

Supporting Information

Tetrapyrrole regulator CrtJ contains a redox active cysteine in a DNA-binding domain that controls activity

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Supplemental Figures

RcCrtJ 1 : -----MRREALQRVSPDLLADIVTSACDIALVSPGRVVEVMVNPQFGSAERFA--AWQGARLSQLFSPESAQKLENRLA
 RsPpsR 1 : -----MLAGGLSPSLAPDLVRDLIATAADITLIVSQEGVREVMANPHHPSFGQLS--EWEGRPLEEVLTAESVAKFRLRSE
 RdPpsR 1 : MTTGGNTFWSSGAVPLLEPEFLSSIIAAASDIALVVSAGETLISVVVNSHSESGFNLK--HMEDRPVSEFTLRESIPKFEKAHA
 RrPpsR 1 : ---MKQRDPPEIVLEGLDPEAVTLVTTATDVLVVDQEGVDRDALGGDGLPVR--KDWLGRPWAETVSAESRPKVEAMLR
 TrPpsR1 1 : ---MSPFRAPKESFGTLDAETAALVEGATDIAVVDQAGIVCDISFGSEDLSSSELT--RDWIGQPWLTTLVPESTRANLEALLS
 TrPpsR2 1 : -----MTHLAQEDVITFDHVGVRRRAALSFAFRGEALD--AVVGRLWAETVDSVGSSELQSLVD
 RgPpsR 1 : ---MRPFGAPEKTFGGVTAATAGSLLSVANDITLVDHAGVWRDLACGGDDFAREAG--AEMIDKRWVDIVTPESRDKVEALLR
 BsPpsR1 1 : ---MRAFPRAPKESLGDVNADVAAMLVAASDIALADAGNQQDLAFQQAGLFLELKNITDDEWIGRWSQATVSEESQTKPELLA
 BsPpsR2 1 : -----MAEFHGPREDDVITLDMDCVIREATLSPNMSAENV--AVLGRAWSEIVDDAS-EKIERIMQ
 DsPpsR 1 : MNTREDFWDDRSAPRIAPEHFNEIVATAADIAVILDLKGVQSLVVNPLNPTIGRLD--HWCKRDIREFLAEDSLAKLEKQLS

RcCrtJ 75 : DGPEPGRS--LQLEITHAAD-AFTLIPVRYTITRSGE---DGTLLILIGRDMQPLAEVQQLVKAQIALALERLYEAQREIETRYRV
 RsPpsR 76 : GLEPGRGS--VAVELNHIDPRSEFFPIRYLLHRLPA---DRSILMLGRDIPRIAEVQQLVAAQLAMERDYETQREMETRYRV
 RdPpsR 83 : AYLLEGEVP-KKQLELNHSDNAVWQYVRYVTFHFRFGH---ENAAILLGRDLRPIAETCQLVQAQIALECCYEAARREFDARYRV
 RrPpsR 80 : KPD--AVDPTKWRQVMHLETETGVFVPLAATVPPG---HGQIVALGRDMRSVATLQRLINAGHSLEHYARIRHMETRYRL
 TrPpsR1 80 : EAS--GTRVTRWRQVNHPSVRGTDIPICWRALRLG-----TSVVALGRNIQGMALCQLVDAQALERWYWRERQVETRYRL
 TrPpsR2 59 : DAR--DSGVSAFRQVNIQIFPSGLRPLTEYTAVRLGG---DAGLVALGRSLQAVTELQTRLVQAQIMERYWKLREVEVETRYRL
 RgPpsR 80 : EAAPDVAVAIRWRHLNFVLPDREVPLFVAVRLGGDGKPGHVAFGRDLRAVAALCQLVQAQIMERYWKFPHHAETRYRH
 BsPpsR1 82 : EAS--ERKVSRRWEVRYPAARGDDIPLEAVVAIKG---AARYHAGVGRDVRATAALCQKLEAQAVALERYSRMRNAESRYRT
 BsPpsR2 60 : DTK--RTGISAFRCITQRFPSGLELPMETTVLLGG---RAGMLAIGKNIQAVAEICARLISAQQTIERYWRREIETRYRL
 DsPpsR 83 : AYQQGVEVPTDAIEVNHFDNANWEFPITRYVTLHTGD---ENLILMLGRDIPVVAELQRLVKAQIALALEKYESHEDYETRYRV

