.** HPLC-MS/MS spectra from wt his<sub>6</sub>-SonA and variants after reaction overnight with wt his<sub>6</sub>-SonM. Reactions were set-up under conditions containing 50 mM HEPES pH 8, 1 mM SAM, 8 μM SAHN, 5 μM his<sub>6</sub>-SonM, and 80 μM his<sub>6</sub>-SonA (mutant or wt). Reactions were quenched with 5x SDS-dye and boiled for 5 min. SonA proteins were prepared for mass spectrometry analysis by in-gel digest (AspN or Trypsin) and the MS/MS scans showing the methylation pattern for each mutant is displayed below. For wt SonA, 0, 1, and 2 methylated species are shown (A-D), for SonA\_K51-L53dup (E), SonA\_K51-L53del (F), \*SonA\_K51-S54dup (G), SonA\_K51-S54del (H), \*SonA\_M47-L53dup (I), SonA\_M47-L53del (J) scans for the 2-methylated species are shown as the methylation data was consistent with wt. For SonA\_K51-S57del (K-O) and SonA\_Q60-Y62del (P-S) 0, 1, and 2 methylated species are shown. Primary amino acid sequences for the digested peptide are written above the spectra with confirmed (closed) or inferred (open) methylated residues circled (orange). Within the amino acid sequence, grey lines denote fragmentation with the masses written above (b-ions) and below (y-ions). Masses of fragments containing methylated residues are denoted with “Me” in brackets. Generally, a cut-off of 10 ppm was used. Samples marked with “\*” used a cut-off of 20 ppm. Difference (ppm) between expected and observed masses are in parentheses.



B

● Methylation localized by LC-MS/MS

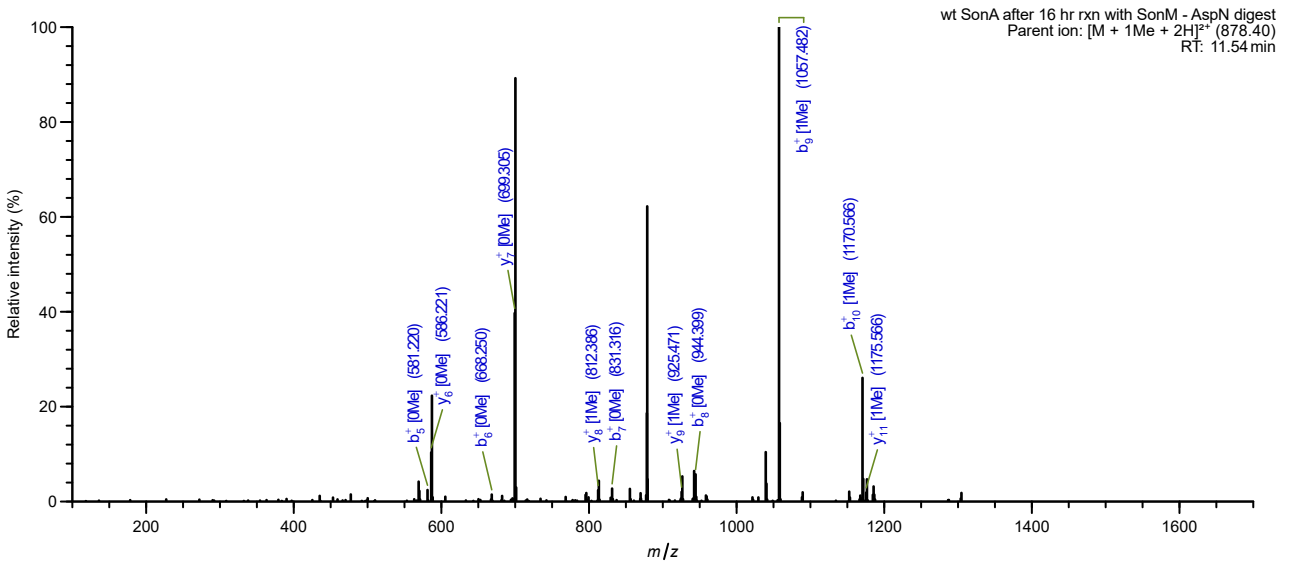
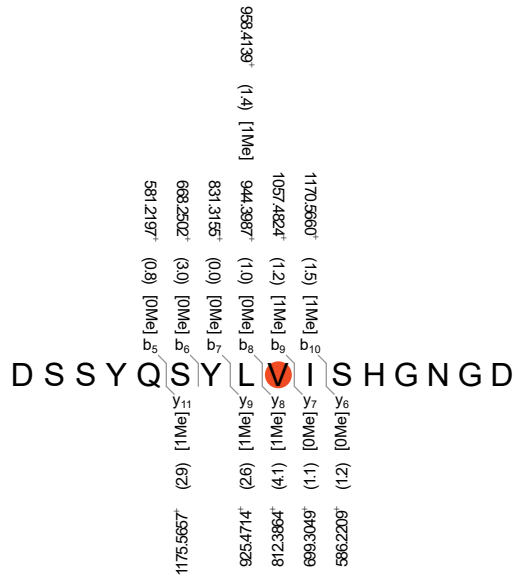
○ Methylation inferred by LC-MS/MS





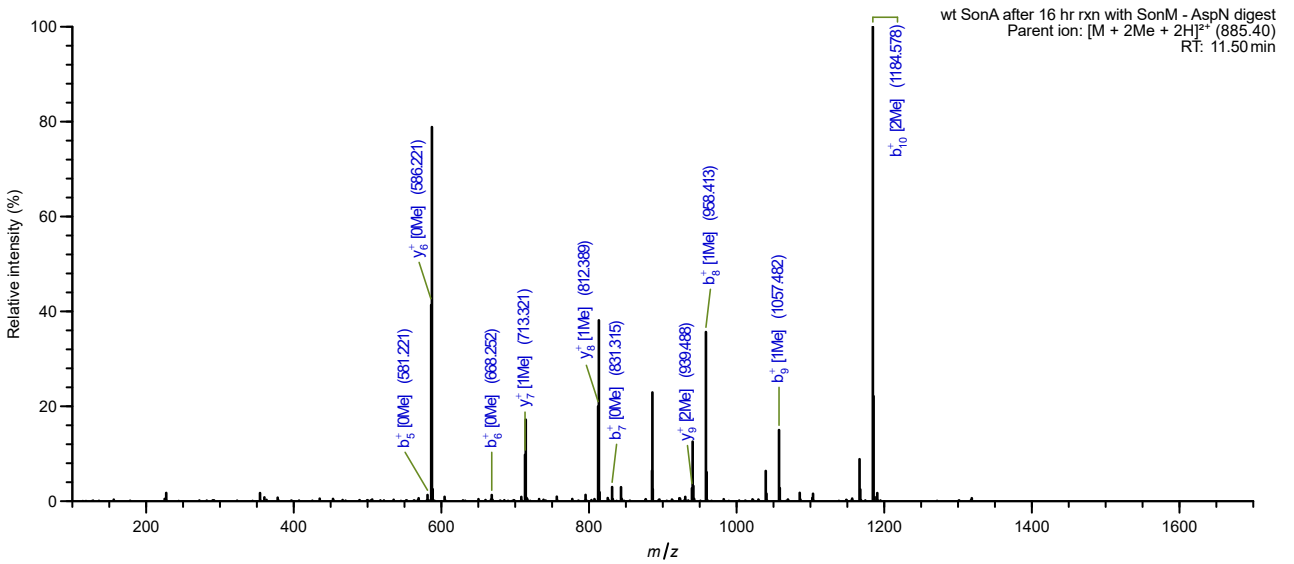
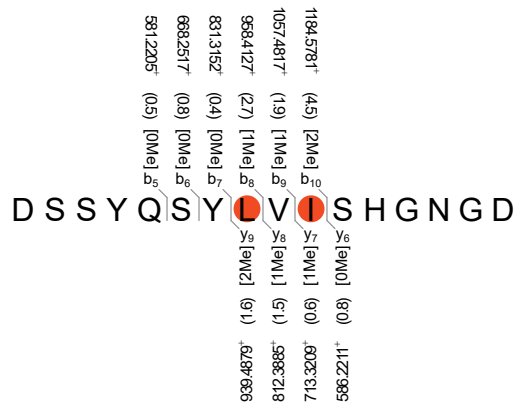
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- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS



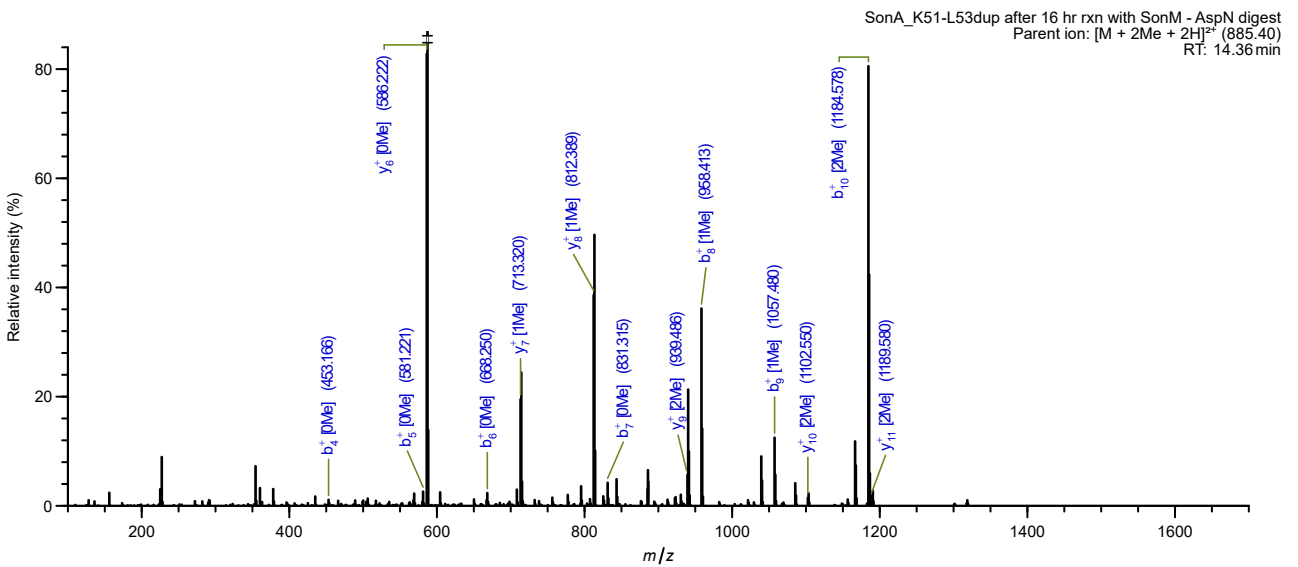
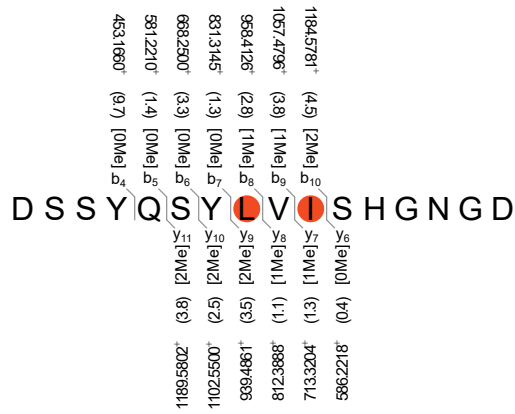
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- Methylation inferred by LC-MS/MS



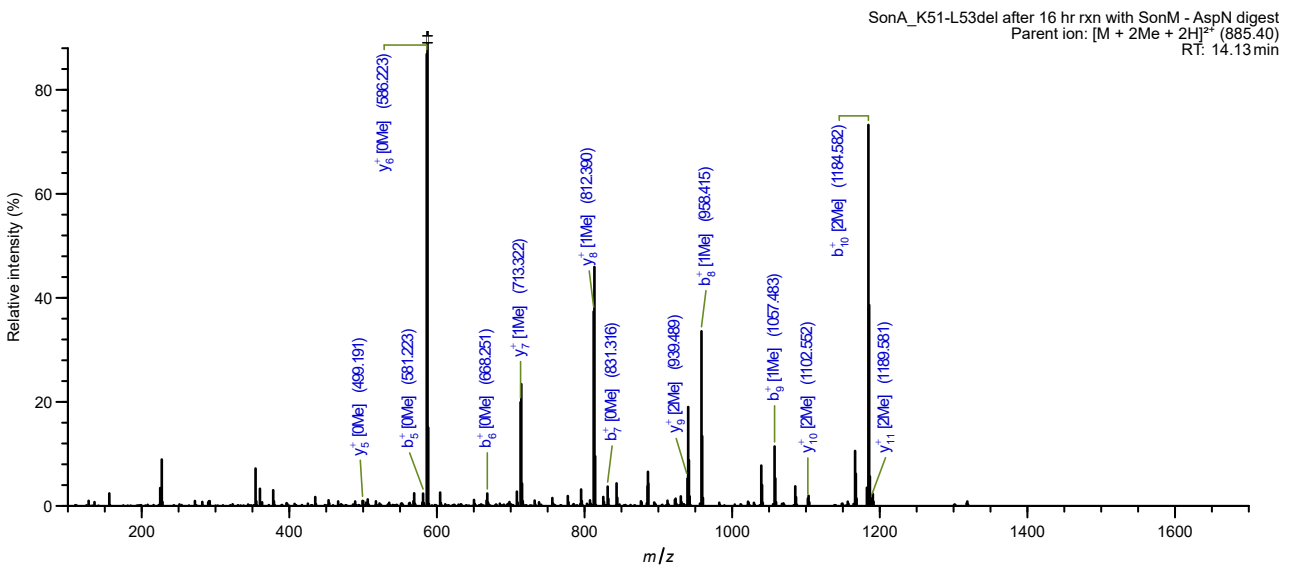
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- Methylation inferred by LC-MS/MS



