

Supplementary Data

Substrate Recognition, Protein Dynamics, and Novel Iron-Sulfur Cluster in

Pseudomonas aeruginosa Adenosine Phosphosulfate Reductase

Justin Chartron, Kate S. Carroll, Carrie Shiau, Hong Gao,

Julie A. Leary, Carolyn R. Bertozzi, C. David Stout

Materials and Methods

Materials

Nonradioactive APS was purchased from Biolog Life Sciences Institute, $\geq 95\%$ (Bremen, Germany). $[^{35}\text{S}]\text{SO}_4^{2-}$ (specific activity 1491 Ci/mmol) was obtained from MP Biochemicals (Irvine, California, United States). Molecular biology grade DTT was from Invitrogen (Carlsbad, California, United States). *E. coli* Trx protein was purchased from EMD Biosciences (San Diego, California, United States). DPCC-treated trypsin was purchased from Sigma. Depending upon availability, PEI-Cellulose TLC plates (20 cm x 20 cm) were purchased from J.T. Baker (Phillipsburg, New Jersey, United States) or EMD Biosciences. ^{35}S -labeled APS and PAPS were prepared by incubating $[^{35}\text{S}]\text{Na}_2\text{SO}_4$, ATP, ATP sulfurylase (Sigma), inorganic pyrophosphatase (Sigma) and recombinant APS kinase together as previously described¹. All other chemicals were purchased from J. T. Baker and were of the highest purity available ($\geq 95\%$).

Protein Expression and Purification

The gene encoding the *M. tuberculosis* APS reductase was amplified from H37Rv *M. tuberculosis* genomic DNA and cloned in a protein expression vector as previously described². The gene encoding *Mycobacterium smegmatis* APS reductase was amplified from *M. Smegmatis* genomic DNA and the gene encoding *P. aeruginosa* APS reductase was amplified from *P. aeruginosa* genomic DNA ATCC 47085D (ATCC, Manassas, Virginia, United States) as previously described¹. Briefly, APS reductase genes were amplified via PCR and cloned into the pET24b vector (Novagen) using the 5' Nde I and 3' Xho I restriction enzyme sites. The expression plasmid encoding

Rhizobium meliloti APS reductase was generated as previously described³.

Proteins were expressed by transforming a reductase-containing plasmid into BL21(DE3) cells (Novagen) grown on LB-agarose containing 50 µg/ml kanamycin. An isolated colony was grown in 5 ml of LB broth containing 50 µg/ml kanamycin. The culture was grown at 37 °C overnight. This culture was used to inoculate 1 L of LB broth containing 50 µg/ml kanamycin. The culture was grown with shaking (250 rpm) at 37 °C to an OD of 0.6, and isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.4 mM and the cells harvested after 4 h. Subsequently, 1 L of cells were collected by centrifugation and resuspended in 30 ml of lysis buffer (20 mM sodium phosphate, pH 7.4, 0.5 M sodium chloride (NaCl), 10 mM imidazole, 1mM methionine) together with an EDTA-free protease inhibitor tablet (Roche, Indianapolis, Indiana, United States). After sonication, DNase and RNase (Sigma) were added to the lysate at 10 µg/ml and 5 µg/ml, respectively, and stirred for 10 min on ice. The cell lysate was cleared by centrifugation and the supernatant was applied to a 5-ml HiTrap Chelating column (Amersham, Piscataway, New Jersey, United States). The column was washed with ten column volumes in 20 mM phosphate, pH 7.4, 0.5 M NaCl and 50 mM imidazole, and was eluted with 20 mM phosphate, pH 7.4, 0.5 M NaCl and 250 mM imidazole. Fractions containing the desired protein were pooled and concentrated using Amicon 10,000-Da molecular weight cut-off centrifugal filters (Millipore, Billerica, Massachusetts, United States) before injection onto a 16/60 Superdex 200 prep grade gel filtration column. The standard gel filtration buffer was 50 mM Tris-HCl, pH 8.0, 10% Glycerol, 5 mM DTT with ionic strength adjusted to 150 mM with NaCl. Fractions

containing APS reductase were pooled, aliquoted into single use portions, snap-frozen in liquid nitrogen and stored at -80 °C. Protein concentrations were determined precisely by quantitative amino acid analysis (AAA Service Laboratory, Boring, Oregon, United States).

Mass Spectrometric Analysis of Dissolved Crystals and Enzyme-Substrate Complexes

Solutions were infused at a rate of 2 µl/min into an Apollo electrospray source (Bruker, Billerica, MA), operated in the positive mode. The syringe and spray chamber were wrapped with ice bags to maintain low temperature, in order to prevent the protein from precipitating. All ions were collected using gated trapping and detected using chirp excitation. Broad band data were acquired using an average of 16-64 time domain transients containing 32 K or 1 M data points. The original time domain free induction decay (FID) spectra were zero filled, Gaussian-multiplied and Fourier transformed. All data were acquired and processed using Bruker Xmass version 6.0.0 software. The parameters of the electrospray ionization (ESI) source, ion optics, and cell were tuned for the best signal-to-noise ratio and were maintained for systematic experiments.

Crystals from 10 drops were harvested and centrifuged, yielding a brown pellet, which was washed three times in reservoir solution. The pellet dissolved readily in 10 µl of 2 mM β-octyl glucoside in H₂O, and the resulting solution was discernibly light green-brown in color. The solution was incubated overnight with Biobeads to remove detergent and frozen. Subsequently, this sample was dialyzed against 50 mM ammonium acetate using Amicon 10,000-Da molecular weight cut-off centrifugal filters

to remove residual salt and detergent, and the protein concentration was determined (20 μM). This solution was then diluted with 80:20 acetonitrile:water containing 1% formic acid for mass spectrometry analysis. The derived molecular weights correspond to the full length polypeptide plus SO_3^- (31359.8 Da; theoretical 31360.1 Da) and the same minus the three N-terminal amino acids (31018.2 Da; theoretical 31018.6 Da) (50% of the protein used for crystallization lacked these residues) (Supplementary Figure 4(a)). The data indicate that the enzyme in the crystals is quantitatively sulfated.

For enzyme-substrate incubation experiments with *M. tuberculosis* APS reductase (Supplementary Figures 4(b), (c)), appropriate volumes of enzyme (after buffer exchange to ammonium acetate) and APS were mixed in ammonium acetate buffer and the mixtures were chilled on ice for at least 15 min before being introduced into the mass spectrometer.

Supplementary References

1. Carroll, K. S., Gao, H., Chen, H., Stout, C. D., Leary, J. A. & Bertozzi, C. R. (2005). A conserved mechanism for sulfonucleotide reduction. *PLoS Biology* **3**, e250.
2. Williams, S. J., Senaratne, R. H., Mougous, J. D., Riley, L. W. & Bertozzi, C. R.

- (2002). 5'-Adenosinephosphosulfate lies at a metabolic branch point in mycobacteria. *J. Biol. Chem.* **277**, 32606-32615.
3. Schwedock, J. & Long, S. R. (1990). ATP sulphurylase activity of the nodP and nodQ gene products of *Rhizobium meliloti*. *Nature* **348**, 644-647.
 4. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876-4882.

Supplementary Figure Legends

Supplementary Figure 1. Routes of sulfate assimilation. Inorganic sulfate is adenylated by ATP sulfurylase (a) to form APS. Higher plants and the majority of sulfate reducing bacteria use APS as their source of sulfite ($c_1 \rightarrow d \rightarrow e$). In some organisms, APS kinase (b) phosphorylates APS at the 3'-hydroxyl to form PAPS for use as a sulfate donor for sulfotransferases or as a source of sulfite. The lower pathway of sulfate reduction ($c_2 \rightarrow d \rightarrow e$) is utilized by γ -proteobacteria such as *E. coli* and some fungi. Depending on the organism, APS or PAPS is reduced to sulfite by APS reductase (c_1) and PAPS reductase (c_2), respectively. Sulfite is reduced to sulfide by sulfite reductase (d) and incorporated into cysteine by O-acetylserine-(thiol) lyase (e). Important

metabolites such as methionine and coenzyme A are, in turn, synthesized from cysteine.

Supplementary Figure 2. Structure based sequence alignment of 38 APS reductases from prokaryotes and plants, and 34 PAPS reductases from prokaryotes and eukaryotes. The Clustal X (v1.81) Multiple Sequence Alignment program⁴ was used first to define profiles for each group, then to align all APS reductase sequences (species names in square brackets) and all PAPS reductase sequences separately, and then to align all 72 sequences as one group. The figure is color coded by residue property. The bar graph indicates the degree of conservation per position and is included in Figure 3 under an abbreviated sequence alignment for four species.

Supplementary Figure 3. Electron density for APS in each of four subunits of *P. aeruginosa* APS reductase. The unbiased σ_A -weighted $|F_o|-|F_c|$ map, based on the final model at 2.70 Å resolution, is contoured at 2.5 σ and 5.0 σ .

Supplementary Figure 4. (a) ESI FT-ICR mass spectrum of dissolved crystals of *P. aeruginosa* APS reductase demonstrating quantitative sulfonation of enzyme in the crystals. The two molecular weights correspond to the full-length enzyme plus SO_3^- , and the same minus the three N-terminal amino acids (~50% of the protein used for crystallization lacked these N-terminal residues). These molecular weights represent the apo-enzymes with the noncovalently bound [4Fe-4S] cluster dissociated from the protein during preparation of the sample for mass analysis (see Methods). **(b)** ESI FT-

ICR mass spectrum of 10 μM *M. tuberculosis* APS reductase with 20 μM APS in ammonium acetate showing that in the presence of excess substrate that the product AMP can be displaced, and that the sulfonated enzyme (E-SO_3^-) can also bind APS, as observed in the crystals. Previously, 15 μM enzyme was incubated with 10 μM APS; under those conditions, only the sulfonated enzyme with AMP bound was observed (Figure 7(a) of ref. 7). **(c)** ESI mass spectrum of the same mixture dissolved in 80:20 acetonitrile:water containing 1% formic acid, illustrating the release of the noncovalently bound nucleotide and the iron-sulfur cluster. The inset shows the deconvoluted mass of the thiosulfonate intermediate in the apo-form.