RcCrtJ 152 : ILEAHPALITIVSMSTGRITADLLAPAAAMGATRAELI---DAPVGCLEDRRRGEFLENLAKIAGSDPLGAVELTIRRSRRKV
 RsPpsR 154 : VLDVSRDPMVIVSMSTGRITVDINSAPGLLGGVQRDILL---GAAIAQEFEGRRRGEFMETMNLNATESAAPVEVLSRRSQKRL
 RdPpsR 162 : ILANTKQAVVVFVSVQSGRVEDANEAAPALLGLNADALR---SSSFAQHFMDRSNVELTESLMNATLAEEEGQVTLTATRTQATV
 RrPpsR 158 : IFKLTTEAVIFIDGASRRVVEANPAALSLFG-ETARH--IVGRAFPFGFSQPSTQEIEITLLAGVRTIGTGDVVFATLAESSRAV
 TrPpsR1 156 : IFRMVSEAILIIDAPTQRVVEANPAAGQLLGESEPTR---VIGRPFPEGFDTEGTQSINGLLAGVRAAGADVLRAKLDGAGQCF
 TrPpsR2 137 : IFGSSSDAVILLRVDGLAIQDVNPAASRALVGVPSGQRPLVKGDFAGELVDGERDLFYAMLQRLDQCSAPDILHKLDAERAAW
 RgPpsR 164 : IFQVASEAVIVVDATQKLEANPAARLLADGGSGS--LVGLTFPTGVEARGAEQLNLLAGVRATGRADEGRAELADGRGEV
 BsPpsR1 160 : IFQTAEFVILIDAAASHRVEANPAAPALLDHGAAN---LIGKLPDALLIDDLAMSQSLSLAIRSQKIERAKVTLDDGGR-DY
 BsPpsR2 138 : VIEDSNEAVILTKVSDLRIVEANRTPAALGVSNRRRDLVGREFLHEIPEKDRPEVLMRLRRVDRDQKAPGIVIHLEDADAAPW
 DsPpsR 163 : VMESTRDLVLDVASSGRITDMNSAPRAQVLGAEDALV---GSLFGHEFNGLKRGELLDQLMGAATSDTNSVTAHTKRTAKEL



RcCrtJ 233 : TVTATLFRRAAGDRLLICRLEGEAEAR--RTRVDDTVLSERLFLKGIICAMVFLDADGTRRAANDAFIYITDAGSAAVQGRSFDAD
 RsPpsR 235 : LVVPRVFRRAAGERLLICQIDPADAT--QPVGDELSENLAELYHEGVDFVSDADGTRGANEAFINMTDSSSLAARIGRSIAD
 RdPpsR 243 : IAHFVFRFAGGQRVLMCRLETKESK---AATPDAAADHALAMFRAGSDAMIFMGPNVITISVNESLIDVGAHSLSDVVGSRFGD
 RrPpsR 239 : LVSAALFRQERATQFVRLTPQAGDRETNQVPAKFMVLDAVEAMEHGGVVTDLLGRULTANASLIDLAQLATQEQARGQLGR
 TrPpsR1 237 : LVASALLRQENLSEFLVRLSPIVAESATFTLPENNARYLRVLENAEDCVVITDADGRVLSANNTIATETAEEQARGESLDR
 TrPpsR2 221 : MVRASRLASEAGSLYLQLTATEAASRQTADG-AFADVLIERLFDGFFVTDRSGLRWRVWVPSLIDVQMPTSVGLQRSLGR
 RgPpsR 246 : TVAVSTYRQDLVSHFVRLSRVQAQAAEMTPTSGSMLLKVQNAHDCIVVTDLBCRWISANSAFVETLAQLTTEEQVRGETLDR
 BsPpsR1 240 : LLDGTFRRQDTSFFLRLRFSPQYQPALPKASDVNTQLVQFVESAEHGGVITDMDGRLHANATPLQIAQLENMHQIMGETLDR
 BsPpsR2 222 : MLRGSMTSDNAPMFLQMAPIGKSIQPGVEVD---DPEGLDNLDFAVLSIDATGTRKRANRACTDVEIGSKEALIGERLRSR
 DsPpsR 244 : KIQRALFRASGDKTLICRLEGAQAQ--EVIAEELSEVLTLLFREGADAVVFTDPTGMIRNVNEAFINMTDVGQLGDIKGKLSLD