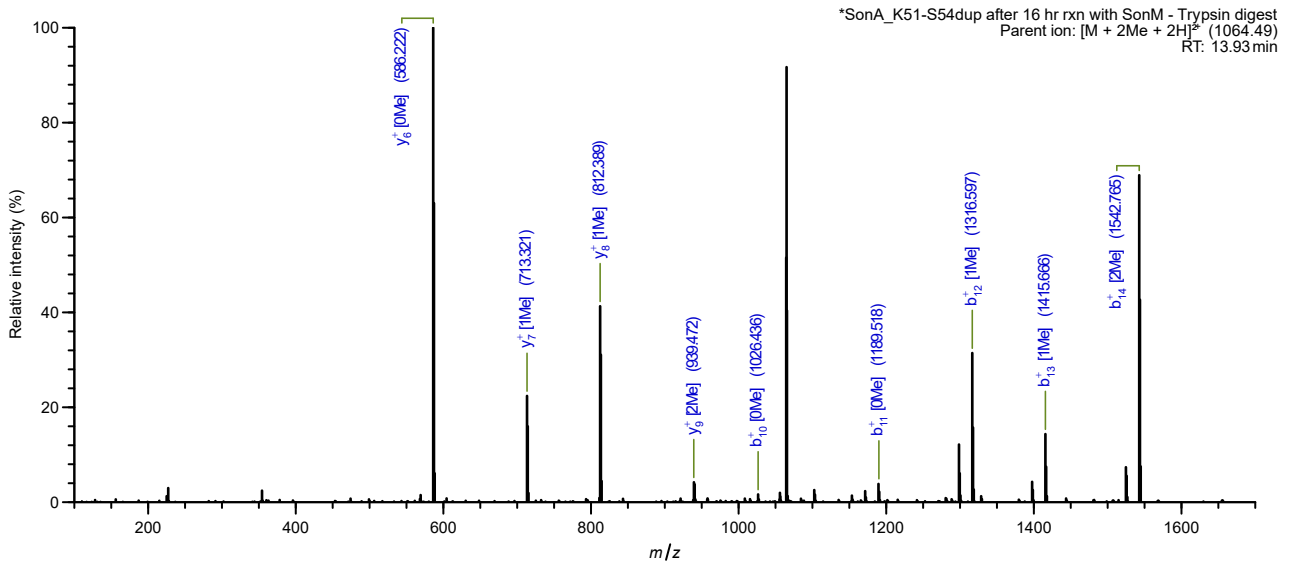
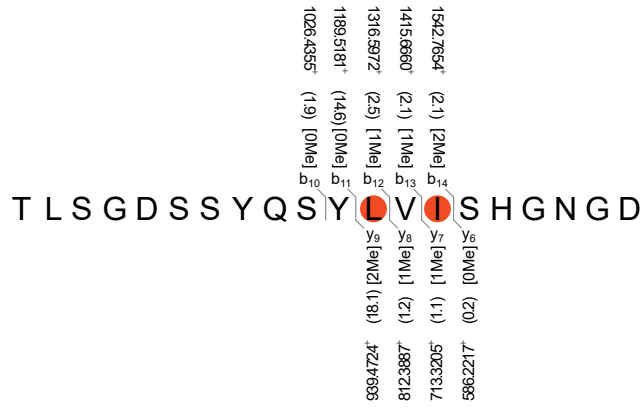
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- Methylation inferred by LC-MS/MS



G

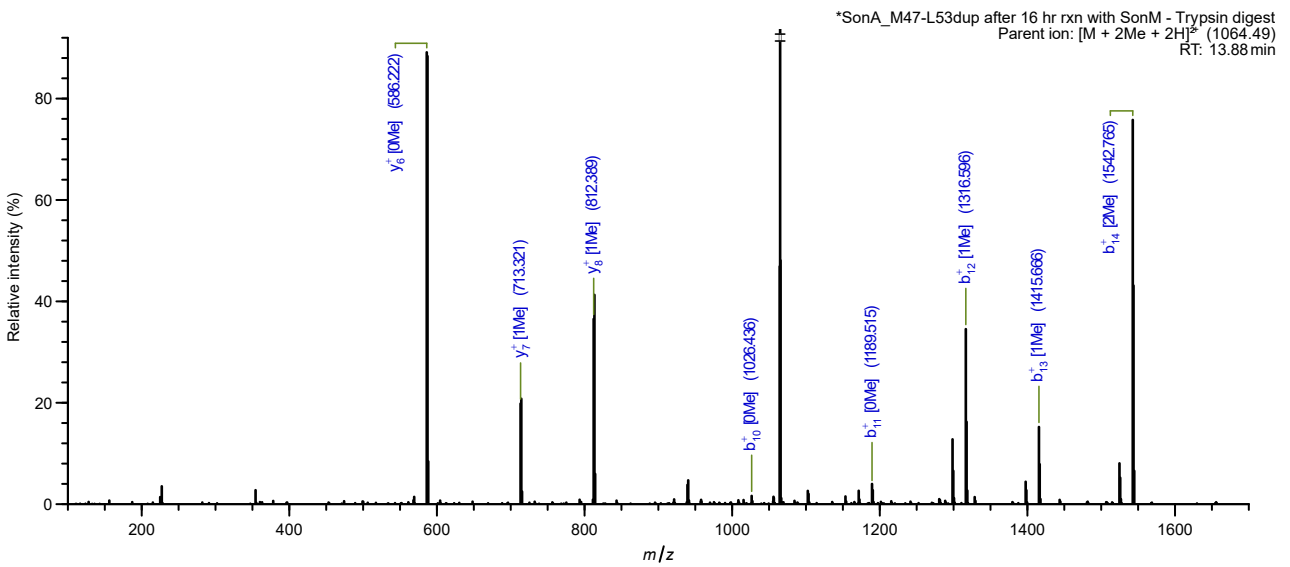
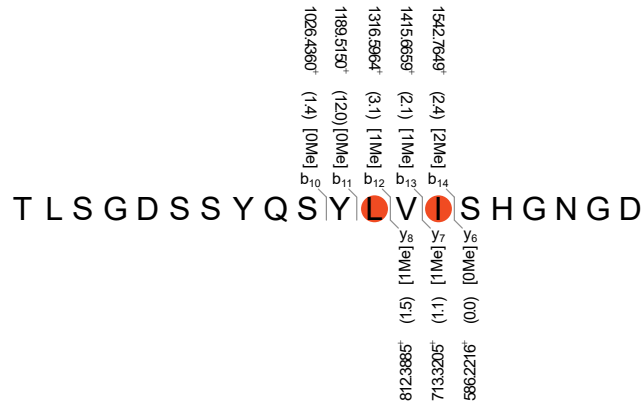
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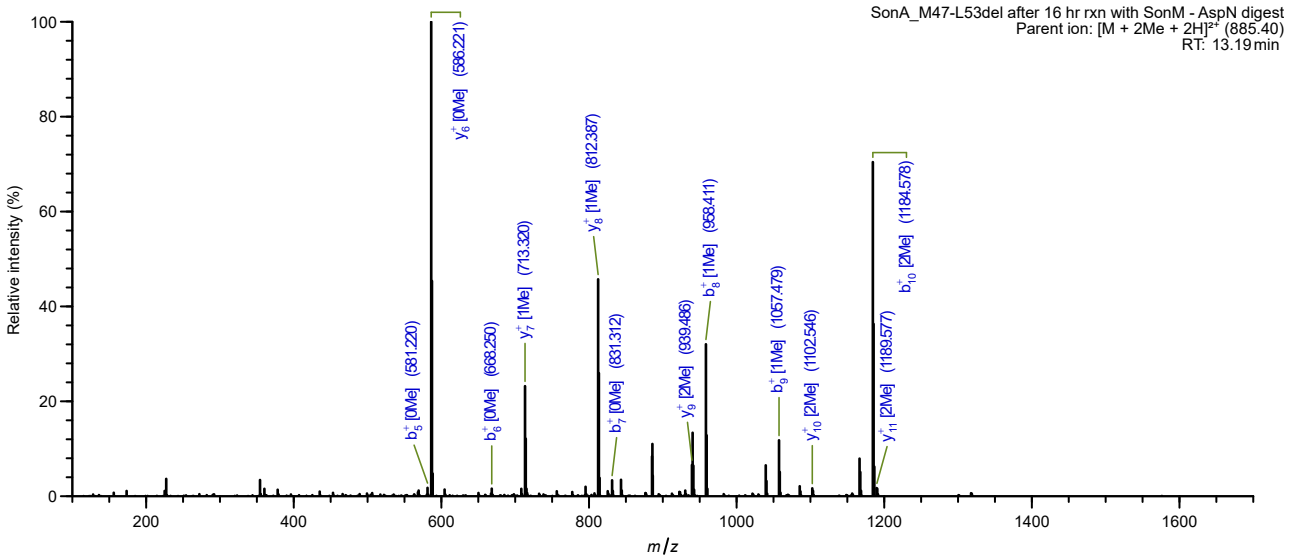
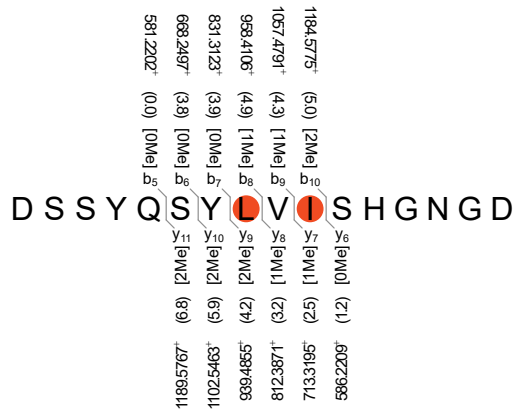
I

- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS



J

- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS

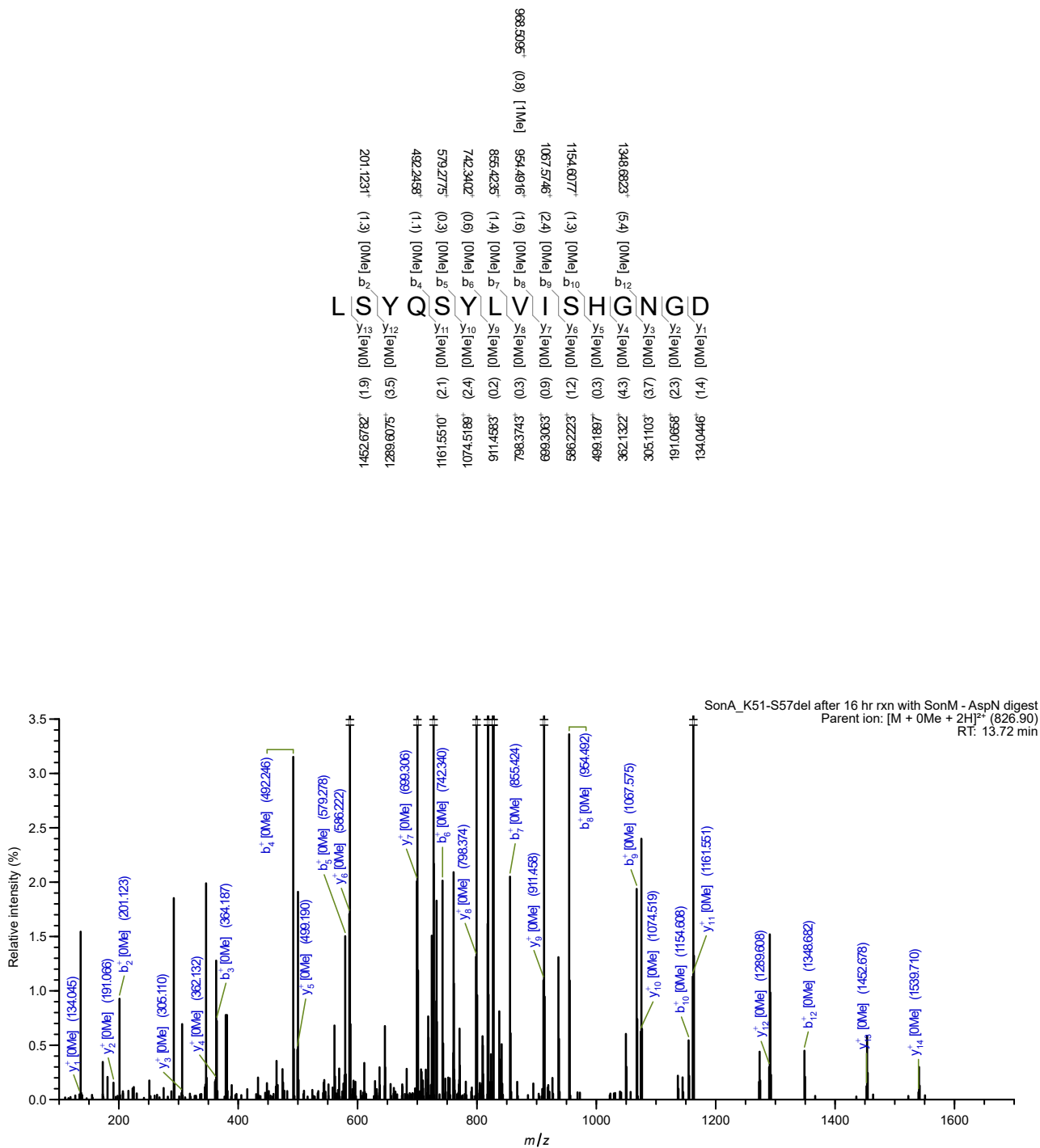




K

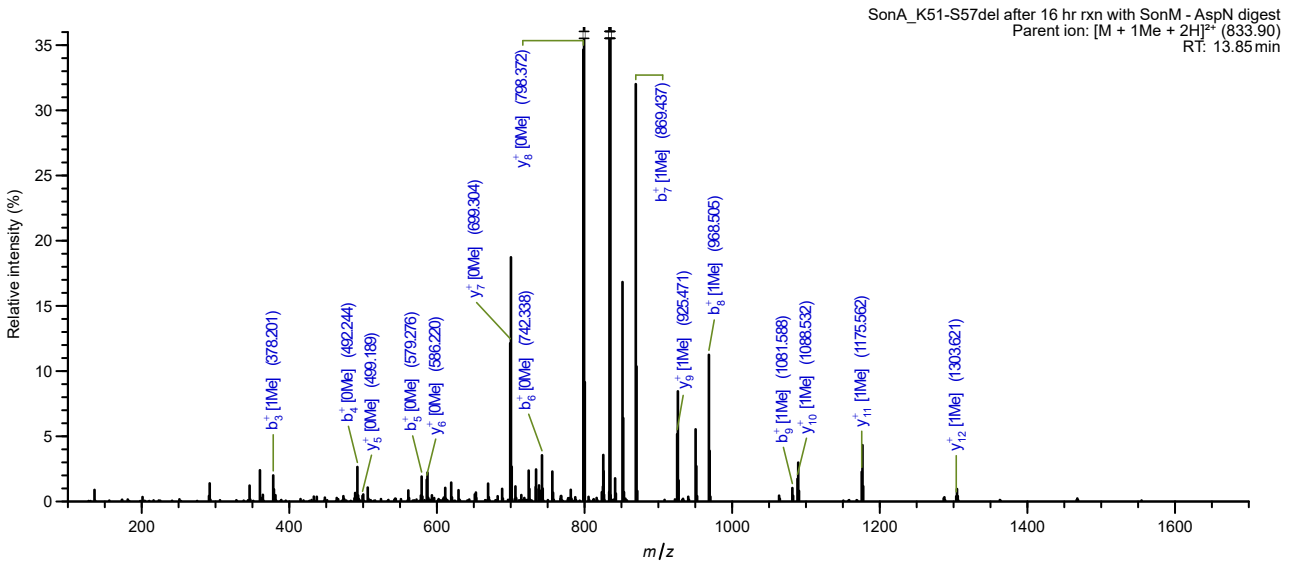
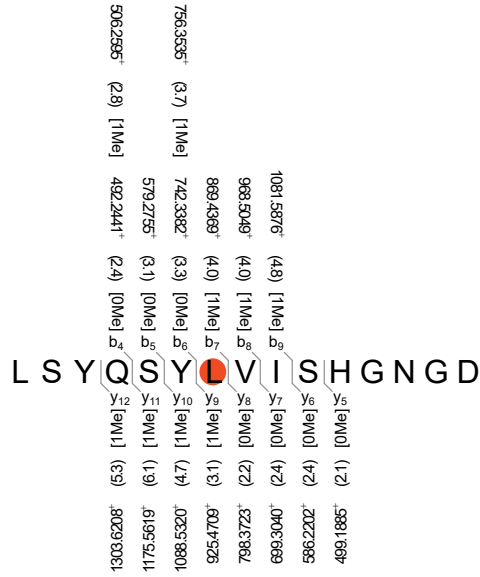
● Methylation localized by LC-MS/MS

○ Methylation inferred by LC-MS/MS



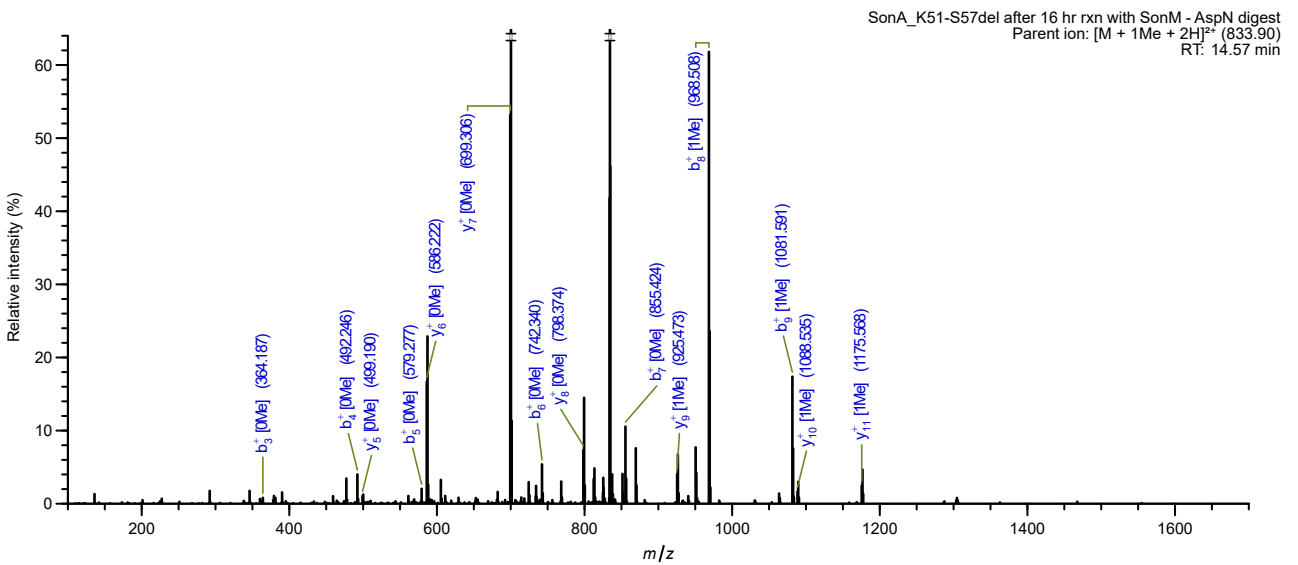
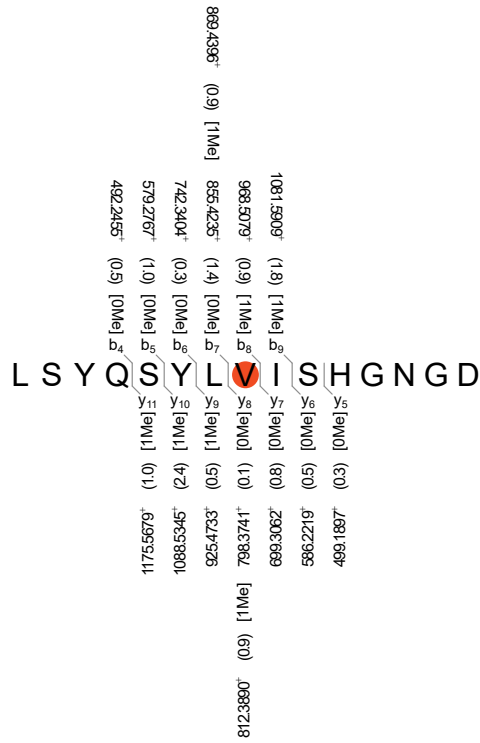
L

- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS



M

- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS

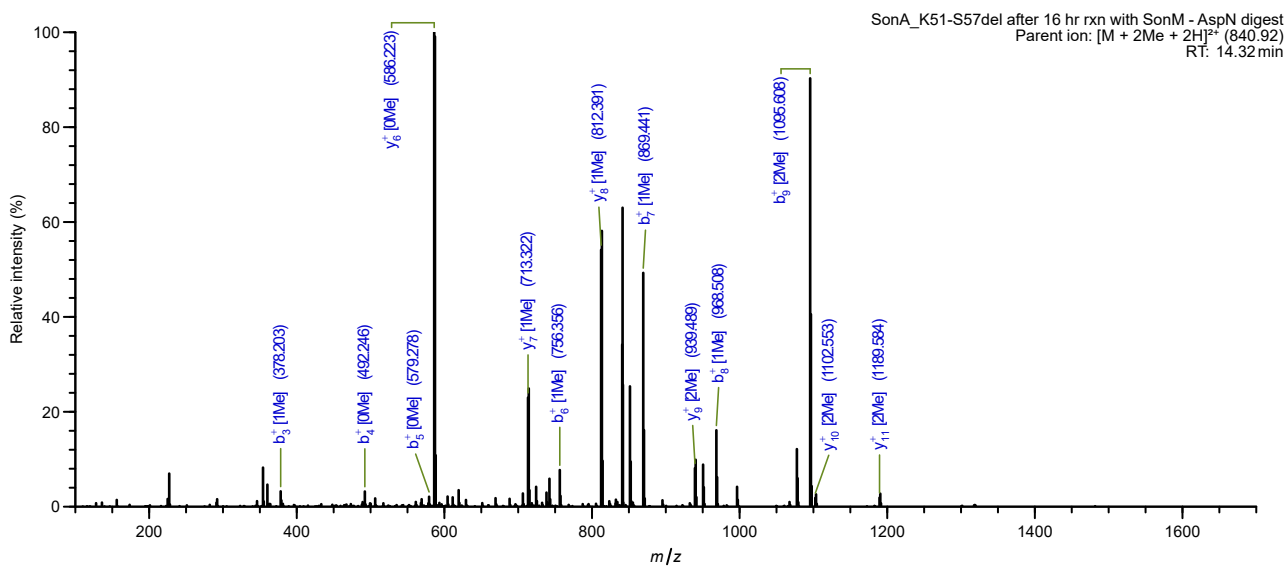
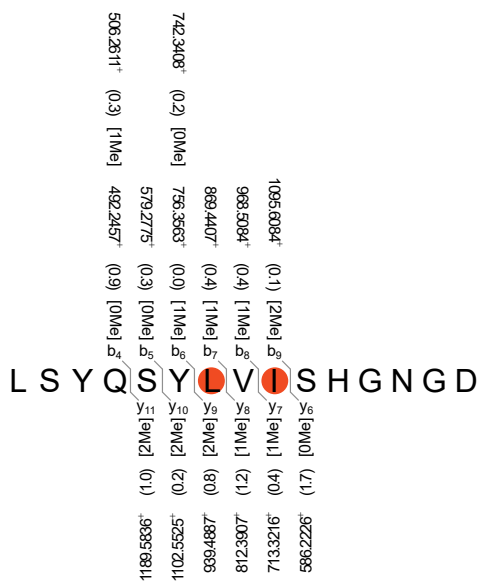




O

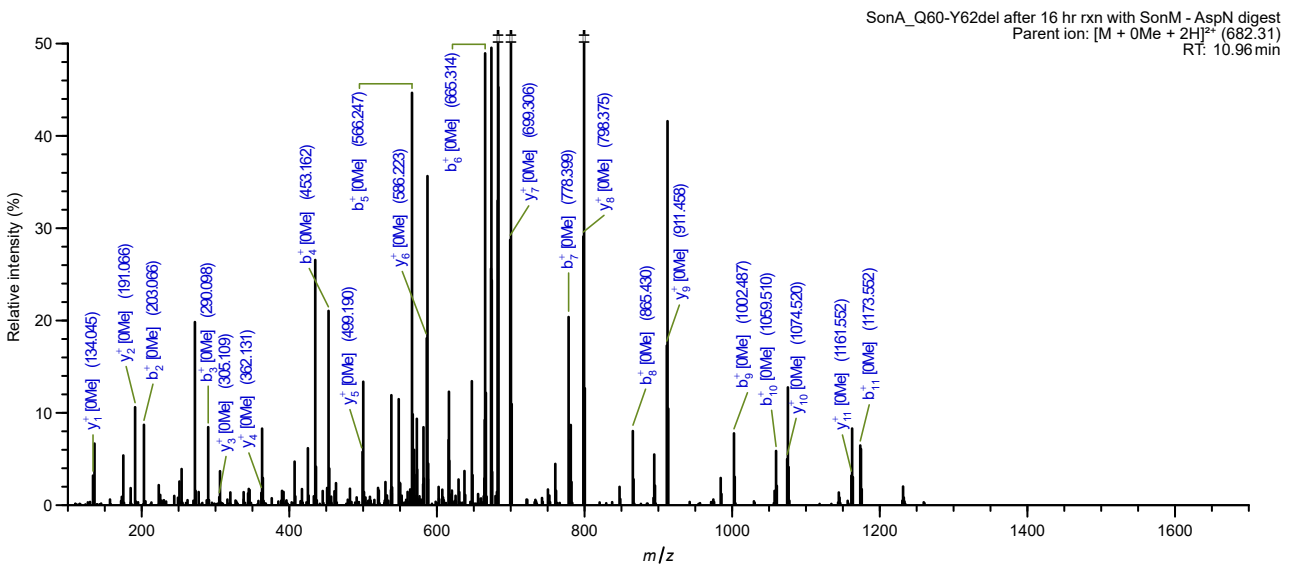
● Methylation localized by LC-MS/MS

○ Methylation inferred by LC-MS/MS



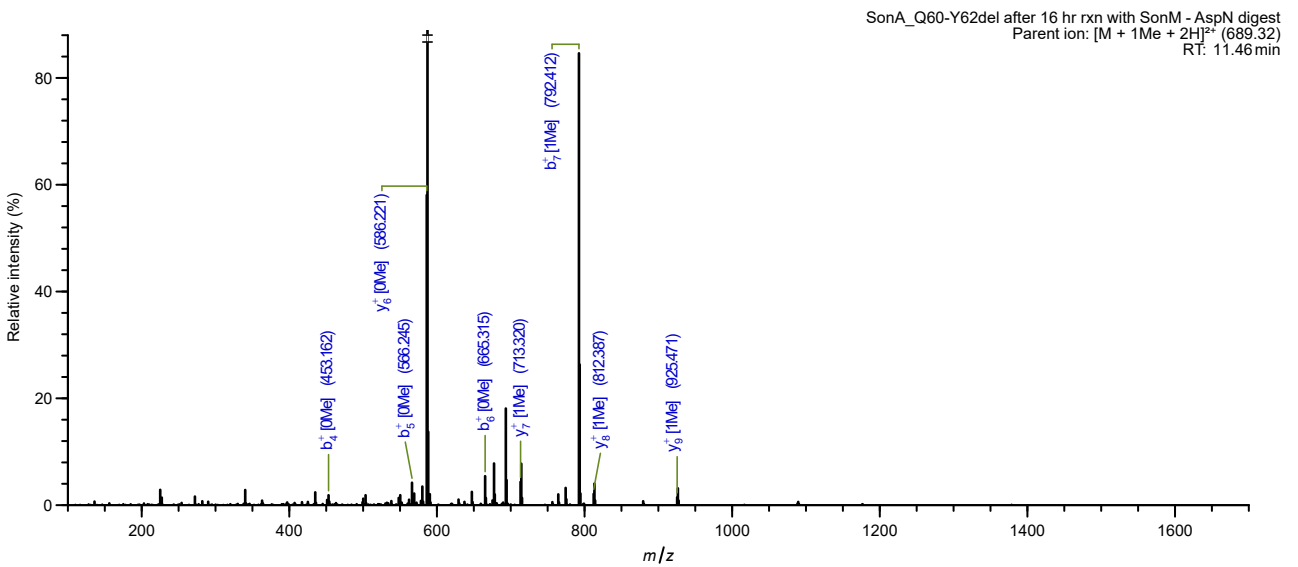
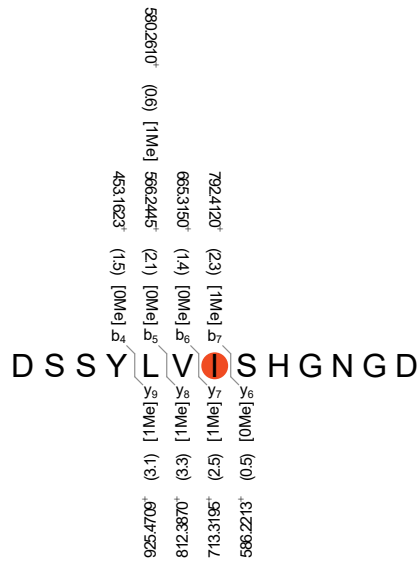
P

- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS



Q

- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS

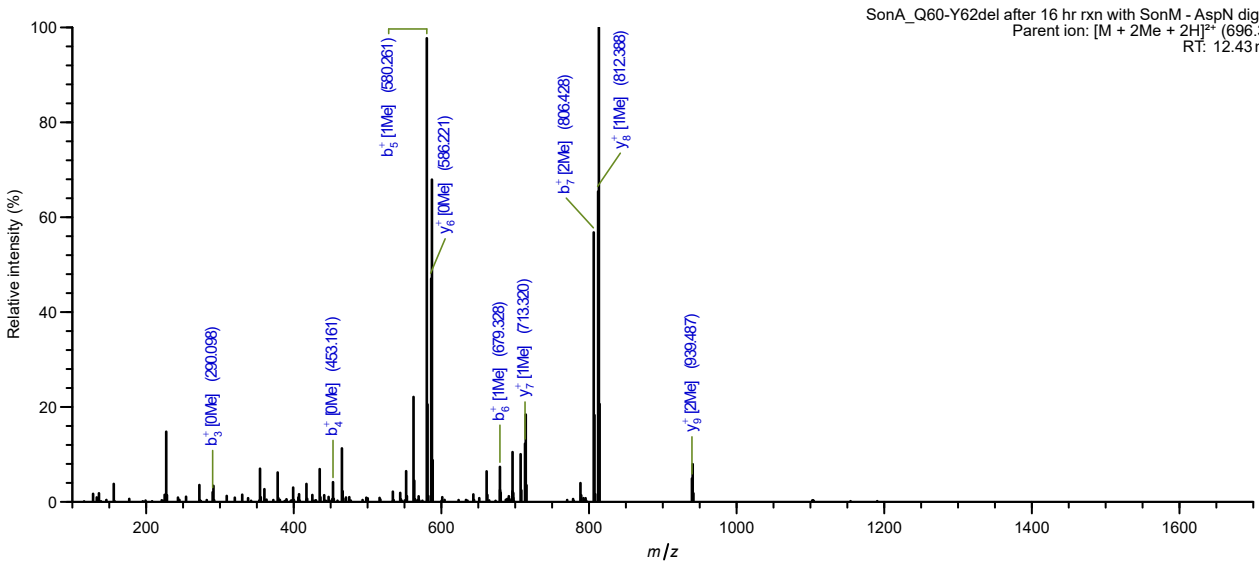
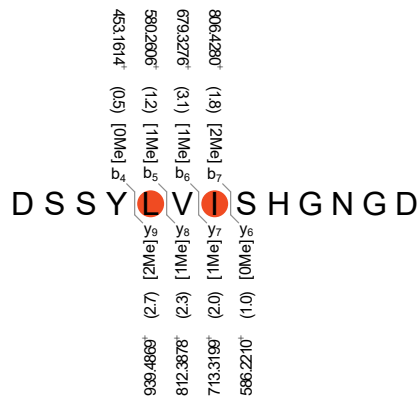




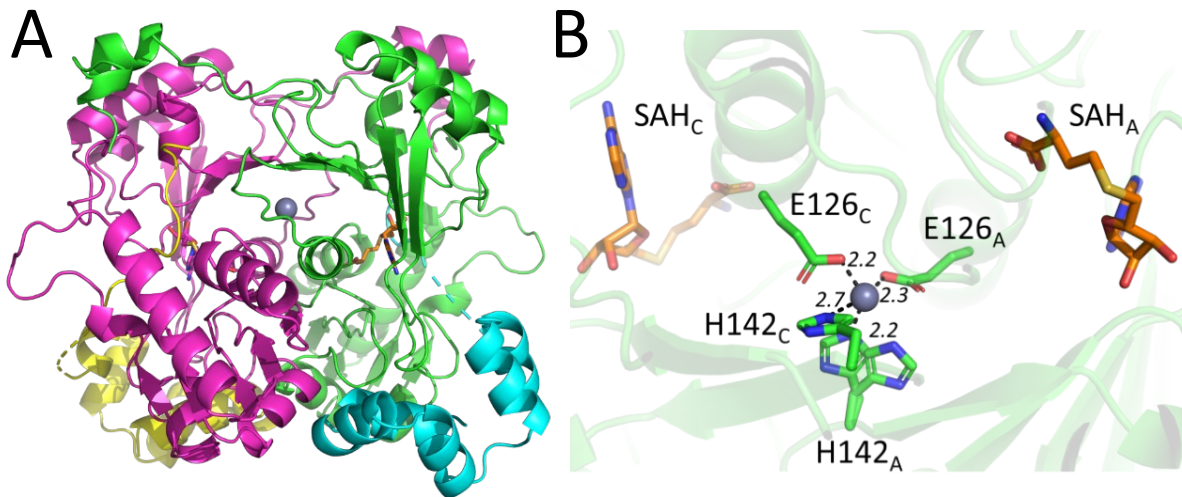


S

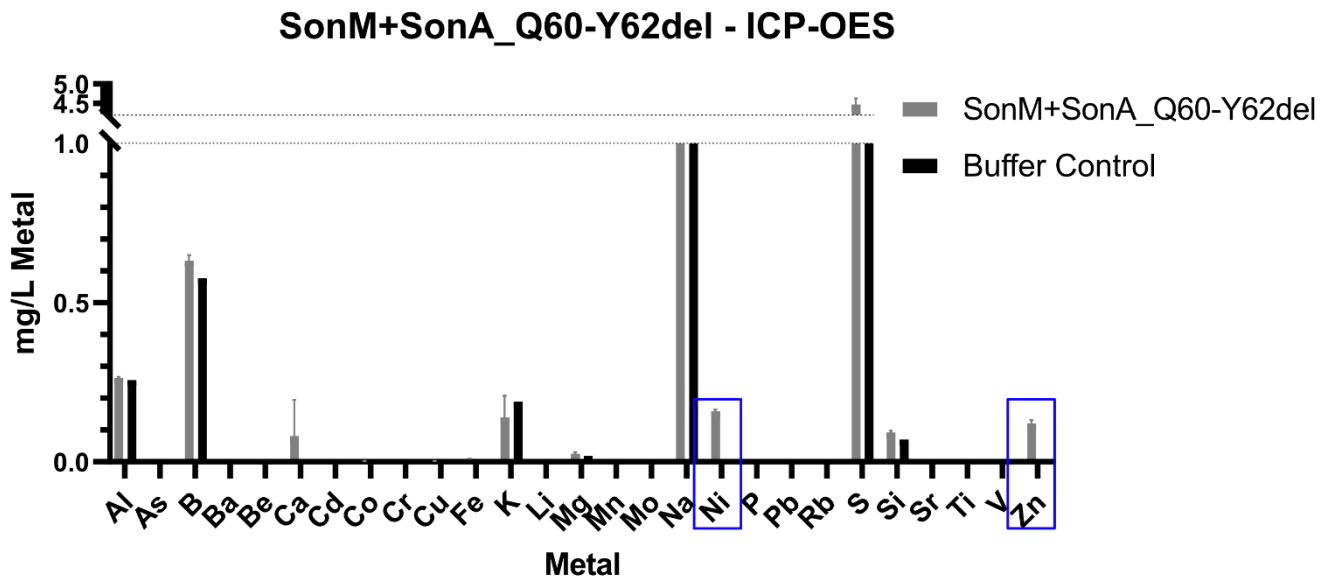
- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS



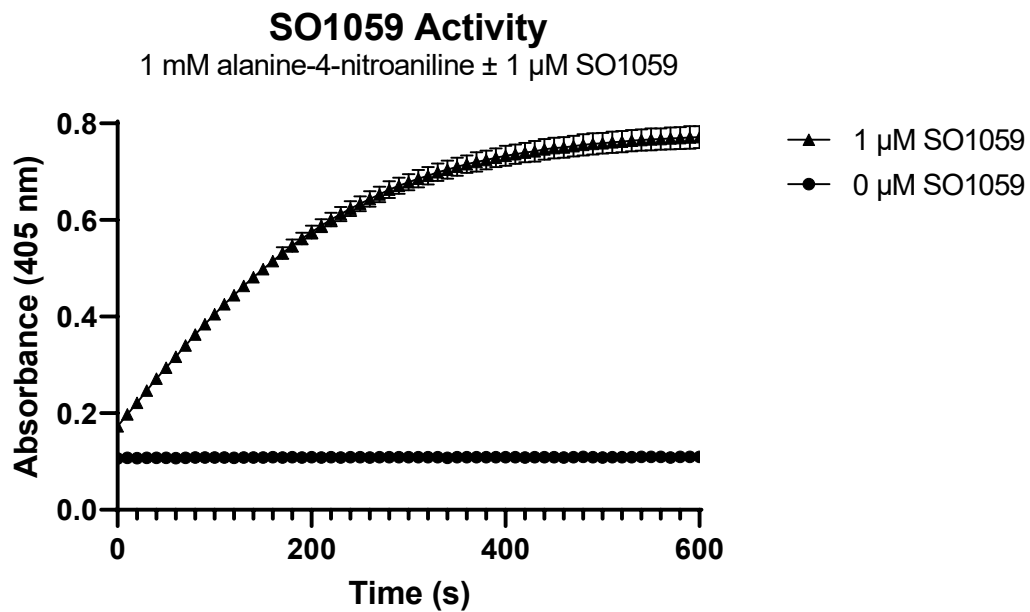
**Figure S2| SonM—SonA\_Q60-Y62del—SAH structure.** PDB code: 8T1S (A) Global orientation of the heterocomplex (green and pink cartoon, SonM, yellow and cyan cartoon, SonA\_Q60-Y62del). Both heterodimers have SAH bound (orange sticks) in the active site. A metal (shown as a grey sphere) is depicted. (B) Close view on the metal binding site at the interface of the two SonM molecules (chain A and C, designated by subscripted letters). Distances are indicated in Ångstroms.



**Figure S3| ICP-OES analysis for SonM+SonA\_Q60-Y62del.** An aliquot of the SonM+SonA\_Q60-Y62del complex used to lay crystal trays was concentrated, acidified, and submitted for ICP-OES at the Research Analytical Laboratory at the University of Minnesota. A fraction containing protein (grey) was run in duplicate and compared to a buffer-only control (black) plotted below. The metal is plotted against the concentration measured in the sample (mg/L). Two metals, zinc and nickel (blue boxes), were present in both replicates at ~1:1 ratio with the concentration of protein complex and absent from the buffer control.

