SUPPORTING INFORMATION

Thioredoxin reductase from *Thermoplasma acidophilum*: a new twist on redox regulation

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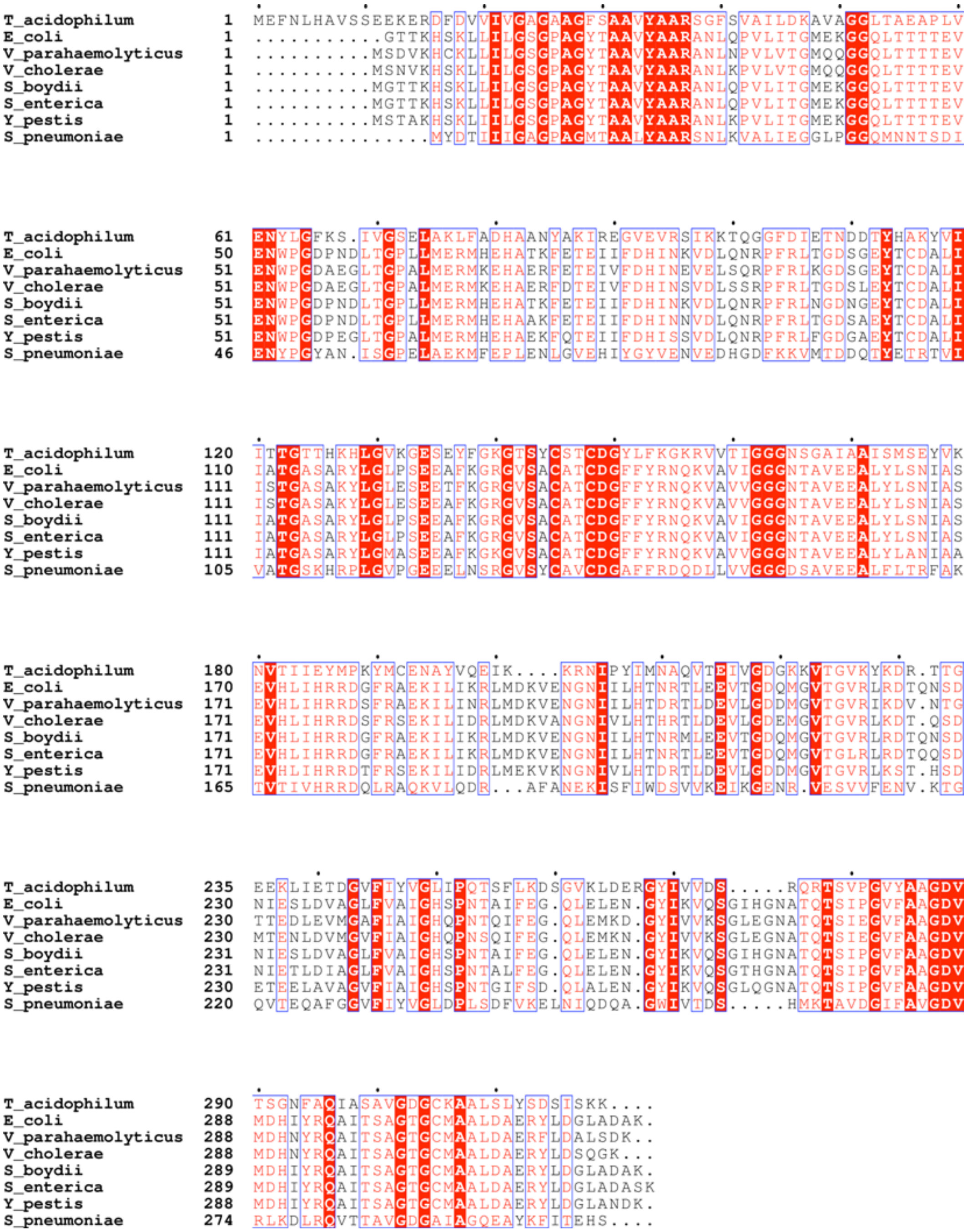
Figure S1. Alignment of selected TrxR sequences from PSI-BLAST (1). Sequences were aligned using the program ClustalW (2) and the output was rendered using the web interface ESPript 2.2 (3). Sequences are *E. coli* (gi:16128855), *V. parahaemolyticus* (gi:28898025), *V. cholerae* 0395 (gi:147674572), *S. boydii* BS512 (gi:82543374), *S. enterica* subsp. *enterica serovar Typhi* str. CT18 (gi:62179482), *Y. pestis* CO92 (gi:16121654), and *S. pneumoniae* R6 (gi:15903355). White letters in red blocks are strictly conserved residues. Red letters in black boxes are more than 50% conserved residues.