RcCrtJ 315 : FLSRGAVDLNVLLDNVKKRIGHLRHYVFRNTDFSGQVTVELSATLHFHDRATPTIALVIRDSNLADATR----IMPGMASNEGL
 RsPpsR 317 : FLARGSVDLRVLDSVRRTCQLRLYATRTTDFAGQIAAEISATWLDREPRPILLVVRDTSRADTMRR---PVPATGVIDEPA
 RdPpsR 325 : FICRGGQIDLAVITEQPQRSGHMRYATKIVNDLCTRLAVEISATHLADADVAAGCMRDVSRTEARGGSGGTISNAAPAEPA
 RrPpsR 323 : FICRAGNEFNSLLVNLRDMDLVTLMNTQGEYGSVADVEISAASAPLAEQPCLGFFVTRDVSQRHD-P----DQKGGDFTRS
 TrPpsR1 321 : WICRPGVDLNVLMANLRQHDTVRLFAITRGEYGSVTDLEISAASAVRNGERYPFYGFFFRVDVGRRLHAE----QP-TSPEQPRWL
 TrPpsR2 304 : WLDPRGADMSVLLTTLARLVVRPFSTRRGELEGGETEVEISAAGDLDETPSSIGILFRDVGRRLLP-----RQDNDERLGGRL
 RgPpsR 330 : WIGRTGVDLSVMTSNLRQRCQVRLFATTRGEYGAVDVVEISATMVAQGDPRFLGFTTRDVGRRLAGH----DARTRELPERSV
 BsPpsR1 324 : WICRSVDFSVMLTNLRQHGTIKLSSVVRGEGSPVDVEISATTVGAPGQARLGFTRNVGPRIS-P-----PDADHAFIPRSR
 BsPpsR2 303 : WITRPGADLAVLLSNVERHGMVRLLSHTHQEELGTETEVEISAASAHGQDQRRIGLVLRNIARRIS----PTAEHNLRALS
 DsPpsR 326 : FLARGSVDLKVMNDNASRHSVPVYSRTRIESAYGSQLSVEISATHLEARQNGGFVAFVRLASRLEVVR--DAAPVNGAVSGEAM



RcCrtJ 394 : RNVMQMVGCIATLRIIVSETTELEKMCIEATVLELTGNNRVAAPPELLSLRQSLYVKLRKFGILSKDAE-----
 RsPpsR 398 : RNVMEIVGNSTLRDIIVAEITDVEKMCIEATVLELTGNNRVAAPPELLSLRQSLYVKLRKFGILNKDE-----
 RdPpsR 409 : RNVMDLVGSAAKDIIVAEITDVEKMCIEATVLELTGNNRVAAPPELLSLRQSLYVKLRKFGILNKNG-----
 RrPpsR 402 : DHIADLVGRVLEKDIITRDITNDITRCLICQAALBLTGDNRASAAEMLGLSRQSLYVKLRKFGHGDLDGDREE-----
 TrPpsR1 400 : DQLTDRVGRVFLKELVRESDTTERLCTEALRKLTCGNRASAPELLGLSRQSLYVKLRKFGHSDQAEPSAPSDK-
 TrPpsR2 382 : NALSGRIGKGFRLRALVDEAIVSLEQHYIEBALTRGNRTATADLLGLSRQSLYVKLRKFGHDPDPTVTDGSR--
 RgPpsR 410 : GCTELVGRVLEKDIIVGETTDLLEQLCIEALBLTRDNRASAPELLGLSRQSLYVKLRKFGHGDLDGSGTPAEAEK-
 BsPpsR1 403 : EQIAELVGRVLEKELVRESDTTERMCTEALRKLTCGNRATAEMLGLSRQSLYVKLRKFGHAEPESEDSQLE---
 BsPpsR2 381 : AGINESVGTFLRDLVRSSTVEVVEQHYVRAALBLANGNRTSAPAILGLSRQSLYVKLRKFGHYNLSQLEENGEETEKGS
 DsPpsR 408 : QNVMEIVGSAPLRDIIVSATTDVEKMCIEATVLELTGNNRVAAPPELLSLRQSLYVKLRKFGILNKGGDE-----

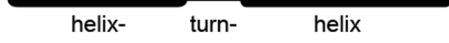


Fig. S1. Alignment of CrtJ homologues [National Center for Biotechnology Information (NCBI) accession numbers are CAA77529.1 (RcCrtJ), AAF24278.1 (RsPpsR), ABG29877.1 (RdPpsR), CAC84414.1 (RrPpsR), AAX53585.1 (TrPpsR1), AAX53580.1 (TrPpsR2), BAA94062.1 (RgPpsR), AAT78846.1(RsPpsR1), YP_001203745.1 (RsPpsR2), ABV95264.1 (DsPpsR). The positions of Cys249 and Cys420 are pointed by arrows. The helix-turn-helix domain is indicated. Red background represents the conserved residues and yellow background represents the similar residues. Sequences were aligned using the MEGA alignment program and modified using GENEDOC. Rc: *Rhodobacter capsulatus*; Rs: *Rhodobacter sphaeroides*; Rd: *Roseobacter denitrificans* OCh 114; Rr: *Rhodospirillum rubrum*; Tr: *Thiocapsa roseopersicina*; Rg: *Rubrivivax gelatinosus*; Bs: *Bradyrhizobium sp.* ORS 278; Ds: *Dinoroseobacter shibae* DFL 12.