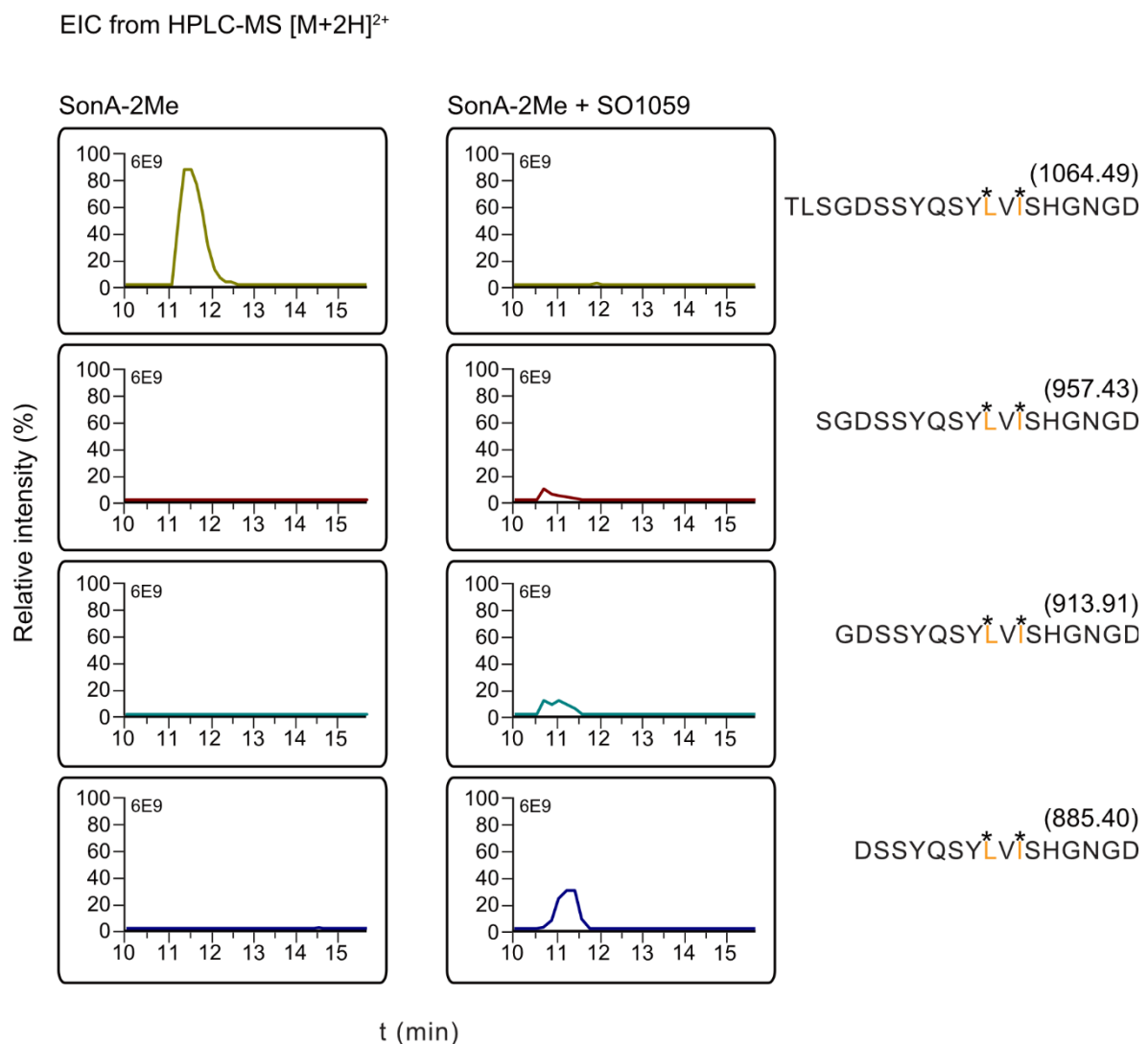


**Figure S4| SO1059 activity with alanine-*p*-nitroaniline.** Gene *so1059* was heterologously expressed and SO1059 was purified from *E. coli* and assayed with 1 mM alanine-4-nitroaniline in 50 mM HEPES buffer pH 8. Change in absorbance over time was monitored at 405 nm with reads every 10 seconds. Three technical replicates were performed for each condition (with and without SO1059). The average value is plotted with error bars representing the standard deviation between replicates. Triangle symbols were used to plot the absorbance for the reaction with SO1059. Circle symbols were used for the reaction without SO1059.



**Figure S5| Mass spectrometric analysis of SO1059 activity with trypsinized SonA-2Me.**

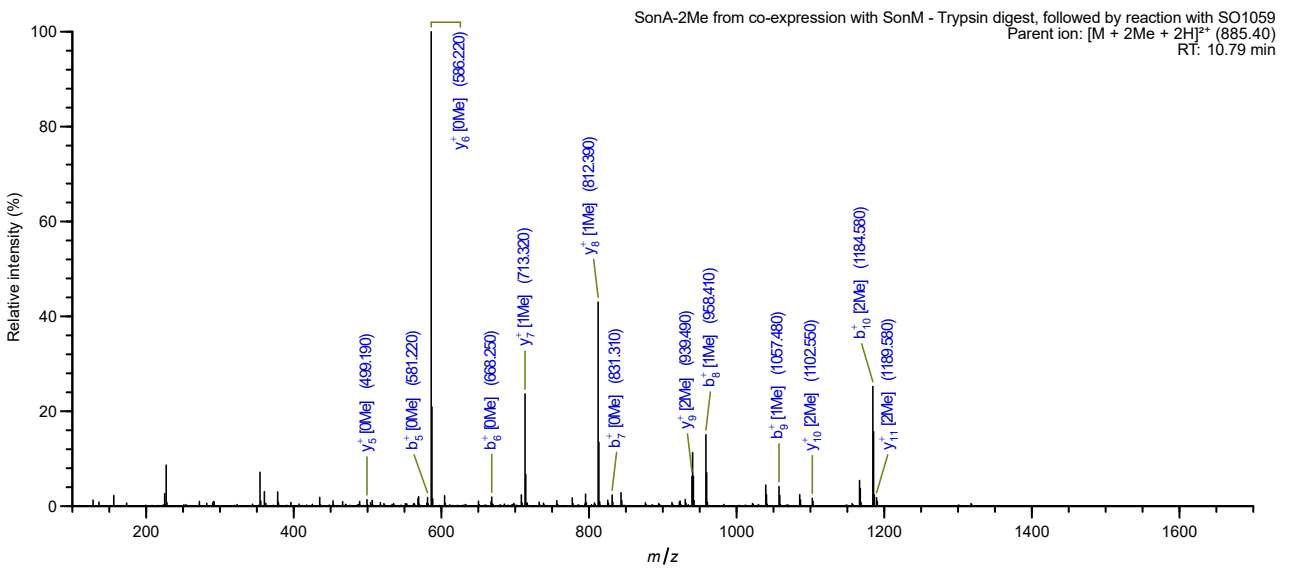
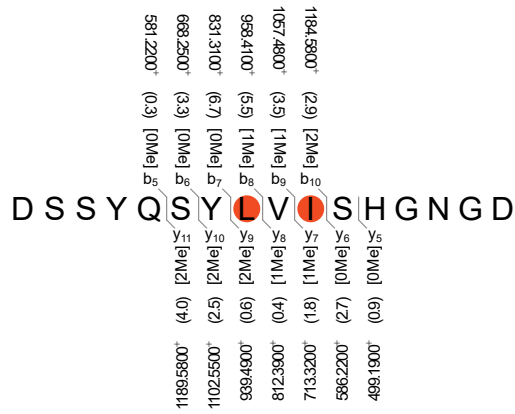
SO1059 was assayed for 50 hours in vitro with overnight trypsin digested SonA-2Me. (A) EIC from HPLC-MS of trypsin digested SonA-2Me with and without SO1059. Sequence is denoted to the right of the chromatogram, where methylated residues are shown in orange with an asterisk. (B) MS/MS spectra of the major species of trypsin digested SonA-2Me after 50-hour incubation at room temperature. (C) HPLC-MS/MS spectra of the major species of trypsin digested SonA-2Me after 50-hour incubation with SO1059 at room temperature. Primary amino acid sequences for the identified peptides are written above the spectra with confirmed (closed) or inferred (open) methylated residues circled (orange). Grey lines within the amino acid sequence denote the fragmentation with the masses written above (b-ions) and below (y-ions). Masses of fragments containing methylated residues are denoted with "Me" in brackets. A cut-off of 20 ppm was used. Difference (ppm) between expected and observed masses are in parentheses.





C

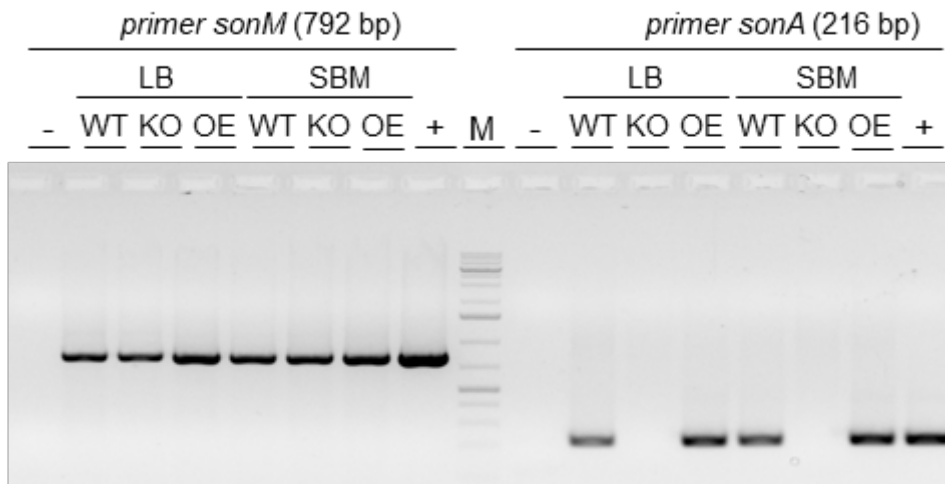
- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS



**Figure S6| Reverse transcription PCR for verification of native transcription conditions of *sonA*.** Extracted RNA was reverse transcribed to cDNA and then amplified with *sonM*- and *sonA*-specific primers. (A) RT-PCR data from *wt S. oneidensis* (WT), the *sonA* knockout strain (KO), and *sonM/A* overexpression strain (OE) cultured for 24 hours in two different media types, LB and SBM. (B) RT-PCR data from the *sonM* knockout strain cultured for 24 hours in SBM as compared to positive control.

A)

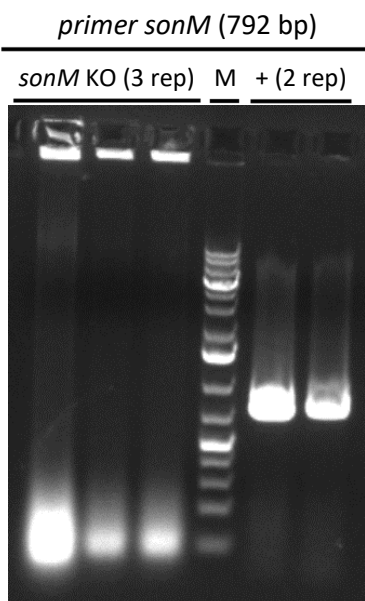
PCR check for *sonM/A*



Template: 1.5  $\mu$ L cDNA  
 Reaction vol. 15  $\mu$ L  
 Q5 DNA polymerase  
 Tm: 64  $^{\circ}$ C

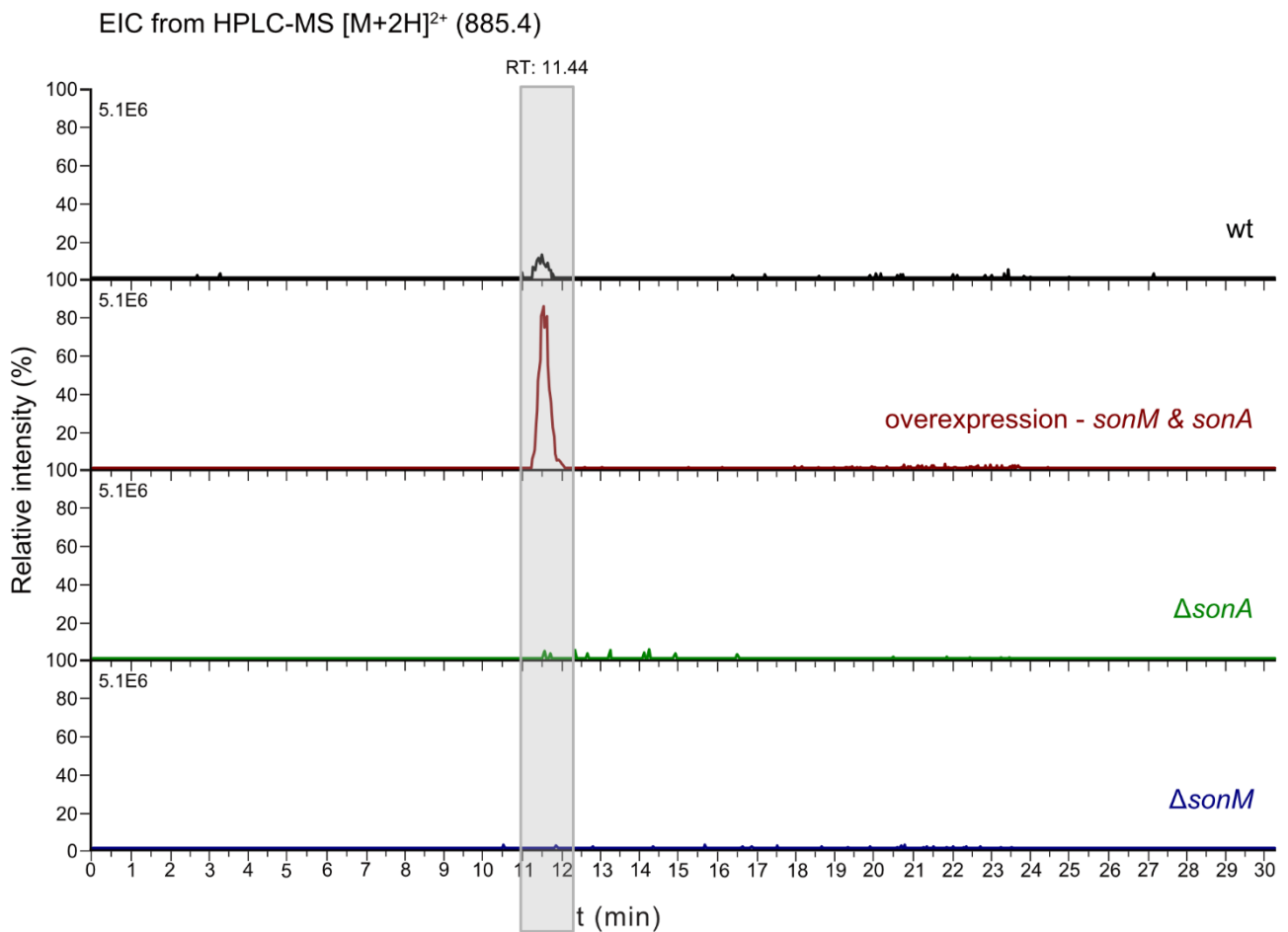
- : no template  
 + : pBBR1-*sonM/A*-C  
 M : DNA marker

B)