Supplementary Figure 5. Partial trypsin proteolysis of *M. tuberculosis* **(a)** and *P. aeruginosa* **(b)** APS reductase showing protection of the C-terminal tail and the Arg-loop upon formation of the thiosulfonate intermediate at equimolar concentration. The time course of the trypsin digestion is shown in the presence (+APS) and absence (-APS) for each enzyme. In **(a)** *M. tuberculosis* APS reductase (50 μM active site concentration) was incubated with or without 50 μM APS for 10 min at RT, and trypsin was added at a final concentration of 10 $\mu\text{g/ml}$ and incubated at 4 $^\circ\text{C}$. In **(b)** *P. aeruginosa* APS reductase (40 μM active site concentration) was incubated with or without 40 μM APS for 10 min at RT, and trypsin was added at a final concentration of 10 $\mu\text{g/ml}$ and incubated at 4 $^\circ\text{C}$. All samples were analyzed by SDS-PAGE using a 4-12% gradient Criterion gel. Trypsin digest fragments were purified by reverse phase HPLC and analyzed by electrospray mass spectrometry. In the presence of APS, the starred fragments, HR/G – End* for *M. tuberculosis* and ER/N – SK/A* for *P. aeruginosa*,

represent the mass of the peptide fragment plus an additional 80 Da for the covalent sulfite adduct. Full length *M. tuberculosis* APS reductase without N-terminal Met, is 28,356.87 Da; full length *P. aeruginosa* APS reductase is 31,279.6 Da.

Supplementary Figure 6. Electron density for the [4Fe-4S] cluster and its Cys ligands in Subunit B of *P. aeruginosa* APS reductase. The σ_A -weighted $2|F_o|-|F_c|$ map, based on the final model at 2.70 Å resolution, is contoured at 1.0 σ and 5.0 σ . The C α -C β -S γ -Fe torsion angle for Cys140 is indicated; this angle is *cis* (+10°) so that the C α and Fe atoms are eclipsed and only 3.5 Å apart.

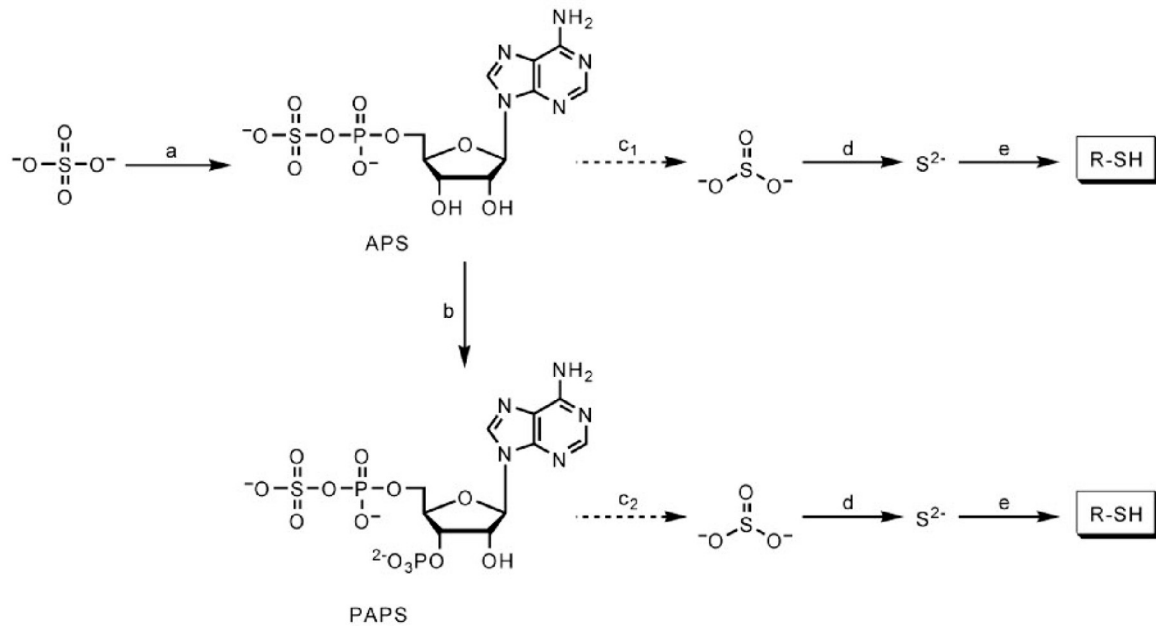
Supplementary Table 1
Effect of reductants on APS reductase activity ^a

Reductant ^b	E ^o , mV	Activity (pmol/min) ^c
Thioredoxin	-260	40
GSH	-230	≤ 0.1
Reduced lipoic acid	-290	≤ 0.1
DTT	-330	≤ 0.1
Dithionite	-527	≤ 0.1

(a) Rate of APS reduction measured with various reductants. Each value reflects the average of at least two independent experiments, and the standard deviation was less than 15% of the value of the mean.

(b) 10 μM thioredoxin or 10 mM chemical reductant was used in each assay (see Methods).

(c) Due to the slow nature of the reactions measured with chemical reductants, reported rates are considered upper limits.