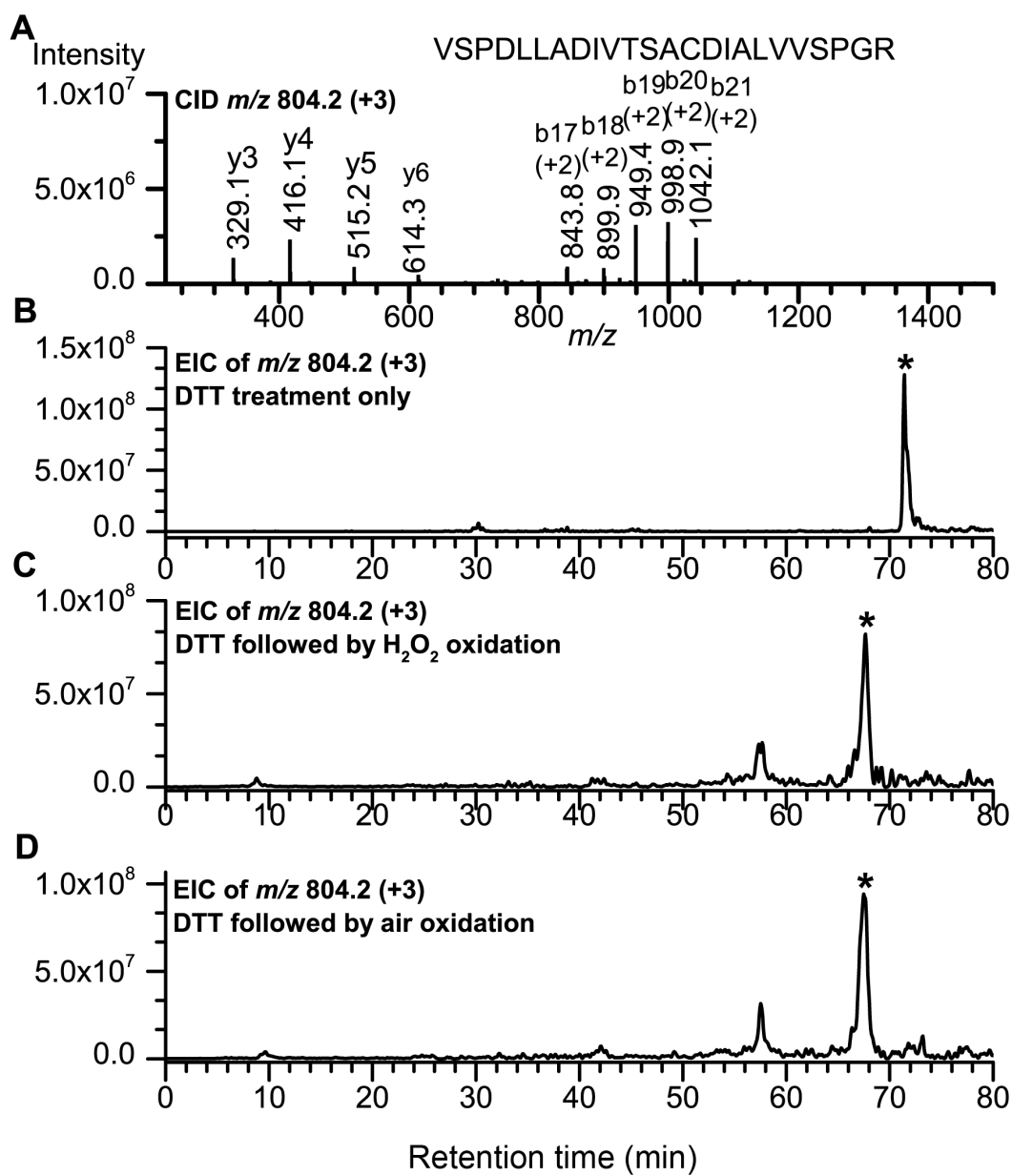


Fig. S2. (A) Collision Induced dissociation (CID) spectrum of the triply-charged peptide ion at m/z 804.2. The peptide sequence and fragment ion assignments are listed on the figure (Roepstorff and Fohlman, 1984). (B-D) The extracted ion chromatograms (EIC) of the triply-charged peptide at m/z 804.2 (the star indicates the peak of interest) obtained from the protein sample treated with (B) DTT only, or (C) DTT followed by H_2O_2 oxidation, or (D) DTT followed by air oxidation.