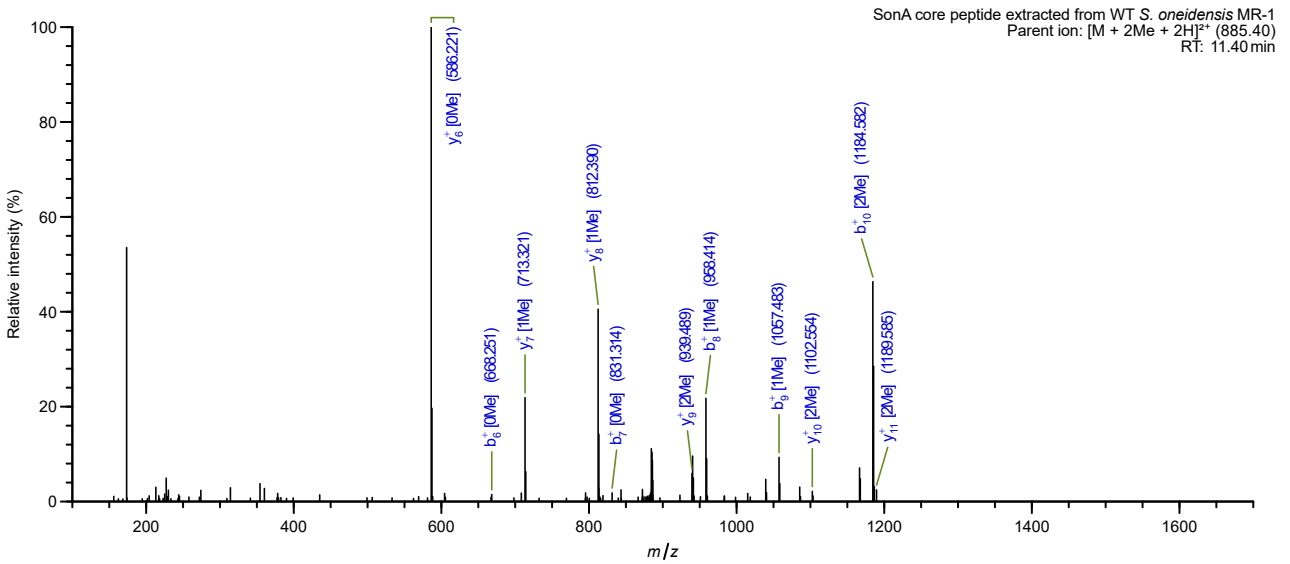
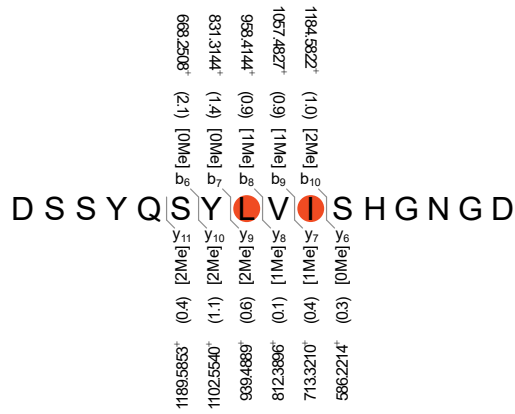


**Figure S7| Mass spectrometric identification of the SonA core peptide.** (A) EIC from HPLC-MS of the identified SonA core peptide 56-DSSYL<sub>Me</sub>VI<sub>Me</sub>SHGNGD-71. The strain is denoted to the right of the chromatogram. The retention time for the mass 885.4 is consistent in both the wt and overexpression strains and absent from the knockout strains. MS/MS spectra of the identified mass from wt (B) and overexpression (C) extracts. Primary amino acid sequences for the identified peptides are written above the spectra with confirmed (closed) or inferred (open) methylated residues circled (orange). Grey lines within the amino acid sequence denote the fragmentation with the masses written above (b-ions) and below (y-ions). Masses of fragments containing methylated residues are denoted with “Me” in brackets. A cut-off of 10 ppm was used. Difference (ppm) between expected and observed masses are in parentheses.



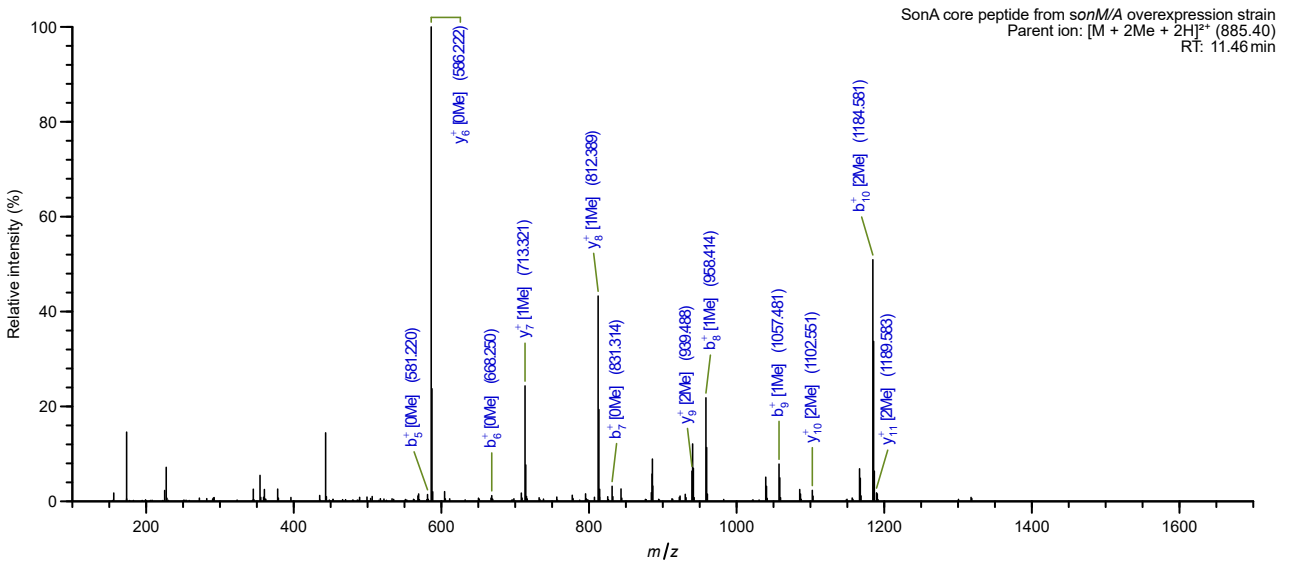
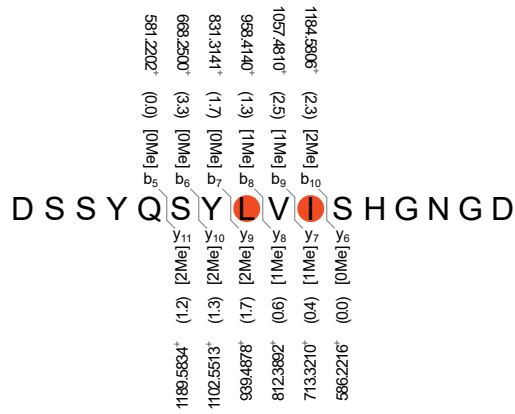
B

- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS

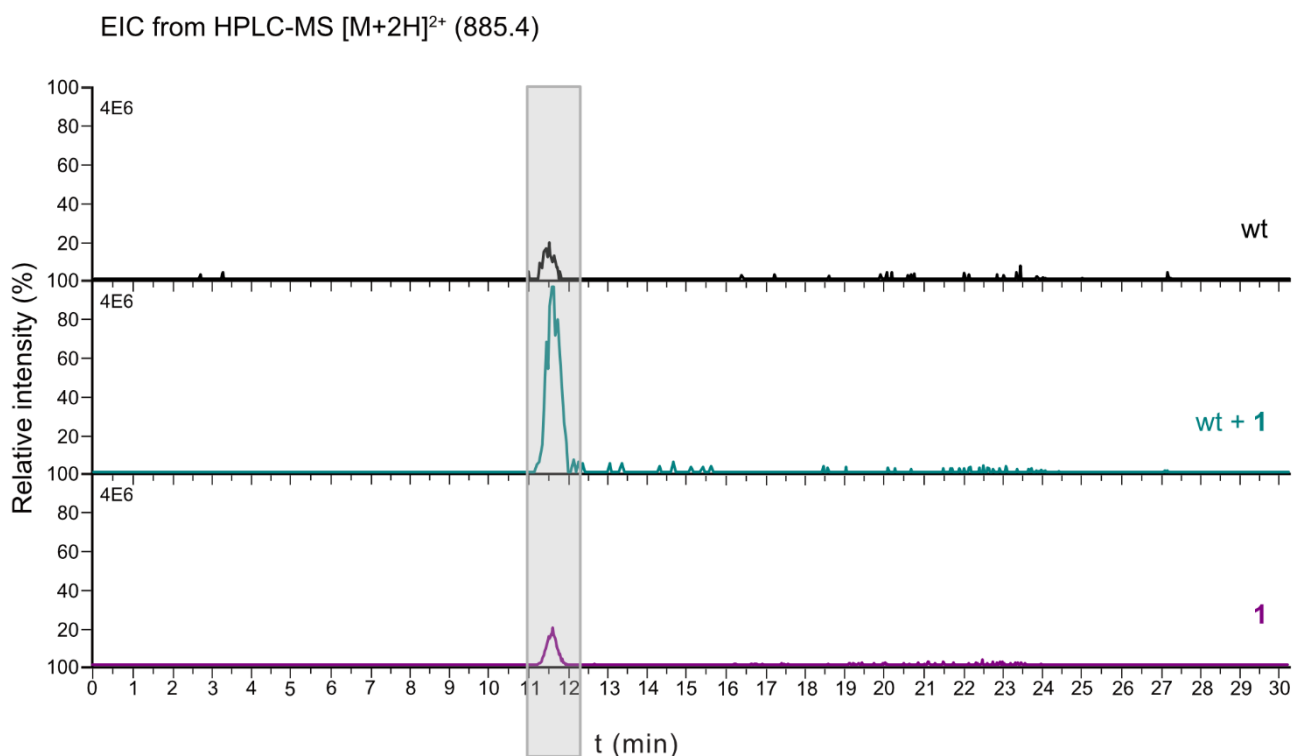


C

- Methylation localized by LC-MS/MS
- Methylation inferred by LC-MS/MS



**Figure S8| Retention time and mass spectrometric analysis of 1, heterologously produced in *E. coli*.** (A) The EIC from HPLC-MS of Shewanellamide A (**1**) heterologously produced in *E. coli* was compared between wt extract (black), co-injection of wt extract and **1** (blue), and **1** (purple). (B) MS/MS spectra of **1**. The primary amino acid sequence for the identified peptide is written above the spectra with confirmed (closed) or inferred (open) methylated residues circled (orange). Grey lines within the amino acid sequence denote the fragmentation with the masses written above (b-ions) and below (y-ions). Masses of fragments containing methylated residues are denoted with “Me” in brackets. A cut-off of 10 ppm was used. Difference (ppm) between expected and observed masses are in parentheses.