Supplementary Fig. 1

Supplementary Fig. 2

Page 1 of APSr and PAPSr sequences

[Mycobacterium tuberculosis]	-----MSGETRLTSPQLRELAARGAAELDGATATDMLRWIDETFGDIGGAGGGVSGHRGWT	57
[Pseudomonas aeruginosa]	MLPFPATIPA-----TERNSAAQHQPSPMSQPFDPALASSLADKS-PQDILKAA-----FEHFQDE	56
[Pseudomonas syringae]	-----MSQPFVVAELATTYANKS-AQDILKLA-----FEHFQDE	33
[Pseudomonas fluorescens]	-----MSPTFDVVELATTYANKS-AQDILKLA-----FAEFQDD	33
[Pseudomonas putida]	-----MSQPFVVAALAAATYANKS-PQDILKLA-----FEHFQDD	33
[Azotobacter vinelandii]	-----MKQAVNFAEALAAAGIGKS-PQDILQIA-----FDHFQDD	33
[Methylococcus capsulatus]	MT-----QIEIDTLQAELAGRS-PRITILEYA-----LSRF-QR	31
[Microbulbifer degradans]	MSN-----TIDFTDIDAKLQQAQ-FOILIYA-----LAE-NDN	32
[Ceratopteris richardii]	VVRAGLFGQRSSENVFQRSCSVQPSDKHRCGLNAAIATPEVQVEDINVEDLALQLVDASPLEIMDRA	111
[Polygonatum pubescens]	LRKRWAVNVMFQKRNDSVAASAVADLAAVVEEKARALEKEVEVADYEKTAVELQNASPLEIMDRA	114
[Zea mays]	AARQQRARAVRSLRAAEPARQPVASAAAAAPAAPVADAAALAAVDYEALEARLEGASPLEIMDRA	112
[Lycopersicon esculentum]	LSSTVLNRRRSVAVKPLIYAEKRNDSIVPSAATIVAVEVGESEVAEDFEKLAKELQNASPLEVMDKA	115
[Catharanthus roseus]	ISPSVNVSRRLAVKFINAEPKRNESIIVPSAATIVAVEVEEKVDVEDYEKLADLQNASPLEIMDKS	114
[Brassica juncea]	VSSVLSLSLQKRSVVKPLNVQSIKESFVSAASEVTEKQDVVEVEDFEELAKLETASPLEIMDKA	116
[Arabidopsis thaliana]	RTNVSAASLSLQKRSVVKALNVQSIKESIVASEVTEKLDVVEVEDFEELAKLENASPLEIMDKA	111
[Lemna minor]	PMRPLCAVDPRKRSQSPVAPLAAVPSSTSDAVTLVQAETSIVDVVDFEKLASLERASPLEIMDKA	116
[Chlamydomonas reinhardtii]	FAAQRPAVQ-----QRRMAVRANVARVQVNAAAVEAKPDWAGLAAEMDNKS-PLIEMDHA	70
[Nocardioides sp. JS614 2]	MTAATTNSARN-----FRGITAGRSSEELRVSHWGALELELAPADIVWA-----VAT-----FGER-----	82
[Desulfitobacterium hafniense]	IIWNRLLGEEPDSFMTWEGIQKQRRRNMEDRSDFLNLDLDIRAFAGED-PRKLLAHV-VEK-IGPA	78
[Pyrobaculum aerophilum]	MRR-----EWTDLVEELSLRFENST-AEHLLSWA-LNE-F-YP	35
[Clostridium thermocellum]	MVQ-----LDLEKLNKEYSDKS-PEDIVRFV-VEN-IGI	32
[Bordetella parapertussis]	MMA-----ARPLAALAGLTAARWHDLSRLAI-IAR-QYP	36
[Burkholderia mallei]	AA-----HAFALT--FALAEEKARLDALLAQ-IAA-RHP	30
[Ralstonia eutropha]	MSGLSDIIV-----VDGGAPASAL-RFTLWTWVTEGSLADLDEKERELARLAG-IAA-RFF	55
[Ralstonia solanacearum]	MSALQEAAV-----SE--VPMSTIGRFVLWSRFVITGTPEGLAVKEALLERLIE-IAA-KHG	54
[Dechloromonas aromatica]	MTP-----SLNLIPELTASVAVKRTVVKALLAD-IAT-NWS	35
[Sinorhizobium meliloti]	MTT-----QSLKAEVALEADWMLQAAKALNDKLESLDLAQRALIA-GLG	48
[Wolfinella succinogenes]	MR-----DAIKESLERLC--EVKGLI-4	20
[Bacillus subtilis]	MLT-----YDNWEEPIITFPEDDPYKQ-ALSVLKWA-YGH-YGDQ	37
[Corynebacterium glutamicum]	MSP-----EVAKHNEELVEKHAALYDAS-AQELIETW-AEH-APGA	39
[Corynebacterium efficiens]	VTVNALKNAGPTQDPEVSPGEPRIITAPLPEEVAHRNRELVDKHADELYHADAATILEWA-AEH-VGR	69
[Nocardioides sp. JS614]	MFK-----TERDLQRLQAVVFEAAEELEQVS-ADSVVRNA-RDT-FGTK	41
[Chloroflexus aurantiacus]	MTT-----QYWDAN-----IDPRDVIRWA-LAG-YPD	25
[Deinococcus geothermalis]	MTALEDRSP-----ASRRPPDLPFTTPTPEIDPLDVVRAW-LKA-HPD	40
[Deinococcus radiodurans]	MMAEVRTP-----EQGGGLTTEPRAPRSPGHADASAPAFGPETDPRDIIRWA-LAA-HPD	56
[Nitrobacter winogradskyi]	MTPLEENS-----VDPGRAAQSAANAANSAMPAAAALESRLDAS-PAVIVSAA-LRA-VGRD	56
[Nitrobacter hamburgensis]	VSYISDGRRTSLPEQKSVASAMTQPAATGTTPALPPAAALNDALRDAR-PADVIAAA-LRT-VGRD	74
[Cytophaga hutchinsonii]	MNK-----EEIHLKLTXYQ-EKGLKFT-4	23
Salmonella typhimurium	MSQ-----LDLNALNELPKVDVRYMALAETNAQLEKLS-AEERVAWA-LEN-LPGE	47
Escherichia coli	MSK-----LDLNALNELPKVDRIILALAEINAELEKLD-AEGRVAWA-LDN-LPGE	47
Erwinia carotovora	MAE-----FNLEALNALPKDEQVAALAAVNGQLEQLS-AQERVSWA-LEN-LPGD	47
Yersinia pseudotuberculosis	MSQ-----FNLESENALPKAKQAAALVVLVNGQLEHLT-AQERVSWA-LDN-LPGE	47
Zymomonas mobilis	MSA-----PHLETKSLPKABEQEALSEANRQLEMS-AEDRLKWA-FDN-LSGQ	47
Photorhabdus luminescens	MSQ-----FSLSQCVSMTARQEQQSIAEINLRLEMMD-AHQRVNWA-LEN-LPGE	47
Vibrio parahaemolyticus	MLDSVA-----SKPELAEALLTLTKTEQILRLAEQINVELEPLS-AQERVKWA-LEN-LDGE	52
Vibrio vulnificus	MLDSVA-----STLQSELLSLTKAEQSLRLAEINVELEMLS-AQERVAWA-LQN-LBGA	52
Vibrio fischeri	MP-----KLQSELLSLTKVEQTLRLAEVNVLEKLT-AQERVVWA-LEN-LSGN	47
Vibrio cholerae	MPN-R-----TVETLEELLTKNVQQTILRLAEVNVHLESLT-AQERVVWG-LEN-LOGN	50
Photobacterium profundum	MPN-----YALADLLEKTKIEQILQAEINAELEALS-AQERVVWA-LAN-LGSE	47
Candidatus Blochmannia	MNKLINVIN-----HCWTVTEFNSLDINEQKLVLVKINRYLESIN-IVDRFKWA-IEY-LPKQ	55
Candidatus Blochmannia florida	MNR-----CWCVEELDLVIGVERALINEINQYLESVT-IEDRIMVW-MKY-LSGQ	48
Buchnera aphidicola	MSKFY-----IENINLNSKKNKNIISELNLNLSNYS-AEERISWA-LSH-LPHT	47
Xanthomonas oryzae	MTA-----LPAASISALADD--LDALNAQLEGLR-ADERVAWA-LQH-GPQD	43
Xanthomonas campestris	MTA-----LPAASISALADD--LDALNAHLETLR-ADERVAWA-LQH-GPQQ	43
Xylella fastidiosa	MTV-----LPAL--PLDD--LETLNVHLETLS-AENRVCAW-LER-GPDP	43
Thiocapsa roseopercidina	MSK-----PDDDAF-LHQDDAALREINRRELSMP-AEDRVVWA-LEH-LPPQ	39
Pseudoalteromonas haloplanktis	MSEF-----KNILQLDKQSQTSMMLADANGILKNMS-AEQRVQWA-LEN-LPET	45
Shewanella oneidensis	MSS-----SELKALLTAPKSVQQAELERINRFLAGLT-AQERVVWG-LAY-LPGN	47
Shewanella frigidimarina	MPNEVVTTAPTGAADVDSVTPAQQLQALLAPKAEQTAELININFLAGLT-AQERVVWG-LAY-LPGD	65
Shewanella denitrificans	MDKDGLSNNSN-----NAVISPALKQLLLAPKDEQVRELERINGPLSSLS-AQERVVWG-LAY-LPGE	61
Shewanella amazonensis	MN-----VHLLSLDAAEQAAALEIDINLQAKAT-PTERVFPA-LRE-LPGE	44
Colwellia psychroerythraea	MLNITTTG-----IADKELSPPEVTFPPHATGEWNSQLEKQT-AIARAQWA-MEN-LPQG	52
Crocospaera watsonii	MPQSNLHLINSHVDN--HQVSEHYNKQTEQAYKPELSLKEVNSQLENAN-AQVVVETW-RET-FGEG	63
Prochlorococcus marinus	MTDQSIDTVST-----ATSTAAGQSPEVQVSTLEMPLEDARIHLANLS-AQKRLWA-LKQ-FQGG	59
Trichodesmium erythraeum	MLDQSLIRLN-----KNGQYNYQDYRYLPELDLGVNVQELSAD-FHTLVQWG-LKI-FKEG	54
Synechococcus elongatus	MFA-----LLPSTLEINAQLADQA-ATLIQWA-ATE-FSGS	42
Anabaena variabilis	MTA-----STAFK-----TQTFNFDLDELNQCFETAL-PKEILAWS-IEH-LPTG	34
Schizosaccharomyces pombe	MSSIDT-----PANKLQARTLPHPEHLEYINKQSELS-PQDILKWC-RWT-LPS	47
Saccharomyces cerevisiae	MKTYH-----LNNDIVTQEQDLHWNEQILKLETPOEIIAWS-IVT-FPH	43
Thermosynechococcus elongatus	MPS-----KMTLTPMMAASVSFDLDALNQRFEAGH-PRDILAWA-VTE-IPQG	45
Neurospora crassa	SQTSTRASSPKLCKFDDNASVDSGFASANSSTANPLSLKPHLDHLNKLENMALDILRFCKI--L-FPN	77
Aspergillus fumigatus	SDAEAAVDSRDLQTESGYVSDSSDVTYVEIVFTKPHLQFLNRQLQFLE-FOEILRWC-----ITS-LPH	73