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V_parahaemolyticus     1   ..........MSDVKHCKLLILGSGPAGYTAAVYAARANLNPVLVTGMQQGGQLTTTTEV
V_cholerae             1   ..........MSNVKHSKLLILGSGPAGYTAAVYAARANLKPVLVTGMQQGGQLTTTTEV
S_boydii               1   ..........MGTTKHSKLLILGSGPAGYTAAVYAARANLQPVLITGMEKGGQLTTTTEV
S_enterica             1   ..........MGTTKHSKLLILGSGPAGYTAAVYAARANLQPVLITGMEKGGQLTTTTEV
Y_pestis               1   ..........MSTAKHSKLIILGSGPAGYTAAVYAARANLKPVLITGMEKGGQLTTTTEV
S_pneumoniae           1   ...............MYDTIIIGAGPAGMTAALYAARSNLKVALIEGGLPGGQMNNTSDI

T_acidophilum         61   ENYLGFKS.IVGSELAKLFADHAANYAKIREGVEVRSIKKTQGGFDIETNDDTYHAKYVI
E_coli                50   ENWPGDPNDLTGPLLMERMHEHATKFETEIIFDHINKVDLQNRPFRLTGDSGEYTCDALI
V_parahaemolyticus    51   ENWPGDAEGLTGPALMERMKEHAERFETEIVFDHINEVELSQRPFRLKGDSGEYTCDALI
V_cholerae            51   ENWPGDAEGLTGPALMERMKEHAERFDTEIVFDHINSVDLSSRPFRLTGDSLEYTCDALI
S_boydii              51   ENWPGDPNDLTGPLLMERMHEHATKFETEIIFDHINKVDLQNRPFRLNGDNGEYTCDALI
S_enterica            51   ENWPGDPNDLTGPLLMERMHEHAAKFETEIIFDHINNVDLQNRPFRLTGDSAEYTCDALI
Y_pestis              51   ENWPGDPEGLTGPALMERMHEHAEKFQTEIIFDHISSVDLQNRPFRLFGDGAEYTCDALI
S_pneumoniae          46   ENYPGYAN.ISGPELAEKMFEPLENLGVEHIYGYVENVEDHGDFKKVMTDDQTYETRTVI

T_acidophilum        120   ITTGTTHKHLGVKGESEYFGKGTSYCSTCDGYLFKGKRVVTIGGGNSGAIAAISMSEYVK
E_coli               110   IATGASARYLGLPSEEAFKGRGVSACATCDGFFYRNQKVAVIGGGNTAVEEALYLSNIAS
V_parahaemolyticus   111   ISTGASAKYLGLESEETFKGRGVSACATCDGFFYRNQKVAVVGGGNTAVEEALYLSNIAS
V_cholerae           111   ISTGASAKYLGLESEEAFKGRGVSACATCDGFFYRNQKVAVVGGGNTAVEEALYLSNIAS
S_boydii             111   IATGASARYLGLPSEEAFKGRGVSACATCDGFFYRNQKVAVIGGGNTAVEEALYLSNIAS
S_enterica           111   IATGASARYLGLPSEEAFKGRGVSACATCDGFFYRNQKVAVIGGGNTAVEEALYLSNIAS
Y_pestis             111   IATGASARYLGMASEEAFKGKGVSACATCDGFFYRNQKVAVVGGGNTAVEEALYLANIAA
S_pneumoniae         105   VATGSKHRPLGVPGEEELNSRGVSYCAVCDGAFFRDQDLLVVGGGDSAVEEALFLTRFAK