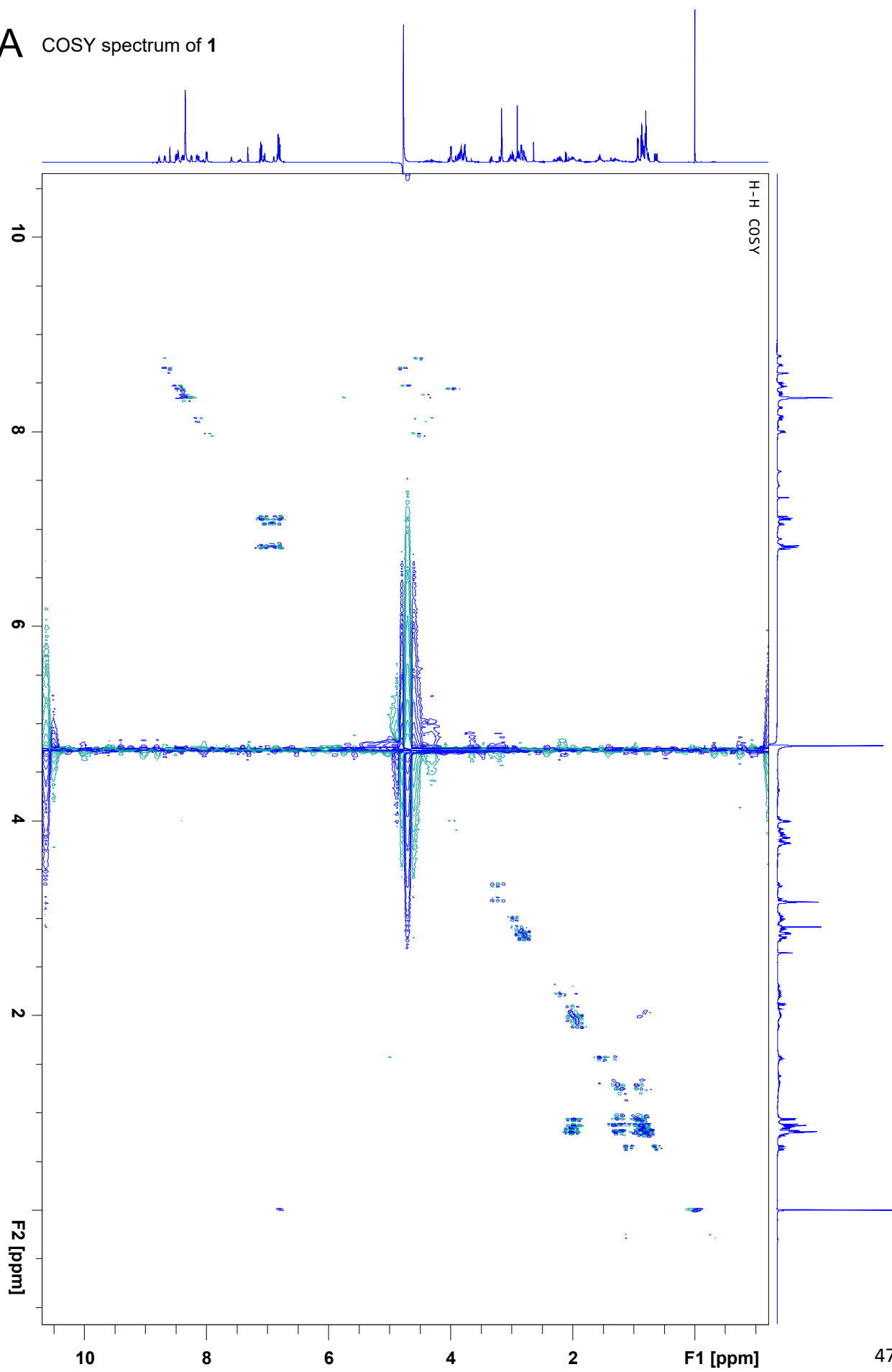




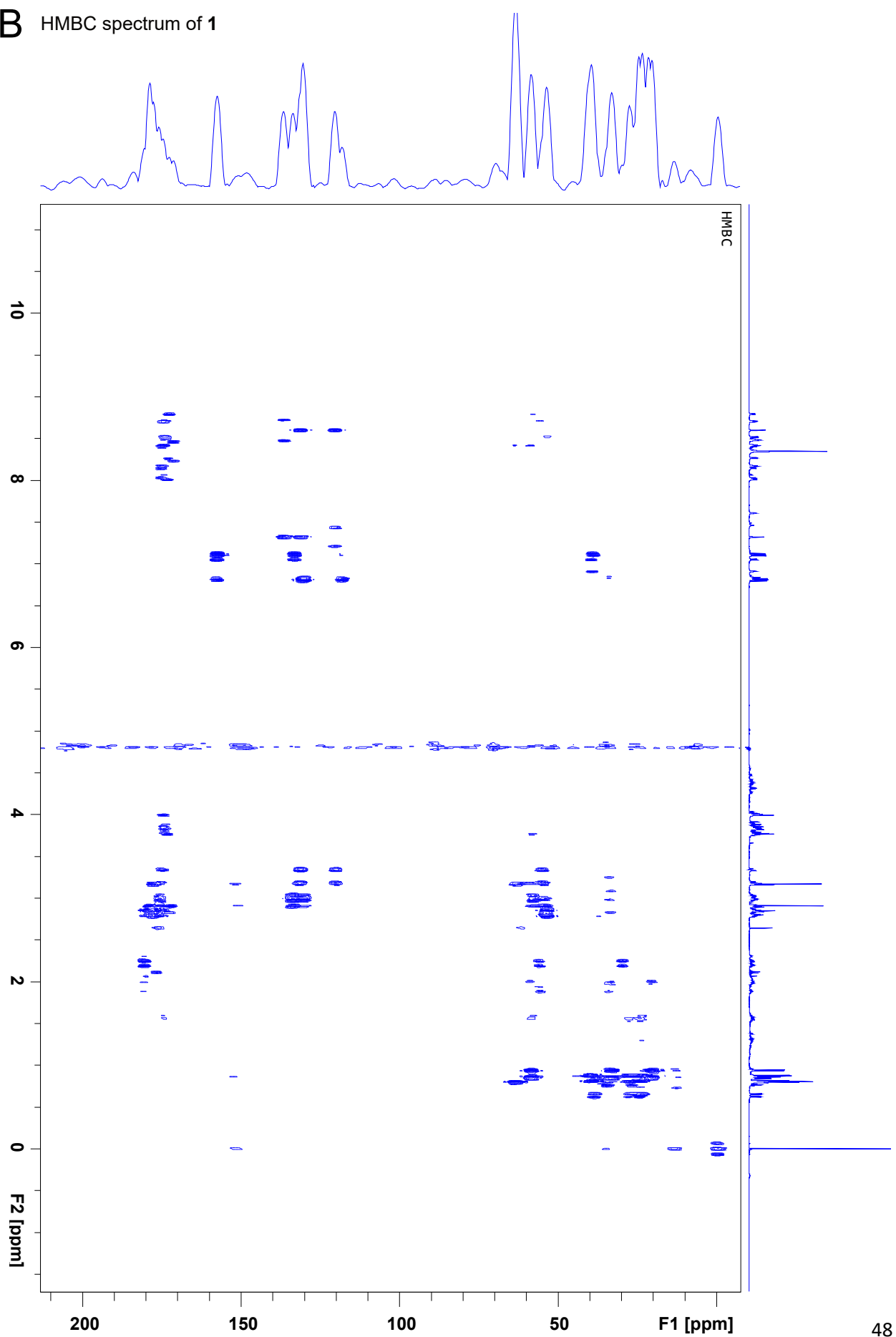
**Figure S9| NMR peak table and spectra of 1. (A) COSY (B) HMBC (C) HSQC (D) ROESY (E) TOCSY.**

AA	position	$\delta_C$ , type	$\delta_H$ , (J in Hz)	HMBC from H to C	AA	position	$\delta_C$ , type	$\delta_H$ , (J in Hz)	HMBC from H to C	
<b>Asp<sup>1</sup></b>	1	172.29, C	-	-	<b>Val<sup>9</sup></b>	1	177.70, C	-	-	
	2	53.55, CH	4.32, m	-		2	58.47, CH	4.48, m	-	
	3	39.65, CH <sub>2</sub>	2.83, m	172.29, 178.98, 53.55		3	33.12, CH	2.00, m	20.69, 21.15, 58.47	
	4	178.98, C	2.89, m	172.29, 178.98, 53.55		4	20.69, CH <sub>3</sub>	0.86, m	21.15, 33.12, 58.47	
	$\alpha$ -NH <sub>3</sub>	-	n. o.	-		4'	21.15, CH <sub>3</sub>	0.93, d (6.7)	20.69, 33.12, 58.47	
				$\alpha$ -NH	-	8.00, d (7.3)	175.29, 58.27, 33.12			
<b>Ser<sup>2</sup></b>	1	174.47, C	-	-	<b>Ile<sup>10</sup></b>	1	174.64, C	-	-	
	2	57.90, CH	4.54, m	-		2	63.55, CH	4.768, m	-	
	3	64.00, CH <sub>2</sub>	3.86, m	58.15, 174.47		3	34.49, CH	2.04, m	58.27,	
	$\alpha$ -NH	-	8.78, d (7.1)	172.29, 64.00, 57.90		4	26.88, CH <sub>2</sub>	1.29, m	-	
<b>Ser<sup>3</sup></b>	1	174.78, C	-	-		5	12.18, CH <sub>3</sub>	0.80, m	63.55	
	2	58.80, CH	4.41, m	-		6	17.29, CH <sub>3</sub>	0.81, m	63.55	
	3	63.03, CH <sub>2</sub>	3.82, m	58.80, 174.78	$\alpha$ -N-CH <sub>3</sub>	33.98, CH <sub>3</sub>	3.17, s	177.7		
	$\alpha$ -NH	-	8.40, d (6.6)	174.47, 63.03, 58.80	<b>Ser<sup>11</sup></b>	1	174.44, C	-	-	
<b>Tyr<sup>4</sup></b>	1	175.65, C	-	-		2	58.04, CH	4.39, m	-	
	2	58.60, CH	4.55, m	-		3	64.58, CH <sub>2</sub>	3.77, m	58.04, 174.64	
	3	38.95, CH <sub>2</sub>	2.98, m	58.20, 130.56, 133.45, 175.65	$\alpha$ -NH	-	8.38, d (6.6)	174.64, 64.58, 58.04		
	4	130.56, C	3.02, m	58.20, 130.56, 133.45, 175.65	<b>His<sup>12</sup></b>	1	174.60, C	-	-	
	5, 9	133.45, CH	7.10, d (8.9)	157.7, 133.45, 118.44, 39.11		2	55.12, CH	4.808, m	-	
	6, 8	118.44, CH	6.82, d (8.9)	157.7, 130.56, 118.44		3	29.34, CH <sub>2</sub>	3.18, m	131.25, 120.40, 174.60	
	7	157.7, C	-	-		4	131.25, C	-	-	
	$\alpha$ -NH	-	8.15, m	174.78		5	120.40, CH	7.32, s	136.51, 131.25	
	OH	-	-	-		6 $\epsilon$ -NH	-	n. o.	-	
				7		136.51, CH	8.60, m	131.7, 120.8		
<b>Gln<sup>5</sup></b>	1	175.17, C	-	-	$\alpha$ -NH	-	8.68, d (7.1)	174.44, 55.12, 29.34		
	2	55.89, CH	4.27, m	-	<b>Gly<sup>13</sup></b>	1	174.12, C	-	-	
	3	29.77, CH <sub>2</sub>	1.89, m	33.88, 55.89, 175.17, 180.73		2	45.46, CH <sub>2</sub>	3.99, m	174.12	
	4	33.88, CH <sub>2</sub>	1.98, m	33.88, 55.89, 175.17, 180.73		$\alpha$ -NH	-	3.99, m	174.12	
	5	180.73, C	2.21, m	29.77, 55.89, 180.73			8.47, m	174.12		
		$\epsilon$ -NH <sub>2</sub>	-	6.83 m	-	<b>Asn<sup>14</sup></b>	1	174.30, C	-	-
		$\alpha$ -NH	-	7.45 s	-		2	53.41, CH	4.75, m	-
		-	8.16, m	175.65	3		39.65, CH <sub>2</sub>	2.78, m	174.30, 177.91, 53.41	
		-	-	-	4		177.91, C	2.86, m	174.30, 177.91, 53.41	
		-	-	-	5 $\delta$ -NH <sub>2</sub>		-	6.90, s	-	
<b>Ser<sup>6</sup></b>	1	173.14, C	-	-	$\alpha$ -NH	-	7.59, s	-		
	2	58.36, CH	4.36, m	-	$\alpha$ -NH	-	8.50, d (7.6)	177.91, 174.30, 53.41, 39.65		
	3	64.00, CH <sub>2</sub>	3.76, m	58.36, 173.14	<b>Gly<sup>15</sup></b>	1	173.3, C	-	-	
	$\alpha$ -NH	-	8.13, d (7.2)	175.17		2	45.54, CH <sub>2</sub>	3.90, m	173.3	
		-	-	$\alpha$ -NH		-	4.02, m	173.3		
<b>Tyr<sup>7</sup></b>	1	175.70, C	-	-			8.47, m	173.3		
	2	54.41, CH	5.02, m	-	<b>Asp<sup>16</sup></b>	1	175.35 C	-	-	
	3	39.11, CH <sub>2</sub>	2.90, m	54.41, 130.56, 133.45, 175.70		2	53.88, CH	4.576, m	-	
	4	130.56, C	2.99, m	54.41, 130.56, 133.45, 175.70		3	39.65, CH <sub>2</sub>	2.79, m	178.59, 175.35, 53.88	
	5, 9	133.45, CH	7.12, d (8.9)	157.7, 133.45, 118.44, 39.11		4	178.59, C	2.85, m	178.59, 175.35, 53.88	
	6, 8	118.44, CH	6.80, d (8.9)	157.7, 130.56, 118.44	$\alpha$ -NH	-	-	-		
	7	157.7, C	-	-	$\alpha$ -NH	-	8.00, d (7.3)	173.3, 53.88		
	$\alpha$ -NH	-	8.24, d (7.6)	173.14						
	OH	-	-	-						
<b>Leu<sup>8</sup></b>	1	175.29, C	-	-						
	2	58.29, CH	4.97, m	-						
	3	39.61, CH <sub>2</sub>	1.57, m	24.0, 24.98, 27.13, 58.29, 175.29						
	4	27.13, CH	1.31, m	24.00, 24.98						
	5	24.00, CH <sub>3</sub>	0.81, m	24.98, 27.13, 39.61						
	5'	24.98, CH <sub>3</sub>	0.87, m	24.00, 27.13, 39.61						
	$\alpha$ -N-CH <sub>3</sub>	34.09, CH <sub>3</sub>	2.91, s	58.29, 173.08, 175.29, 175.70						