[Mycobacterium tuberculosis]	TCNVVASNMADAVLVDLAAKVRPG	VPVIFLDLGYHFEVETIGTRDAISSVVDVR	-VLNVTPEHIVAEQDELL	128
[Pseudomonas aeruginosa]	-LWISFS-GAEDVVLVDMMAKLNKN	-VKVFSLDLTRLHPETVYRFIDQVREHKGIAIDVLS	DDPRLLEPLVVKRGG	127
[Pseudomonas syringae]	-LWISFS-GAEDVVLVDMMAKLNKN	-VKVFSLDLTRLHPETVYRFIDQVREHKGIDIVLS	DDPRLLEPLVVKRGG	104
[Pseudomonas fluorescens]	-LWISFS-GAEDVVLVDMMAKLNKN	-VKVFSLDLTRLHPETVYRFIDQVREHKGIDIVLS	DDPRLLEPLVVKRGG	104
[Pseudomonas putida]	-LWISFS-GAEDVVLVDMMAKLNKN	-VKVFSLDLTRLHPETVYRFIDQVREHKGIDIVLS	DDPRLLEPLVVKRGG	104
[Azotobacter vinelandii]	-LWISFS-GAEDVVLVDMMAKLNKN	-VKVFSLDLTRLHPETVYRFIDQVREHKGIDIVLS	DDPRLLEPLVVKRGG	104
[Methylobacterium capsulatus]	-IAVFSF-GAEDVVLVEMAARLRFG	-IEVFLDLDLTRLHPETVYRFIDQVREHKGIDIVLS	DDPRLLEPLVVKRGG	102
[Microbulbifer degradans]	-LWISFS-GAEDVVLVDMASKIKPG	-VQVCFLLDTRLHAEITYQFIEQVRKHDKIDKLDVLP	DDPRLLEPLVVKRGG	103
[Ceratopteris richardii]	-IAIAFS-GAEDVALIEYA-KLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	182
[Polygonatum pubescens]	-IAIAFS-GAEDVALIEYA-KLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	183
[Zea mays]	-IAIAFS-GAEDVALIEYA-KLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	185
[Lycopersicon esculentum]	-IAIAFS-GAEDVALIEYA-HLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	186
[Catharanthus roseus]	-IAIAFS-GAEDVALIEYA-HLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	185
[Brassica juncea]	-IAIAFS-GAEDVALIEYA-HLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	187
[Arabidopsis thaliana]	-IAIAFS-GAEDVALIEYA-HLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	182
[Lemna minor]	-IAIAFS-GAEDVALIEYA-HLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	187
[Chlamydomonas reinhardtii]	-VIAAFS-GAEDVALIEYA-HLTGR	-PFRVFSLDLTRLNPETVYRFDFVEKHYGIRIEM	YMPDASEVEALVRSKG	141
[Nocardioideus sp. JS614.2]	FC-VTSS--MADAVLAHLVSRVAFG	-IDVFLDLDGYHFAETIGTRDAVQATLQVNV	--LIITVQVQSAQDA	159
[Desulfobacterium hafnense]	RIALAASLSIHDQVLTQMLKIDAR	--IRFFIIDLGRNFQOTYDLMEETMSRHEGFIH	YVAPENSELFLPAIEY	141
[Pyrobaculum aerophilum]	NIALAFSGAEDVVVDLIMKRVAPD	-KIRVFMLDTRLPEEIEVLVDKVRHHGVEIEI	YVYVPTKEIEEFVKRGG	109
[Clostridium thermocellum]	KVALAASLSIHDQVLTDLIKINP	-KRVVFLDTRLGRHPQOTYDLMEETMSRHEGFIH	YVAPENSELFLPAIEY	105
[Bordetella parapertussis]	DAALASLSAARDMVLTHAIYAGG	-LDLEVFLLDTRLHAEITLGLVDAVATRYGRAIT	YVYRPPDAAEQVAVDHAG	109
[Burkholderia mallei]	KVKFASLSAARDMVLTHAIYAGG	-VAIGFISLDTGRHAEITLGLVDAVATRYGRAIT	YVYRPPDAAEQVAVDHAG	103
[Ralstonia eutropha]	RARFATLSAARDMVLTHAIYAGG	-RAGIRVFLDTRLHAEITLGLVDAVATRYGRAIT	YVYRPPDAAEQVAVDHAG	102
[Ralstonia solanacearum]	TAKFASLSAARDMVLTHAIYAGG	-RAHLPFLDTRLHAEITLGLVDAVATRYGRAIT	YVYRPPDAAEQVAVDHAG	131
[Dechloromonas aromatica]	SAAFANLSAARDMVLTHAIYAGG	-LPIEFSLDTRLPLEYDILAAVDKHHGLRLKLL	YVYRPPDAAEQVAVDHAG	108
[Sinorhizobium meliloti]	RAVFTLSLGIHQDQVLTAAIGSNR	-LDIEVALDTRLHAEITLGLVDAVATRYGRAIT	YVYRPPDAAEQVAVDHAG	121
[Wolinella succinogenes]	--LAFSHQARDVVVDLIMKRVAPD	-SFEVFLDTRLHAEITLGLVDAVATRYGRAIT	YVYRPPDAAEQVAVDHAG	91
[Bacillus subtilis]	-LVYACSFQIEGIVLIDLILKYVKK	-DAEIVFLDTRLHAEITLGLVDAVATRYGRAIT	YVYRPPDAAEQVAVDHAG	109
[Corynebacterium glutamicum]	IA-VTSS--MENTVLAELAAARHLPE	-ADPFLDLDGYHFEVETIGTRDAISSVVDVR	-SKLVYVPLIKRTEQSDIY	108
[Corynebacterium efficiens]	VA-VTSS--MENTVLAELAAARHLPE	-QADFLDLDGYHFEVETIGTRDAISSVVDVR	-SKLVYVPLIKRTEQSDIY	136
[Nocardioideus sp. JS614]	LC-ATSS--MSDAATHLASSCAPG	-LDVFLDLDGYHFEVETIGTRDAISSVVDVR	-SKLVYVPLIKRTEQSDIY	105
[Chloroflexus aurantiacus]	-LLMSTAFNLNGVVLIELA-AQAG	-FA-GEVVFVDTGYHFPPELAWRDLITRPPV	LLSADLPPVWGE----	94
[Deinococcus geothermalis]	-LLMSTAFNLNGVVLIELA-AQAG	-YR-GEVVFVDTGYHFPPELAWRDLITRPPV	LLSADLPPVWGE----	111
[Deinococcus radiodurans]	-LLMSTAFNLNGVVLIELA-AQAG	-YR-GEVVFVDTGYHFPPELAWRDLITRPPV	LLSADLPPVWGE----	127
[Nitrobacter winogradskyi]	RLALVSSFGTSSAALLKVM-ADVDP	-A-IPVIFLDTGWLFEETLITRDTLAAALGLD	KVDSRIFPSSDALKQEDPDR	129
[Nitrobacter hamburgensis]	RLALVSSFGTSSAALLKVM-ADVDP	-A-IPVIFLDTGWLFEETLITRDTLAAALGLD	KVDSRIFPSSDALKQEDPDR	145
[Cytophaga hutchinsonii]	----SSPQTHSIVLHLL-SEDFP	-S-IPVIFLDTGWLFEETLITRDTLAAALGLD	KVDSRIFPSSDALKQEDPDR	91
Salmonella typhimurium	-FVLSSTFGICAAVSLHLV-NQIR	-PDIPVILDDTGYLFPETVYRFIDELTKKLN	--LKVYRAESPAWQEAR	116
Escherichia coli	-FVLSSTFGICAAVSLHLV-NQIR	-PDIPVILDDTGYLFPETVYRFIDELTKKLN	--LKVYRAESPAWQEAR	116
Erwinia carotovora	-FVLSSTFGICAAVSLHLV-TQQR	-PDIPVILDDTGYLFPETVYRFIDELTKKLN	--LKVYRAESPAWQEAR	116
Yersinia pseudotuberculosis	-FVLSSTFGICAAVSLHLV-TQQR	-PDIPVILDDTGYLFPETVYRFIDELTKKLN	--LKVYRAESPAWQEAR	116
Zymomonas mobilis	-FVLSSTFGICAAVSLHLV-TRKK	-ADIPVILDDTGYLFPETVYRFIDELTKKLN	--LQVYRAESPAWQEAR	116
Photobacterium luminescens	-FVLSSTFGICAAVSLHLV-TQXY	-PDIPVILDDTGYLFPETVYRFIDELTKKLN	--LQVYRAESPAWQEAR	116
Vibrio parahaemolyticus	-FVLSSTFGICAAVSLHLV-TQEX	-PDIPVILDDTGYLFPETVYRFIDELTKKLN	--LQVYRAESPAWQEAR	121
Vibrio vulnificus	-HAVSSSTFGICAAVSLHLV-SKQQ	-ADIPVILDDTGYLFPETVYRFIDELTKKLN	--LKVYRAESPAWQEAR	121
Vibrio fischeri	-FVLSSTFGICAAVSLHLV-TEVK	-SDIPVILDDTGYLFPETVYRFIDELTKKLN	--LKVYRAESPAWQEAR	116
Vibrio cholerae	-HALSSSTFGICAAVSLHLV-TSVK	-SDIPVILDDTGYLFPETVYRFIDELTKKLN	--LKVYRAESPAWQEAR	119
Photobacterium profundum	-FVLSSTFGICAAVSLHLV-TNES	-PKVIVLDDTGYLFPETVYRFIDELTKKLN	--LKVYRAESPAWQEAR	116
Candidatus Blochmannia	-AILSSSTFGICSSVSLHLV-THYI	-ENIPVILDDTGYLFPETVYRFIDELTKKLN	--LHVFSPQSAWQEAR	124
Candidatus Blochmannia florida	-LVLSSSTFGICHSVSLHLV-TNXY	-PSVIVLDDTGYLFPETVYRFIDELTKKLN	--LHVFSPQSAWQEAR	117
Buchnera aphidicola	-QIMSSSTFGICSVLHLL-IKKQ	-PDIPVILDDTGYLFPETVYRFIDELTKKLN	--LKVFRSTISSAWQEAR	116
Xanthomonas oryzae	-AALSSSTFGICSAVLLHLL-SQQR	-PDIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYRPLVSRAMWQEAR	112
Xanthomonas campestris	-AALSSSTFGICSAVLLHLL-TQQR	-PDIPVILDDTGYLFPETVYRFADALTEKLSL	--LQYVPLVSRAMWQEAR	112
Xylella fastidiosa	-PALSSSTFGICSAVLLHLL-TRFA	-PDIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYQPLVSRAMWQEAR	108
Thiopsis roseopercarina	-FVLSSTFGICSAVLLHLL-SRQM	-PEIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYRPLVSRAMWQEAR	112
Pseudoalteromonas haloplanktis	-HPLSSSTFGICSAVLLHLL-TLQQ	-PDIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYRPLVSRAMWQEAR	114
Shewanella oneidensis	-HALSSSTFGICSAVLLHLL-SVQQ	-SDIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYQAPVSRAMWQEAR	116
Shewanella frigidimarina	-HALSSSTFGICSAVLLHLL-TQAA	-SDIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYQAGQVSRAMWQEAR	114
Shewanella denitificans	-HGLSSSTFGICSAVLLHLL-TQVQ	-SDIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYQAGQVSRAMWQEAR	130
Shewanella amazonensis	-HILSSSTFGICSAVLLHLL-THYK	-SDVPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYRPAVSRAMWQEAR	113
Colwellia psychroerythraea	-FVLSSTFGICSAVLLHLL-TQVD	-SNIPVILDDTGYLFPETVYRFADALTEKLSL	--LQYVQAKESPAWQEAR	121
Crocospaera watsonii	-LVMSSSTFGICSAVLLHLL-TEVV	-PDIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYQSPVSRAMWQEAR	132
Prochlorococcus marinus	-FVLTSSSTFGICSAVLLHLL-HGLDGG	--SVVPPVVDVDTGYLPAETVYFADALTEKLSL	--LKVYQSTMSPARMEAL	130
Trichodesmium erythraeum	-LVMSSSTFGICSAVLLHLL-TSVI	-PNIPVIVIDDTGYLPAETVYFAELIERKLSL	--VKVYQSHISPARMEAL	123
Synechococcus elongatus	-LVLSSSTFGICSAVLLHLL-TQVQ	-PDIPVILDDTGYLFPETVYRFADALTEKLSL	--LKVYQSEISSPARMEAL	103
Anabaena variabilis	-LVQTSFANVVDLVIITHLISELKL	-QVPPVIFLDTLHHPQOTLLELVAKAKHINLND	--LQYVYKTPDVTDR-RAF	111
Schizosaccharomyces pombe	-LQQTAFGLTGLVLDML-SKMD	-MNVPLIFLDTLHHPQOTLLELVAKAKHINLND	--LQYVYKTPDVTDR-RAF	116
Saccharomyces cerevisiae	-LQQTAFGLTGLVLDML-SKLS	-EKVYVPELFLDTLHHPQOTLLELVAKAKHINLND	--LQYVYKTPDVTDR-RAF	118
Thermosynechococcus elongatus	-LVQTSFANVVDVITDILLRDLRP	--NPPVPLVFLDTLHHPQOTLLELVAKAKHINLND	--LQYVYKTPDVTDR-RAF	116
Neurospora crassa	-LQQTAFGLTGLVLDML-SKLAKE	-PDAAVVDLIFLDTLHHPQOTLLELVAKAKHINLND	--LQYVYKTPDVTDR-RAF	152
Aspergillus fumigatus	-LQQTAFGLTGLVLDML-SKLVQV	--PRFQMVLDLIFLDTLHHPQOTLLELVAKAKHINLND	--LQYVYKTPDVTDR-RAF	146