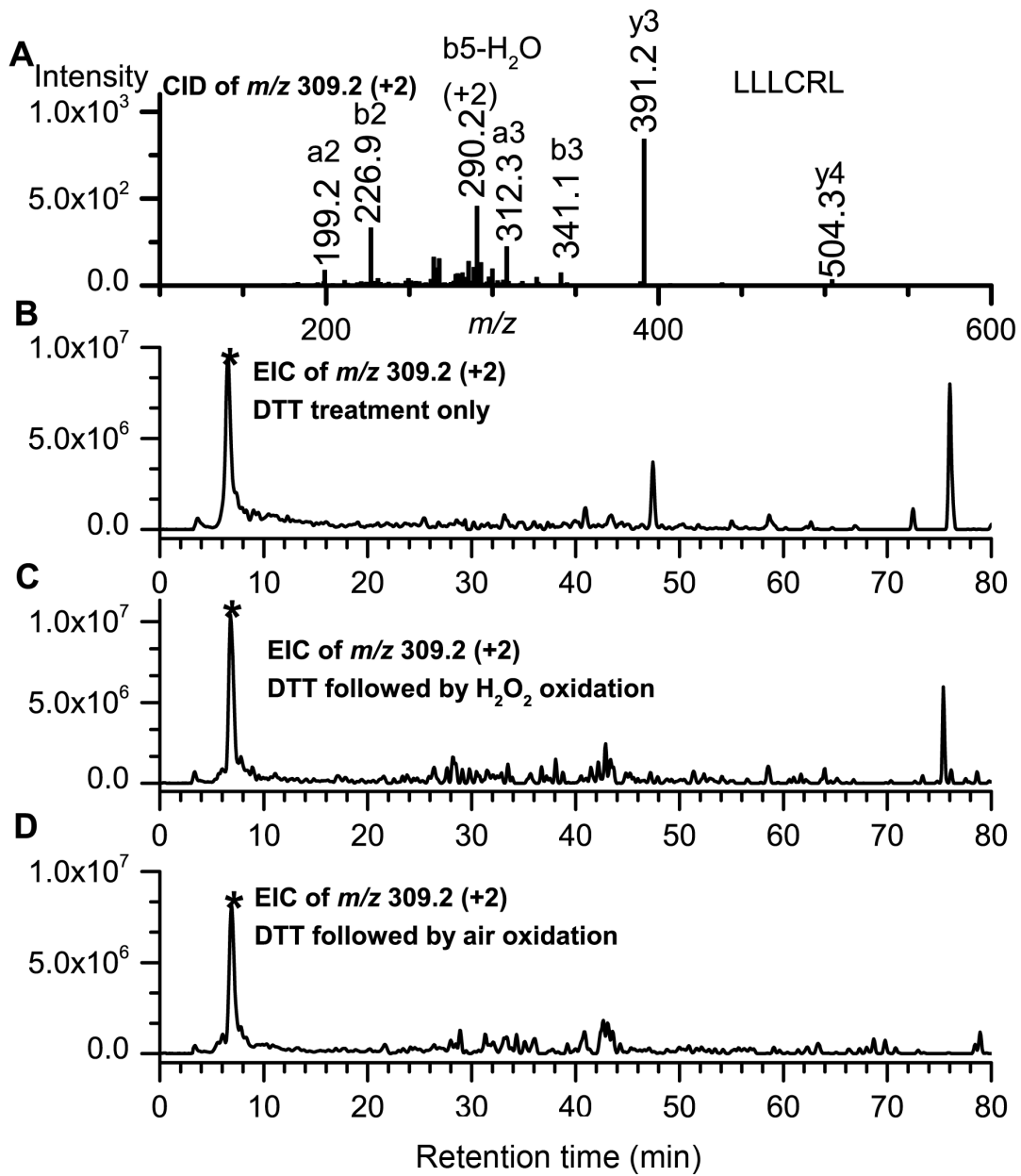


Fig. S3. (A) Collision Induced dissociation (CID) spectrum of the doubly-charged peptide ion at m/z 309.2. The peptide sequence and fragment ion assignments are listed on the figure (Roepstorff and Fohlman, 1984). (B-D) The extracted ion chromatograms (EIC) of the doubly-charged peptide at m/z 309.2 (the star indicates the peak of interest) obtained from the protein sample treated with (B) DTT only, or (C) DTT followed by H_2O_2 oxidation, or (D) DTT followed by air oxidation.