A COSY spectrum of 1

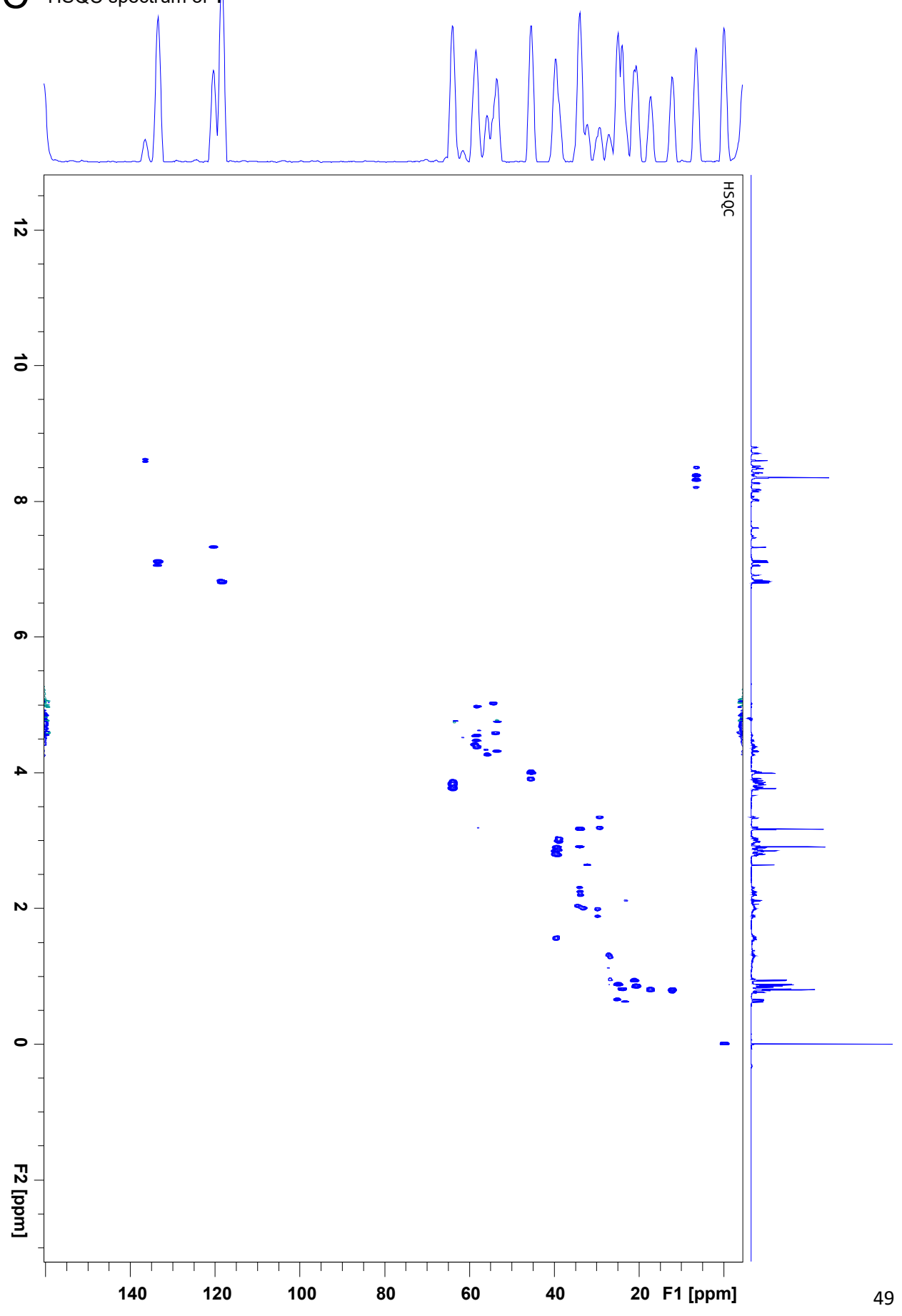


**B** HMBC spectrum of **1**

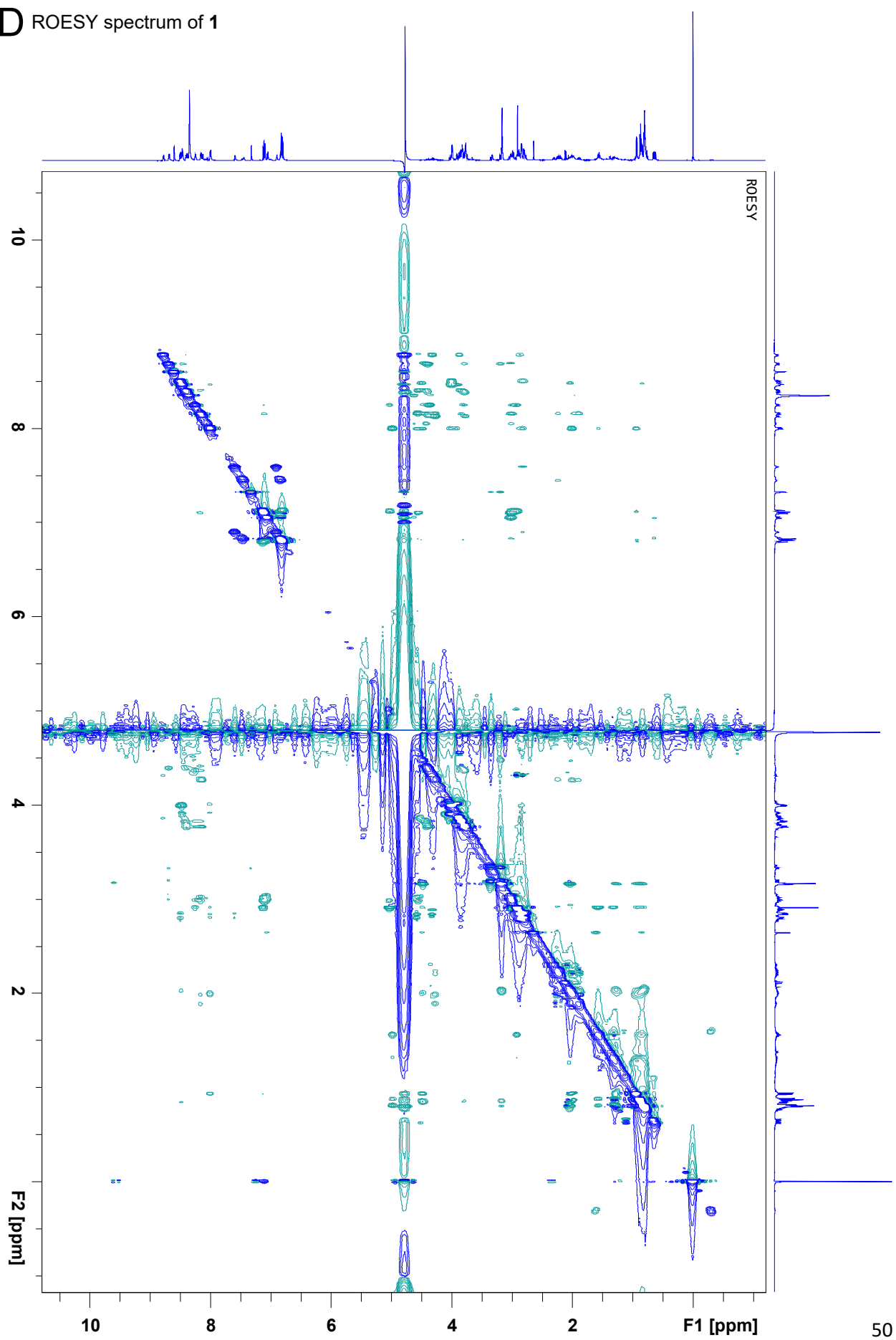




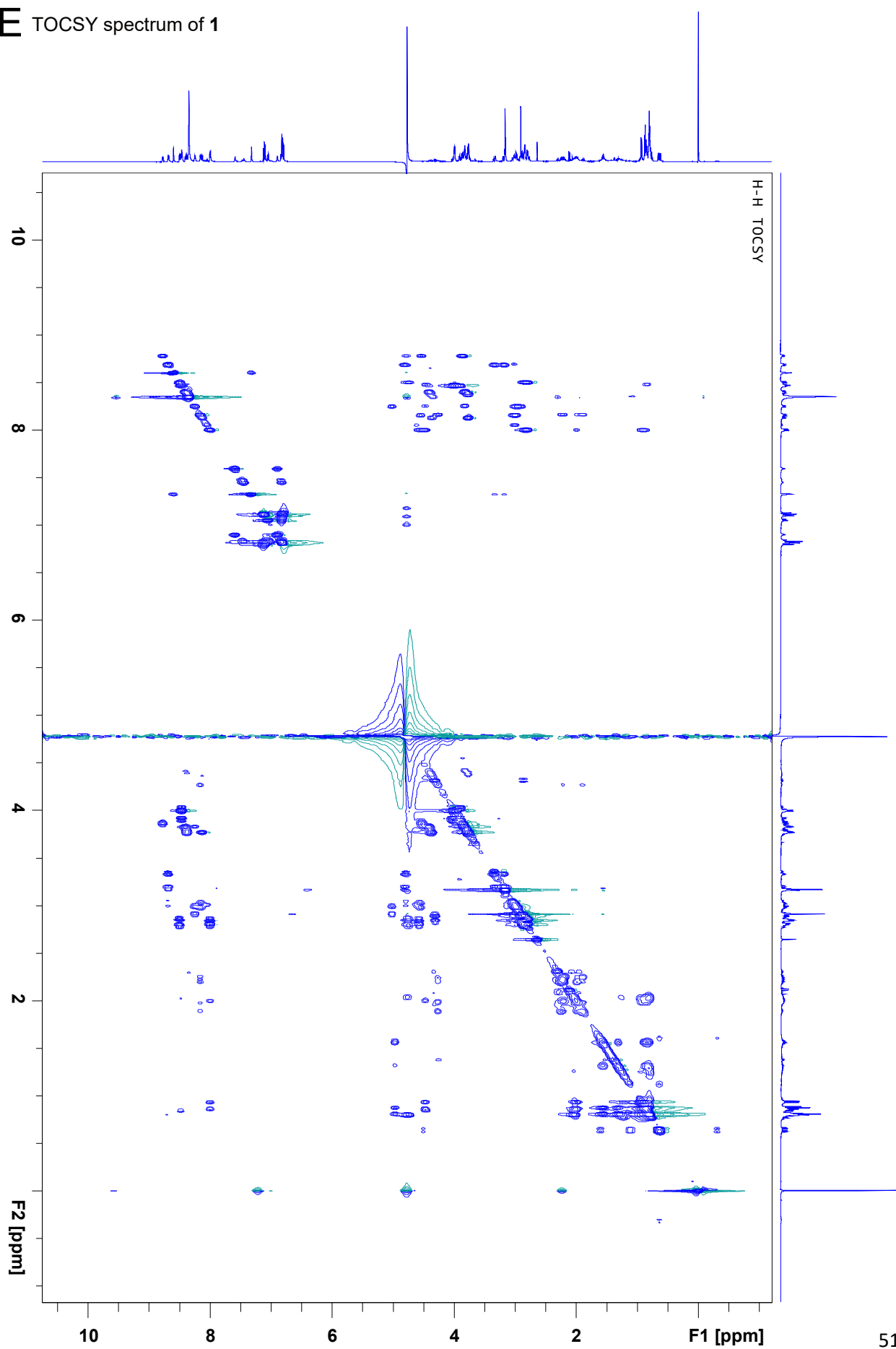
C HSQC spectrum of 1



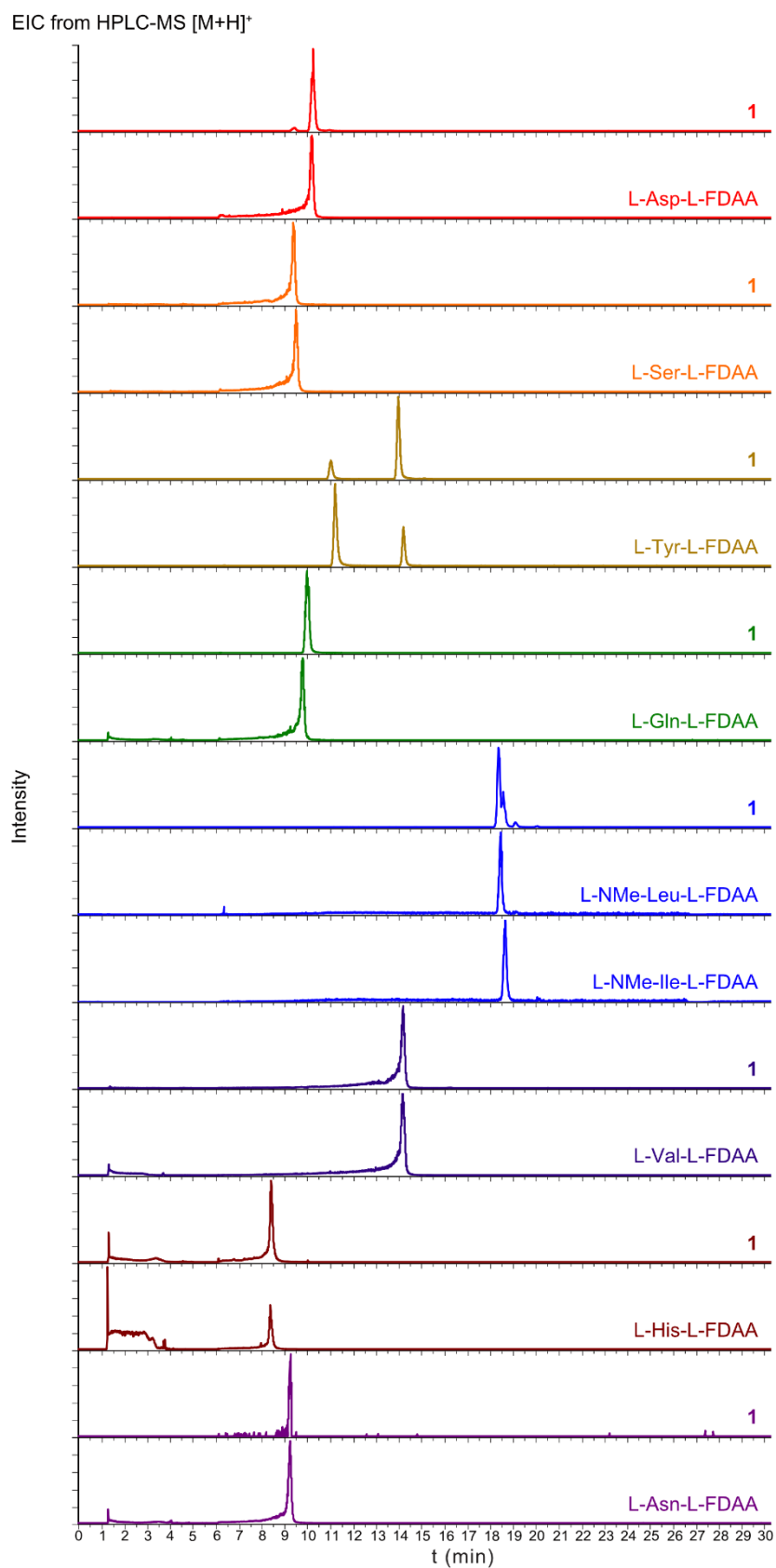
D ROESY spectrum of 1



**E** TOCSY spectrum of **1**



**Figure S10| Extracted ion chromatogram for Marfey's analysis of 1.** EIC from HPLC-MS of Shewanellamide A compared with amino acid standards derivatized under identical conditions. Relative peak intensities and retention times for the same masses are plotted in matching colors.



**Figure S11| Biological activity testing of 1.** Shewanellamide A was assayed against bacteria and yeast for a minimum inhibitory concentration. Under the conditions assayed, no growth inhibition was observed.

	Tested strains	<b>1</b> MIC (μM)
yeast	<i>Saccharomyces cerevisiae</i> YPH499	>50
	<i>Pichia pastoris</i> X-33	>50
gram-negative bacteria	<i>Escherichia coli</i> TOP10	>50
	<i>Pseudomonas putida</i> KT2440	>50
	<i>Shewanella oneidensis</i> MR-1	>50
gram-positive	<i>Bacillus subtilis</i> WB800N	>50