CLUSTAL X (1.81) MULTIPLE SEQUENCE ALIGNMENT

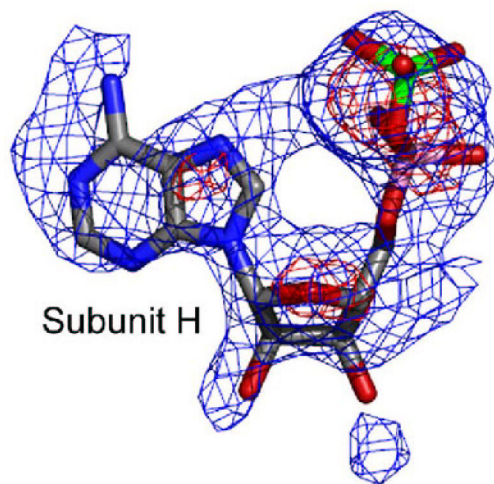
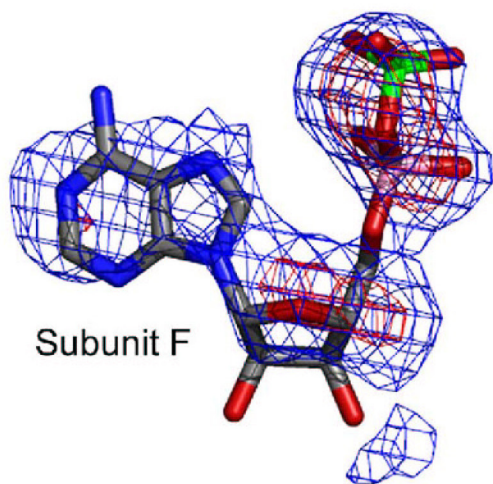
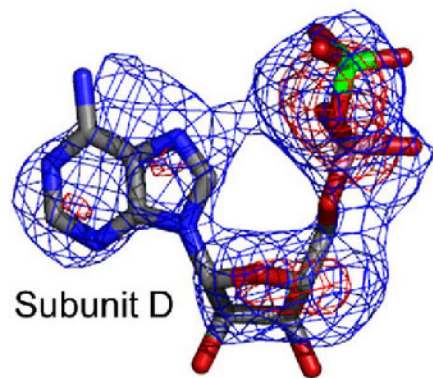
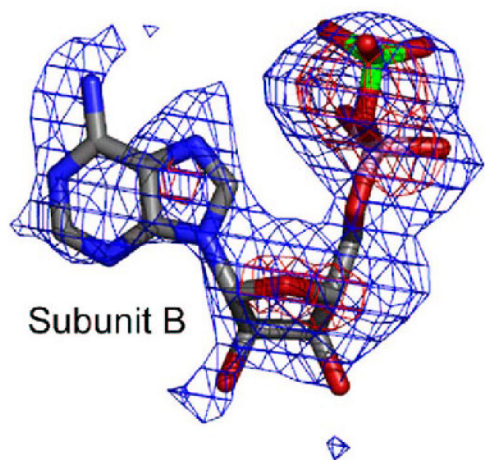
[Mycobacterium tuberculosis]	GKDLFAR---NPHECCRLRQVPLGKLRG---YSAWVGLRRVDAP--TRANAPLVVSFDET-----FKLVKVNPLAA	193
[Pseudomonas aeruginosa]	LFSFYRD--G--HGECGIRKIEPLRKLKLAG--VRAWAGQRRDDSPGTRSQVAVLEIDGAFSTPHK---PLYKFNPLSS	198
[Pseudomonas syringae]	LFSFYRD--G--HGECGIRKIEPLRKLKLAG--VKAWAGQRRDDSPGTRSAVAALVDSAFSTAES---TLYKFNPLAQ	175
[Pseudomonas fluorescens]	LFSFYKD--G--HGECGIRKIEPLRKLKLAG--VKAWAGQRRDDSPGTRSAVAAMEIDTAFSTPHR---TLYKFNPLAQ	175
[Pseudomonas putida]	LFSFYKD--G--HGECGIRKIEPLRKLKLAG--VSAWAGQRRDDSPGTRSQVAALVDSAFSTPHR---TLYKFNPLAQ	175
[Azotobacter vinelandii]	LFSFYKD--G--HGECGIRKIEPLRKLKLAG--VSAWAGQRRDDSPGTRSQVAVFEVVDGAFSTPHR---PLYKFNPLAQ	175
[Methylococcus capsulatus]	LFSFYRD--G--HGECGIRKVRPLRKLKLAG--LDAMIWGQRRDDQNP--TRQAVPEVREADPAFATGHH---PLVKFNPLAA	172
[Microbulbifer degradans]	LFSFFED--N--HQECCGIRKRVGLRKLKLE--VDAWVQGRKDDSPGTRASIPVIGDNRVRFARPGD---TLTKFNPLVN	174
[Ceratopteris richardii]	MFSFYED--GHQECRIRKVRPLRRLALRG--LRAWITGQRKDDSPGTRSNIPVIGDNRVRFARPGD---TLTKFNPLVN	256
[Polygonatum pubescens]	LFSFYED--GHQECRIRKVRPLRRLALRG--LRAWITGQRKDDSPGTRASIPVIGDNRVRFARPGD---TLTKFNPLVN	259
[Zea mays]	LFSFYED--GHQECRIRKVRPLRRLALRG--LRAWITGQRKDDSPGTRASIPVIGDNRVRFARPGD---TLTKFNPLVN	257
[Lycopersicon esculentum]	LFSFYED--GHQECRIRKVRPLRRLALRG--LRAWITGQRKDDSPGTRSEIPVIGDNRVRFARPGD---TLTKFNPLVN	260
[Catharanthus roseus]	LFSFYED--GHQECRIRKVRPLRRLALRG--LRAWITGQRKDDSPGTRSEIPVIGDNRVRFARPGD---TLTKFNPLVN	259
[Brassica juncea]	LFSFYED--GHQECRIRKVRPLRRLALRG--LRAWITGQRKDDSPGTRSEIPVIGDNRVRFARPGD---TLTKFNPLVN	261
[Arabidopsis thaliana]	LFSFYED--GHQECRIRKVRPLRRLALRG--LRAWITGQRKDDSPGTRSEIPVIGDNRVRFARPGD---TLTKFNPLVN	256
[Lemna minor]	LFSFYED--GHQECRIRKVRPLRRLALRG--LRAWITGQRKDDSPGTRSEIPVIGDNRVRFARPGD---TLTKFNPLVN	261
[Chlamydomonas reinhardtii]	LYSFTE--GHTECCIRKRVPLRKLKLVG--LRAWITGQRKDDSPGTRSEIPVIGDNRVRFARPGD---TLTKFNPLVN	215
[Nocardioideis sp. JS614 2]	YGDLYK---TDPDLCCALRQVPLADALSG--YDAWAGLRRRATQ--HRVIAFVIGWADK---RQKVKVNSPLAR	215
[Desulfitobacterium hafnienae]	PNAFYDK--VELRQKCCRLRQVPLRKLKLAG--VDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	218
[Pyrobaculum aerophilum]	INPFYRD--VELRHLCCKIRKVNPLRRLALSG--LDAMIWGQRRDDSPGTRATRKIIDID--HDIYHG---LLKISPID	177
[Clostridium thermocellum]	PNPFYDS--VELRKKCCIRKVNPLRKLKLAG--VDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	172
[Bordetella parapertussis]	MHAFYDS--VALRKACCEIRKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	176
[Burkholderia mallei]	LNAFYDS--VELRKKCCIRKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	170
[Ralstonia eutropha]	LNAFYDS--IDLKDCCKIRKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	199
[Ralstonia solanacearum]	LNAFYDS--IDLKDCCKIRKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	198
[Dechloromonas aromatica]	INAFYDS--VTLRKACCYARKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	175
[Sinorhizobium meliloti]	MNGFYDS--VBARHACCGVRKLEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	188
[Wollinella succinogenes]	EWGMRES--LNNRRCCKRVRKIEPLRKLKLAG--VDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	158
[Bacillus subtilis]	DKLWER---EPNQCYYLRKVVPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	173
[Corynebacterium glutamicum]	LNLYRS---NPAACCRMRKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	171
[Corynebacterium efficiens]	YGNLYR---SNFTACCRMRKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	201
[Nocardioideis sp. JS614]	IPPWST---DSNDCCKRRLRQVPLRKLKLAG--VDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	169
[Chloroflexus aurantiacus]	EQFRS---DPDCCCRVRKVVPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	156
[Deinococcus geothermalis]	DLTAS---DPDACCARVVTPLQAYLHKKASALLNARSDDQAA--TRADIPFVREGG---TRVKNINPLAY	174
[Deinococcus radiodurans]	DLTAS---DPDACCARVVTPLQAYLHKKASALLNARSDDQAA--TRADIPFVREGG---TRVKNINPLAY	190
[Nitrobacter winogradskyi]	ELWFS---DPDSCCRIRKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	190
[Nitrobacter hamburgensis]	DRDLWFS---DPDACCIRKVEPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	208
[Cytophaga hutchinsonii]	RLLFIS---DPDYCCYLNRKVVPLRRLALSG--LDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	154
Salmonella typhimurium	YGLLWEQ--GVEGIEKYNKINKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Escherichia coli	YGLLWEQ--GVEGIEKYNKINKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Erwinia carotovora	YGLLWDQ--GVEGIERYNLNLKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Yersinia pseudotuberculosis	YGLLWEQ--GVEGIERYNLNLKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Zymomonas mobilis	YGLLWEQ--GVEGIERYNLNLKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Photobacterium luminescens	YGLLWEQ--GVEGIERYNLNLKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Vibrio parahaemolyticus	YGLLWEQ--GVEGIEKYNKINKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	189
Vibrio vulnificus	YGLLWEQ--GIEGIEKYNKINKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	189
Vibrio fischeri	YGLLWEQ--GVEGIEKYNKINKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Vibrio cholerae	YGLLWEQ--GVEGIERYNLNLKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	187
Photobacterium profundum	BGKLWEQ--GVDGIKQYNRLNKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Candidatus Blochmannia	YGLLWTD--GIEGIEKYNLNLKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	192
Candidatus Blochmannia	YGLLWTD--GIEGIEKYNLNLKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	185
Buchnera aphidicola	YGLLWEK--GIEGIDFYNNLNKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Xanthomonas oryzae	BGRLWEQ--GMVGDQYNNLRKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	180
Xanthomonas campestris	BGRLWEQ--GMVGDQYNNLRKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	180
Xylella fastidiosa	BGRLWEQ--GIDGIDQYNNLRKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	176
Thiocapsa roseopercinina	LGRLEWQ--GADGIERYNRLNKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	180
Pseudoalteromonas haloplanktis	FGKLWEQ--GEAGIKQYNLNLKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	182
Shewanella oneidensis	FGQLWEQ--GVEGLERYNRLNKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	184
Shewanella frigidimarina	FGKLWEQ--GIDGLEKYNRLNKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	202
Shewanella denitrificans	FGKLWEQ--GLDGLEQYNRLNKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	198
Shewanella amazonensis	FGKLWEQ--GLDGLEKYNRLNKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	181
Colwellia psychrotolerans	YGDEWAQ--GEDALKANRRNKVPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	189
Crocospaera watsonii	YGLLWQD--DLNSLNRKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	201
Prochlorococcus marinus	YGLLWET--GSKVDLETYHRIRKVEPLRKLKLAG--VDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	199
Trichodesmium erythraeum	YGLLWAEKDL--EALNRKQDQIRKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	192
Synechococcus elongatus	YGLLWESV--DFNRYDQMRKVEPMRRALDELNVGTFWFGRLRRDQSK--SRAGLPILSISQ---NGVFKFLPVID	172
Anabaena variabilis	YAKYGEALWDKDI--TQFHQVTRIEPLRKLKLAG--VDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	181
Schizosaccharomyces pombe	AQKFGKLEWET--DESRVDFLAKVEPAHRAVSDLNVAVFTGRRRS--GG--ERGLPIVGLD---GPVLKINPLAN	185
Saccharomyces cerevisiae	ASKYGDFLWEK--DDEKDYDLAKVEPAHRAVSDLNVAVFTGRRRS--GG--ERGLPIVGLD---GPVLKINPLAN	189
Thermosynechococcus elongatus	AARYGDKLWET--VVEQFHHLLTIEPLRKLKLAG--VDAMIWGQRRDDSPGTRQETGLLEWDSR---HAIFYKFNPLAA	186
Neurospora crassa	EALYGEKLYET--SEELDWIAKVEPNRAVYQELKVAAVFTGRRRS--GG--ARGSPVILBEE---RGVTKINPLAN	223
Aspergillus fumigatus	SKYTGAKLWET--DDQFVWVAKVEPAORAYRELNVHVAVFTGRRRS--GG--KRGDLDVIEVDBAG---LIKVNPLAN	216