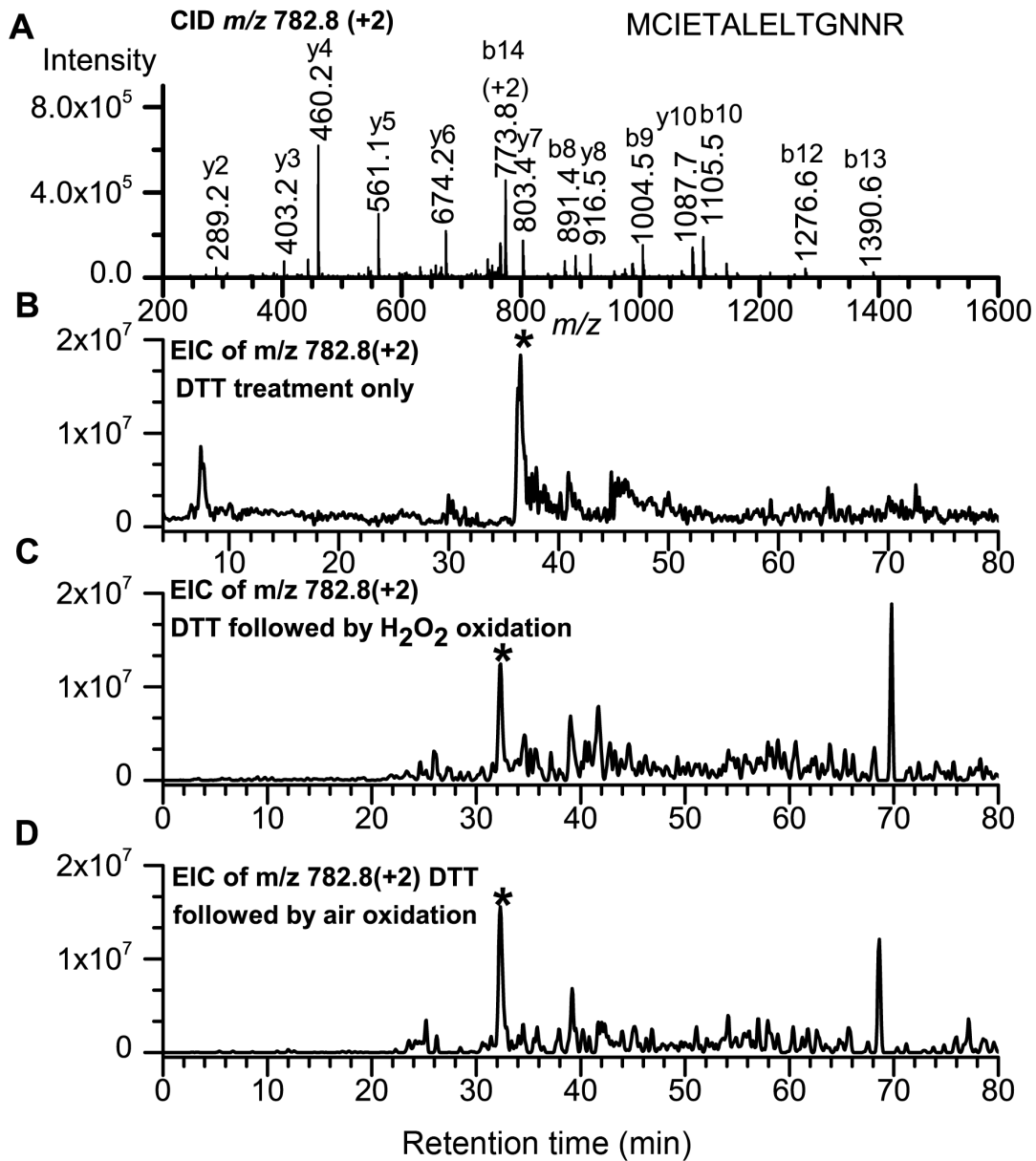


Fig. S4. (A) Collision Induced dissociation (CID) spectrum of the doubly-charged peptide ion at m/z 782.8. The peptide sequence and fragment ion assignments are listed on the figure (Roepstorff and Fohlman, 1984). (B-D) The extracted ion chromatograms (EIC) of the doubly-charged peptide at m/z 782.8 (the star indicates the peak of interest) obtained from the protein sample treated with (B) DTT only, or (C) DTT followed by H_2O_2 oxidation, or (D) DTT followed by air oxidation.