CLUSTAL X (1.81) MULTIPLE SEQUENCE ALIGNMENT

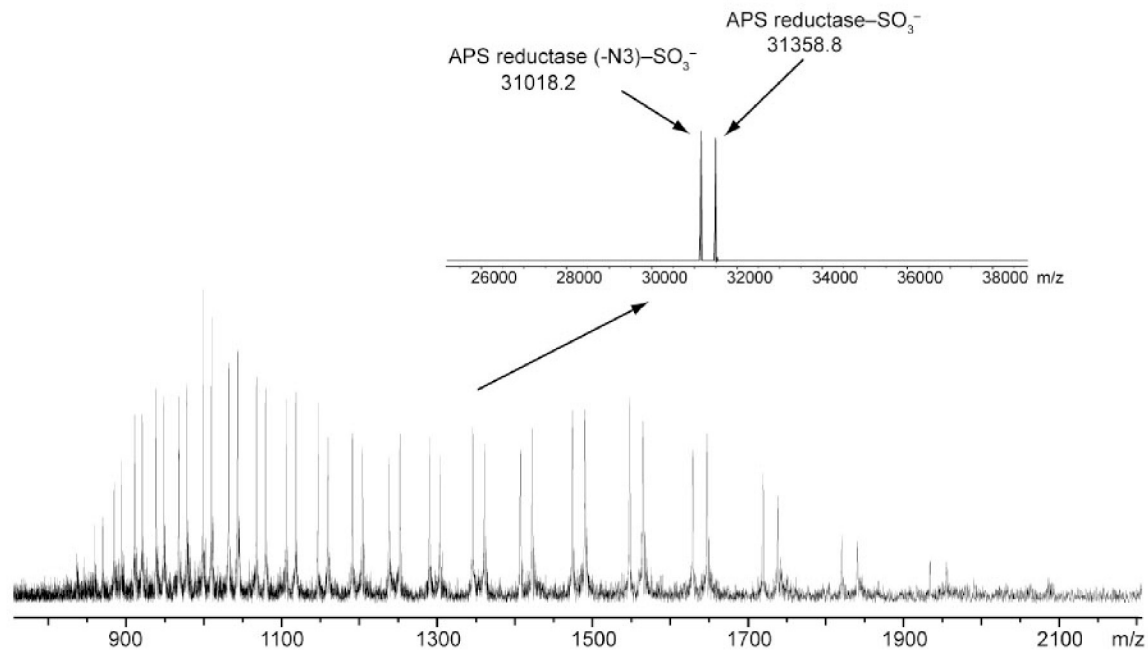
Page 4 of APSr and PAPSr sequences

[Mycobacterium tuberculosis]	WDQDQVEYIADNDVLPVNLVREYFISIGCAPCHA	KFAEG	---	ADFRSGRWQL	---	AKTECGLHAS	254
[Pseudomonas aeruginosa]	MTSEEWVGMIRMLELPYNLSLHERGYIIGCEPCTR	PVLP	---	NQHEREGRWWWE	---	EATKKECGLHAGNLIASKA	267
[Pseudomonas syringae]	MSSEEWVGMIRMLELPYNLSLHERGFISIGCEPCTR	PVLP	---	NQHEREGRWWWE	---	EATKKECGLHAGNLIIVRD	244
[Pseudomonas fluorescens]	MTSEEWVGMIRMLELPYNLSLHERGYIIGCEPCTR	PVLP	---	NQHEREGRWWWE	---	EATKKECGLHAGNLIASKA	244
[Pseudomonas putida]	MSSEEWVGMIRMLELPYNLSLHERGFISIGCEPCTR	PVLP	---	NQHEREGRWWWE	---	ESTKKECGLHAGNLIASKA	244
[Azotobacter vinelandii]	MSSEEWVGMIRMLELPYNLSLHERGFISIGCEPCTR	PVLP	---	NQHEREGRWWWE	---	EATKKECGLHAGNLIASKA	244
[Methylococcus capsulatus]	WTSQAQVMDVITACEIPFNALHLKDFGVYIGCEPCTR	PVPR	---	QHHEREGRWWWE	---	DALKKECGLHIGDASRV	240
[Microbulfiber degradans]	WTSQAQVMDVITACEIPFNALHLKDFGVYIGCEPCTR	PTGP	---	QHHEREGRWWWE	---	EATKKECGLHAGNLIIVRD	241
[Ceratopteris richardii]	VTGTDIWSFLRTMNPVNLVSLHSGYVIGCEPCTR	AVLPG	---	MHEREGRWWWE	---	AKAKECGLHAGNLIASKA	325
[Polygonatum pubescens]	VDGKDVNFRITMNPVNLVSLHSGYVIGCEPCTR	PVLPFG	---	QHHEREGRWWWE	---	AKAKECGLHAGNLIASKA	328
[Zea mays]	VDGKDIWFLRTMNPVNLVSLHSGYVIGCEPCTR	PVLPFG	---	QHHEREGRWWWE	---	AKAKECGLHAGNLIASKA	326
[Lycopersicon esculentum]	VDGKDIWFLRTMNPVNLVSLHSGYVIGCEPCTR	PVLPFG	---	QHHEREGRWWWE	---	AKAKECGLHAGNLIASKA	329
[Catharanthus roseus]	VEGKDIWFLRTMNPVNLVSLHSGYVIGCEPCTR	PVLPFG	---	QHHEREGRWWWE	---	AKAKECGLHAGNLIASKA	328
[Brassica juncea]	VEGNDVWNFLRTMNPVNLVSLHSGYVIGCEPCTR	AVLPG	---	QHHEREGRWWWE	---	AKAKECGLHAGNLIASKA	330
[Arabidopsis thaliana]	VEGNDVWNFLRTMNPVNLVSLHSGYVIGCEPCTR	AVLPG	---	QHHEREGRWWWE	---	AKAKECGLHAGNLIASKA	325
[Lemna minor]	VDGQDIWFLRTMNPVNLVSLHSGYVIGCEPCTR	PVLPFG	---	QHHEREGRWWWE	---	AKAKECGLHAGNLIASKA	330
[Chlamydomonas reinhardtii]	MTSAEAVNFRIVMKNVPTNKLKNCGYIIGCEPCTR	PVLPFN	---	QAHEREGRWWWE	---	AAAKECGLHAGNLIASKA	284
[Nocardiooides sp. JS614 2]	WTDEEIERIADHDVLPVNLVPLAYDGYPSIGCWPCR	RVAPG	---	EDFRSGRWAGT	---	TKTECGIHS	275
[Desulfitobacterium hafniense]	WSSEQVWVYIRRENIPYSSLNKGFYRIGCQPCR	AVAFG	---	ADVRSGRWWWE	---	DFSKKKEGLHPSLNPPAK	287
[Pyrobaculum aerophilum]	WTDWVWQYIKKYNLPYCKLYDRGYIIGCEPCTR	PTFVSLGDV	---	GEELRKGGRWWWE	---	KNAPKECGIHCSLEAGS	251
[Clostridium thermocellum]	WSEDRVWEIKIKYINIPYNSLHSGYRIGCQPCR	AVRF	---	GEDVRSGRWWWE	---	DFDKKKEGLHAGNLIASKA	239
[Bordetella parapertussis]	WNEADVMSVIRALGIPYNSLHSGYRIGCQPCR	AVRF	---	GEDVRSGRWWWE	---	SSDSKKECGLHAGNLIASKA	245
[Burkholderia mallei]	WTESDVAWYLNADFVNPVNLVREYFISIGCEPCTR	AIRP	---	GEDSRAGRWWWE	---	SRDTKKECGLHITITVFP	238
[Ralstonia eutropha]	WTEAEVWYALKRRNVPVNLVSLHSGYRIGCEPCTR	AVRAG	---	EDLRAGRWWWE	---	SKDSKKEGLHAGNLIASKA	266
[Ralstonia solanacearum]	WTEAEVWYALKRRNVPVNLVSLHSGYRIGCEPCTR	AVRAG	---	EDLRAGRWWWE	---	SRDSKKECGLHAGNLIASKA	267
[Dechloromonas aromatica]	WTEKEVWTYIKQNAVYPVNLVREYFISIGCAPCHA	AISP	---	GEDIAGRWWWE	---	NPEKKECGLHAGNLIASKA	239
[Sinorhizobium meliloti]	WGETTQAAVAAEGIPVNLVREYFISIGCEPCTR	AIKP	---	GEPERAGRWWWE	---	NDEKKECGLHAGNLIASKA	257
[Wolinella succinogenes]	WSLETEVFEYIKKHALPLHPLVTEYRIGSPCTR	AIEAG	---	EELRAGRWWWE	---	NPEKKECGLHAGNLIASKA	227
[Bacillus subtilis]	WTWKDIWRYSRNELDVNPVNLVREYFISIGCAPCHA	PAFTA	---	EDLRSGRWNG	---	MAKTECGLHE	233
[Corynebacterium glutamicum]	WSLEEINEFIADNLDHPLHSGYRIGCAPCHA	PVAEG	---	QDFRAGRWAG	---	NAKTECGLHE	231
[Corynebacterium efficiens]	WSLEEINEFIADNLDHPLHSGYRIGCAPCHA	PVAEG	---	QDFRAGRWAG	---	NAKTECGLHE	261
[Nocardiooides sp. JS614]	WTDEALAAHLHEHALPQNRLLGEYFISIGCEPCTR	LVFV	---	GTARSGRWNG	---	QHKTECGLHAS	229
[Chloroflexus aurantiacus]	WRREQIEAFQAHLVNPVNLVREYFISIGCWPCR	AVRPG	---	EDVRSGRWNG	---	KGKIECGLWIGEQSL	221
[Deinococcus geothermalis]	WTRERLETYAREHDLVNPVNLVREYFISIGCWPCR	AVRPG	---	EDARAGRWAG	---	KGKIECGLWIGEQSL	239
[Deinococcus radiodurans]	WTREQLEAYAAEHDLVNPVNLVREYFISIGCWPCR	AVKPG	---	EDARAGRWAG	---	KGKIECGLWIGEQSL	255
[Nitrobacter winogradskyi]	ASREDEIAYASAKLPPHPLVAGSFRVSGMPCPTS	RQAG	---	EDARAGRWRG	---	RAKTECGIHTMKI	254
[Nitrobacter hamburgensis]	ASRADTEIAYASAKLPPHPLVAGSFRVSGMPCPTS	RAQAG	---	EDARAGRWRD	---	AKTECGIHTMKI	272
[Cytophaga hutchinsonii]	WDVVMIEKIKKHKLPVNPVNLVREYFISIGCEPCTR	KFDLET	---	YNRQGRWFG	---	LNKTECGLHAGNLIASKA	224
Salmonella typhimurium	WDNRTVYQYLKQKGLKYLHPLWDEGYLAVGDTHTR	KWEPG	---	MABEETRFVGL	---	KRECGLHEG	240
Escherichia coli	WDNRTVYQYLKQKGLKYLHPLWDEGYLAVGDTHTR	KWEPG	---	MABEETRFVGL	---	KRECGLHEG	244
Erwinia carotovora	WDNRTVYQYLKQKGLKYLHPLWDEGYLAVGDTHTR	KWEPG	---	MSEETRFVGL	---	KRECGLHEG	244
Yersinia pseudotuberculosis	WDNRQVYQYLKQKGLKYLHPLWDEGYLAVGDTHTR	KWEPG	---	MSEETRFVGL	---	KRECGLHEG	244
Zymomonas mobilis	WDNRKIHVYKLENDLPHVPLWDEGYLAVGDTHTR	KWEPG	---	MSEETRFVGL	---	KRECGLHEG	244
Photorhabdus luminescens	WDNRVHVQYLTKHGLEHPLWDEGYLAVGDTHTR	KWEPG	---	MSEETRFVGL	---	KRECGLHEN	244
Vibrio parahaemolyticus	WTNKKDVHYLKEHGLVYHPLWDEGYLAVGDTHTR	KWEPG	---	MSEETRFVGL	---	KRECGLHEDDQNEQD	255
Vibrio vulnificus	WTNKKDVHYLKEHGLVYHPLWDEGYLAVGDTHTR	KWEPG	---	MSEETRFVGL	---	KRECGLHEDDQNEQD	254
Vibrio fischeri	WTNKKDVHYLKEHGLVYHPLWDEGYLAVGDTHTR	KWEPG	---	MSEETRFVGL	---	KRECGLHEDDQNEQD	249
Vibrio cholerae	WTNKKDVHYLKEHGLVYHPLWDEGYLAVGDTHTR	KWEPG	---	MSEETRFVGL	---	KRECGLHEDDQNEQD	252
Photobacterium profundum	WTDNDIEQYLNQYDLVYHPLWDEGYLAVGDTHTR	KWEPG	---	MTEETRFVGL	---	KRECGLHEDDQNEQD	249
Candidatus Blochmannia	WNSLQIHRVYIEKHSLEYHPLWQGYVSVGDVHSTK	KWEPG	---	MKEDTRFVGL	---	QRECGLHIE	253
Candidatus Blochmannia florida	WDHNQVHQYIKRVDLEYHPLWQGYVSVGDVHSTK	KKEPG	---	MKEDTRFVGL	---	QRECGLHIE	246
Buchnera aphidicola	WSKDKIKDLKENNLDLPHVPLWQGYVSVGDVHSTK	KMPG	---	MLEETRFVGL	---	KRECGLHEN	244
Xanthomonas oryzae	WTDREVWQYLQAHALPYHPLWQGYVSVGDVHSTK	RWEPG	---	MREDTRFVGL	---	KRECGIHEDI	241
Xanthomonas campestris	WTDREVWQYLQAHALPYHPLWQGYVSVGDVHSTK	RWEPG	---	MREDTRFVGL	---	KRECGIHEDI	241
Xylella fastidiosa	WTDREIWEYMKHHDLPYHPLWQGYVSVGDVHSTK	PLEPG	---	MREDTRFFFG	---	KRECGIHENI	237
Thiocapsa roseopercarina	WHRPFRARLRHDLPHPLWQGYVSVGDVHSTK	PLLPG	---	MLEETRFVGL	---	KRECGIHR	239
Pseudoalteromonas haloplanktis	WTDREIWEYMKHHDLPYHPLWQGYVSVGDVHSTK	KLEPG	---	MTEETRFVGL	---	NRECGLHIDGDI	246
Shewanella oneidensis	WSNKKDVHLYLQYDLVYHPLWQGYVSVGDVHSTK	PLELG	---	MTEETRFVGL	---	KRECGLHIEI	245
Shewanella frigidimarina	WSNKKDVHLYLQYDLVYHPLWQGYVSVGDVHSTK	PLELG	---	MSEETRFVGL	---	QRECGLHEDI	263
Shewanella denitrificans	WSNKKDVHLYLQYDLVYHPLWQGYVSVGDVHSTK	PLELG	---	MSEETRFVGL	---	KRECGLHIEI	259
Shewanella amazonensis	WSNKKDVHLYLQYDLVYHPLWQGYVSVGDVHSTK	PLELG	---	MSEETRFVGL	---	KRECGLHIEI	242
Colwellia psycherythraea	WSNKKDVHLYLQYDLVYHPLWQGYVSVGDVHSTK	PLTIG	---	MSEEDTRFVGL	---	QRECGLHIDGDI	253
Crocospaera watsonii	WDSRDYHLYLQYDLVYHPLWQGYVSVGDVHSTK	PLMADD	---	SEEDSRFVGG	---	KOEGLHLNLSFGAA	268
Prochlorococcus marinus	WTPKEVYVYMQEHQLPQHPLEFKGYVSVGDVHSSA	PDGGG	---	ASGRETRFVGL	---	KOEGLHLPLQVLEGG	265
Trichodesmium erythraeum	WNARDIYQYLTANHPLPYHPLWQGYVSVGDVHSSR	PLTDD	---	QDRDTRFVGL	---	KOEGLHLPLQVLEGG	259
Synechococcus elongatus	WHSRDVYQYLQYDLVYHPLWQGYVSVGDVHSSR	PLQAD	---	SDRSTRFVGL	---	KOEGLHL	232
Anabaena variabilis	WTRQDQVAVAEHGVYVPLWQGYVSVGDVHSTK	KVGGG	---	EDERAGRWRGSR	---	KTECGIHI	241
Schizosaccharomyces pombe	WSPTVEVNYIITNVPVNLVREYFISIGCEPCTR	PVREG	---	EDERAGRWRGSR	---	KTECGIHSHPQSKFA	252
Saccharomyces cerevisiae	WTFEQVKQYIDANNVPVNLVREYFISIGCEPCTR	PVREG	---	EDERAGRWRGSR	---	KTECGIHSHPQSKFA	256
Thermosynechococcus elongatus	WTRKQVAVAEHGVYVPLWQGYVSVGDVHSTK	PIAEG	---	EDERAGRWRGSR	---	KTECGIHI	246
Neurospora crassa	WSFQVQVAVAEHGVYVPLWQGYVSVGDVHSTK	PVAAG	---	EDERAGRWRGSR	---	NKTECGIHNKQSRVYQ	290
Aspergillus fumigatus	WTFEQVKQYIKENDVPVNLVREYFISIGCEPCTR	PVKEN	---	EDFRSGRWKQ	---	EKTECGIHNPRSKVYQ	283