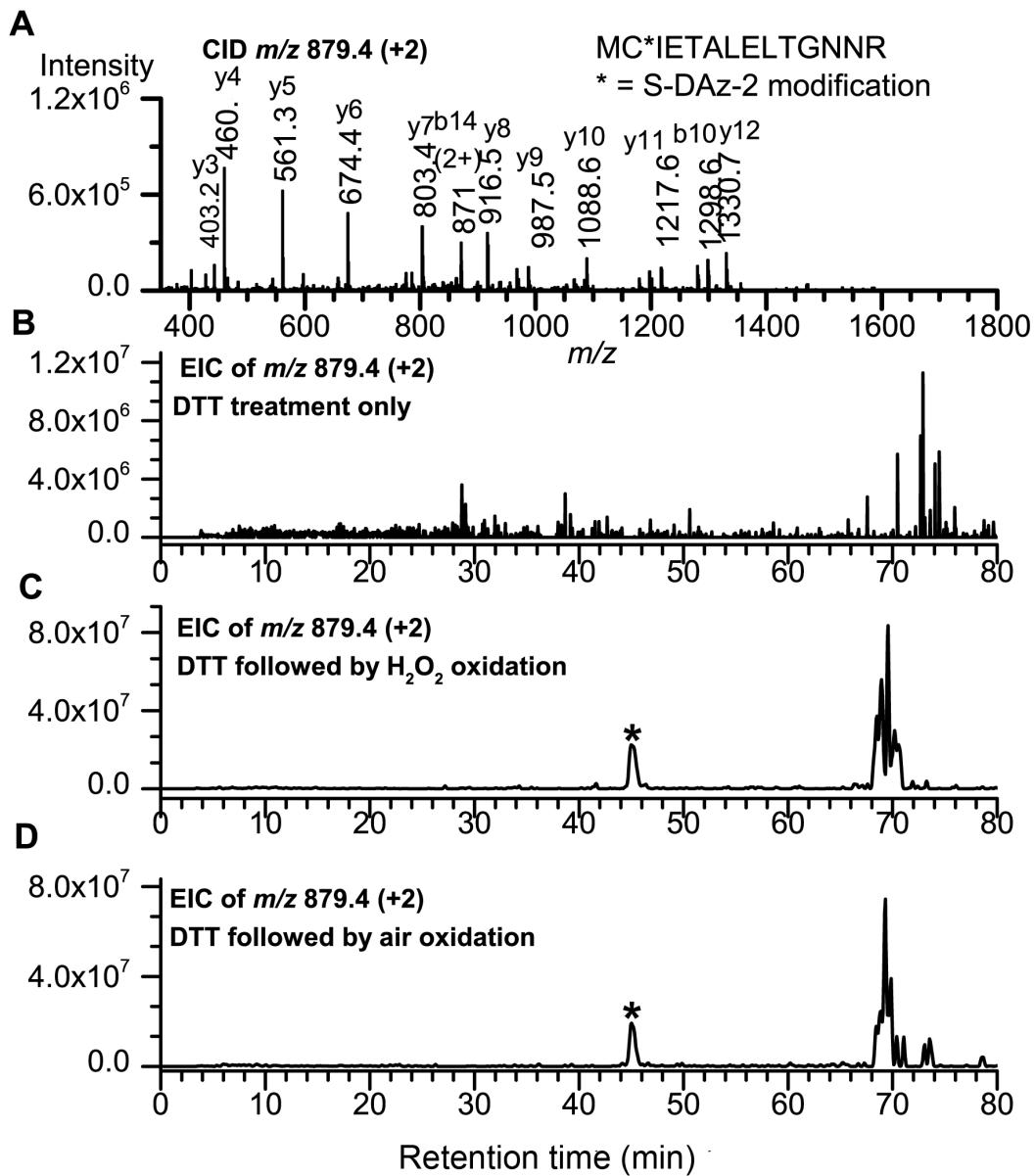


Fig. S5. (A) Collision Induced dissociation (CID) spectrum of the doubly-charged and DAz-2 labeled peptide ion at m/z 879.4. The peptide sequence and fragment ion assignments are listed on the figure (Roepstorff and Fohlman, 1984). (B-D) The extracted ion chromatograms (EIC) of the doubly-charged peptide and DAz-2 labeled peptide at m/z 879.4 (the star indicates the peak of interest) obtained from the protein sample treated with (B) DTT only, or (C) DTT followed by H_2O_2 oxidation, or (D) DTT followed by air oxidation.