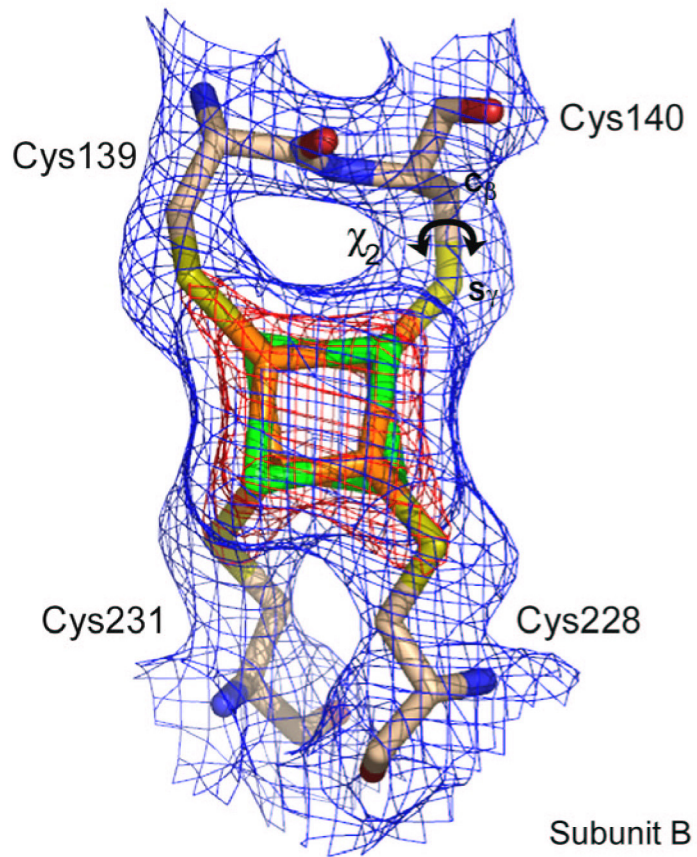


Supplementary Fig.3

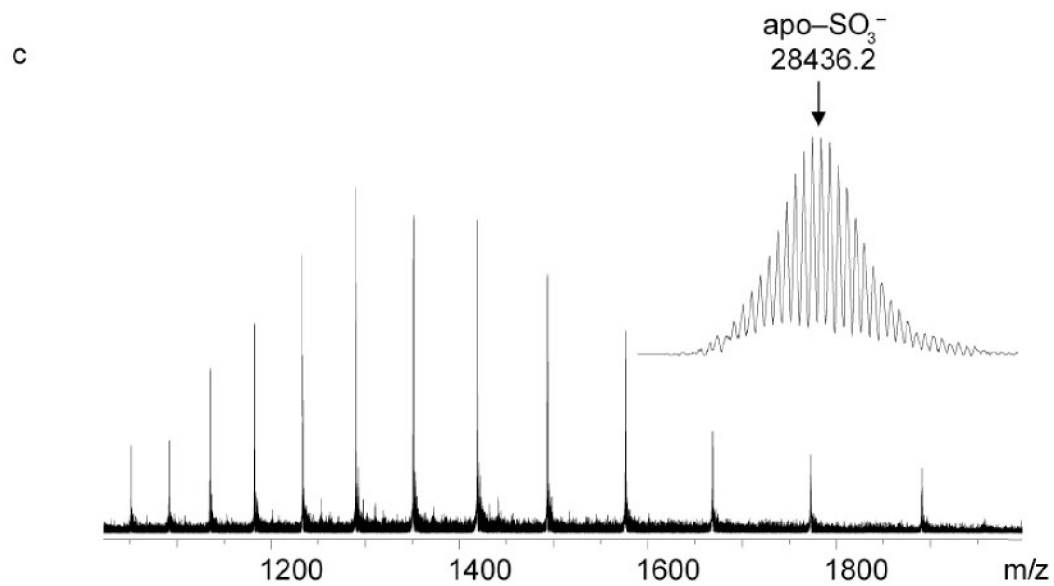
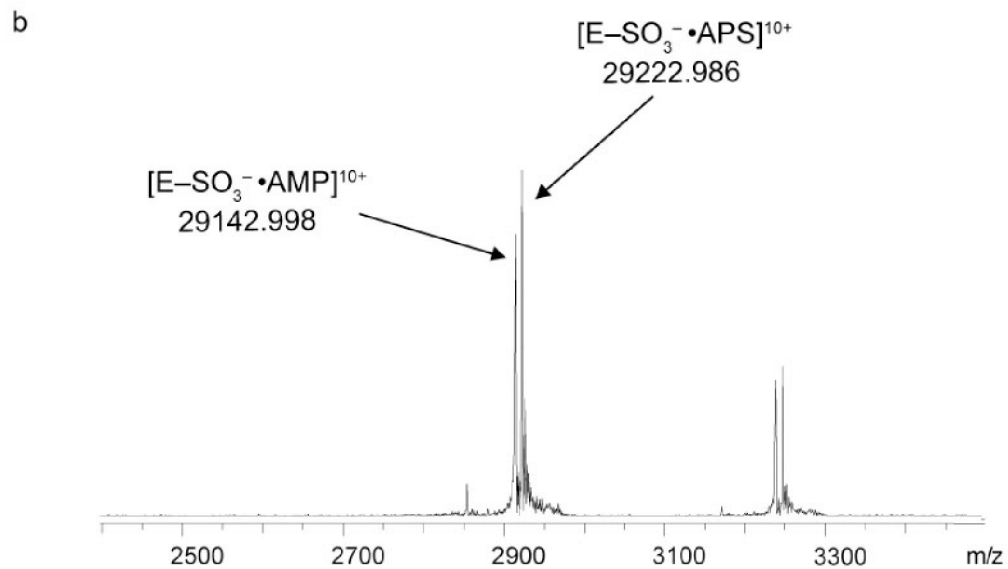
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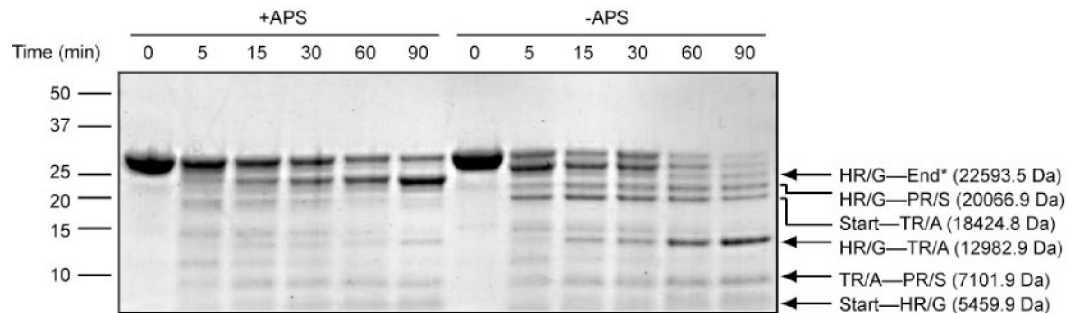
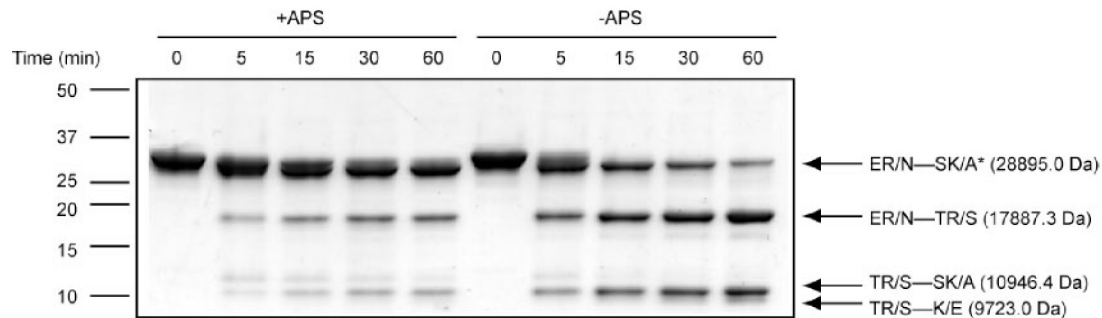
Supplementary Fig. 4a



Supplementary Fig. 6



Supplementary Figs. 4 b and c

a**b****Supplementary Fig. 5**