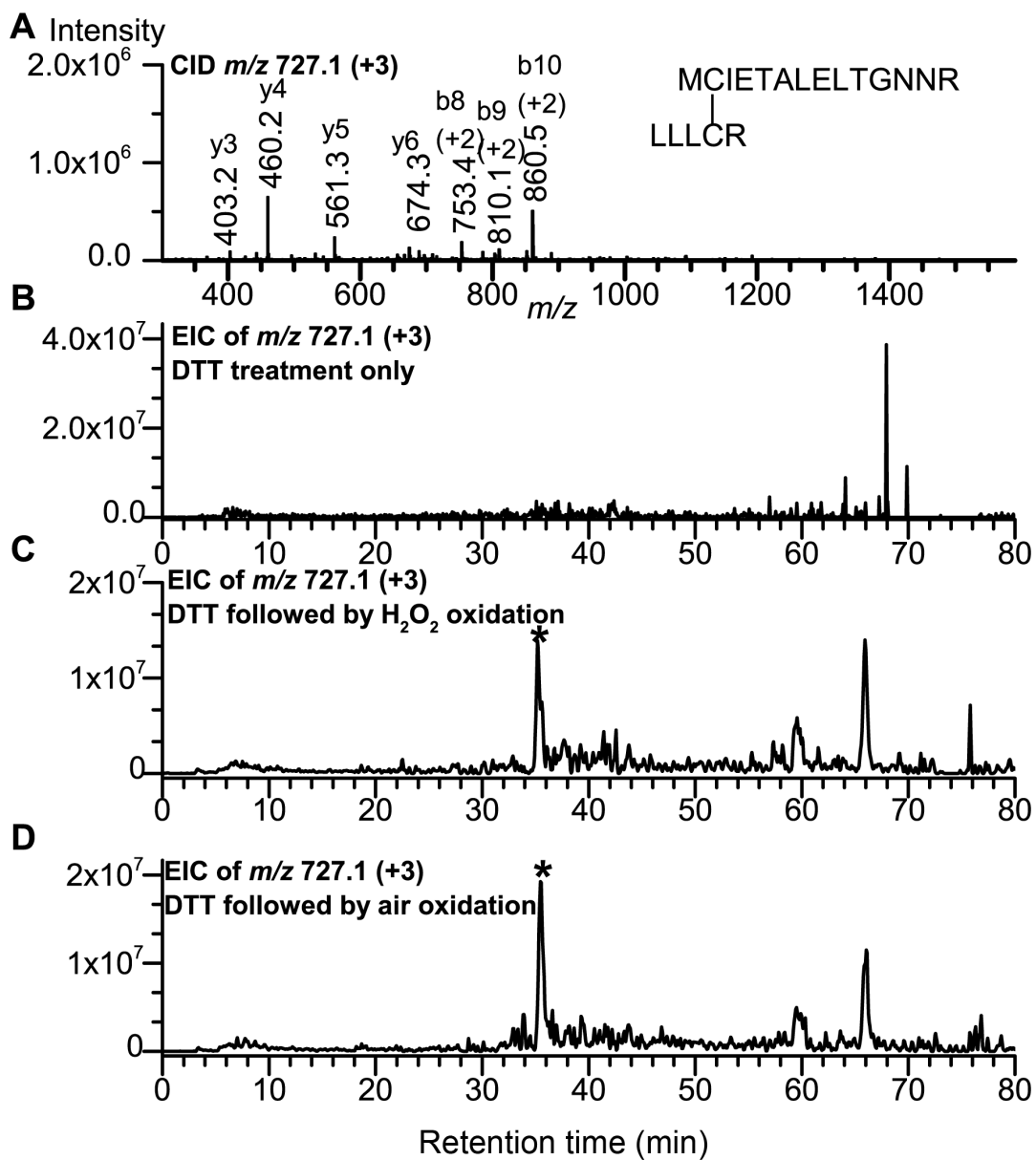


Fig. S6. (A) Collision Induced dissociation (CID) spectrum of the triply-charged cross-linked peptide ion at m/z 727.1. The peptide sequence and fragment ion assignments are listed on the figure (Roepstorff and Fohlman, 1984). ((B-D) The extracted ion chromatograms (EIC) of the triply-charged cross-linked peptide at m/z 727.1 (the star indicates the peak of interest) obtained from the protein sample treated with (B) DTT only, or (C) DTT followed by H_2O_2 oxidation, or (D) DTT followed by air oxidation.

Supplemental Table

Table S1: Primers used in QRT-PCR assays

Primer	Sequence
<i>rpoZ</i>	F: 5' gtgacgggtgaagattgcgttgac 3' R: 5' gatctgggtctggttgcttctgat 3'
<i>bchC</i>	F: 5' cctgtcgctgaaagaaggctagaaga 3' R: 5' gcgatggccgagaggatgtg 3'
<i>pucB</i>	F: 5' ggccgggttgacaatgctc 3' R: 5' gcccgaggcgtcatagatgg 3'

References

Roepstorff, P. and Fohlman, J. (1984) Proposal for a common nomenclature for sequence ions in mass spectra of peptides. *Biomedical mass spectrometry* 11: 601.