T_acidophilum        180   NVTIIEYMPKYMCENAYVQEIK....KRNIPYIMNAQVTEIVGDGKKVTGVKYKDR.TTG
E_coli               170   EVHLIHRRDGFRAEKILIKRLMDKVENGNIILHTNRTLEEVTGDQMGVTGVRLRDTQNSD
V_parahaemolyticus   171   EVHLIHRRDSFRAEKILINRLMDKVENGNIILHTDRTLDEVLGDDMGVTGVRIKDV.NTG
V_cholerae           171   EVHLIHRRDSFRSEKILIDRLMDKVANGNIVLHTHRTLDEVLGDEMGVTGVRLKDT.QSD
S_boydii             171   EVHLIHRRDGFRAEKILIKRLMDKVENGNIILHTNRMLEEVTGDQMGVTGVRLRDTQNSD
S_enterica           171   EVHLIHRRDGFRAEKILIKRLMDKVENGNIILHTNRTLEEVTGDQMGVTGLRLRDTQQSD
Y_pestis             171   EVHLIHRRDTFRSEKILIDRLMEKVKNGNIVLHTDRTLDEVLGDDMGVTGVRLKST.HSD
S_pneumoniae         165   TVTIVHRRDQLRAQKVLQDR...AFANEKISFIWDSVVKEIKGENR.VESVVFENV.KTG

T_acidophilum        235   EEKLIETDGVFIYVGLIPQTSFLKDSGVKLDERGYIVVDS.....RQRTSVPGVYAAGDV
E_coli               230   NIESLDVAGLFVAIGHSPNTAIFEG.QLELEN.GYIKVQSGIHGNATQTSIPGVFAAGDV
V_parahaemolyticus   230   TTEDLEVMGAFIAIGHQPNTQIFEG.QLEMKD.GYIVVKSGLEGNATQTSIEGVFAAGDV
V_cholerae           230   MTENLDVMGVFIAIGHQPNSQIFEG.QLEMKN.GYIVVKSGLEGNATQTSIEGVFAAGDV
S_boydii             231   NIESLDVAGLFVAIGHSPNTAIFEG.QLELEN.GYIKVQSGIHGNATQTSIPGVFAAGDV
S_enterica           231   NIETLDIAGLFVAIGHSPNTALFEG.QLELEN.GYIKVQSGTHGNATQTSIPGVFAAGDV
Y_pestis             230   ETEELAVAGVFIAIGHSPNTGIFSD.QLALEN.GYIKVQSGLQGNATQTSIPGVFAAGDV
S_pneumoniae         220   QVTEQAFGGVFIYVGLDPLSDFVKELNIQDQA.GWIVTDS.....HMKTAVDGIFAVGDV

T_acidophilum        290   TSGNFAQIASAVGDGCKAALSLYSDSISKK....
E_coli               288   MDHIYRQAITSAGTGCMAALDAERYLDGLADAK.
V_parahaemolyticus   288   MDHNYRQAITSAGTGCMAALDAERFLDALSDK..
V_cholerae           288   MDHNYRQAITSAGTGCMAALDAERYLDSQGK...
S_boydii             289   MDHIYRQAITSAGTGCMAALDAERYLDGLADAK.
S_enterica           289   MDHIYRQAITSAGTGCMAALDAERYLDGLADASK
Y_pestis             288   MDHIYRQAITSAGTGCMAALDAERYLDGLANDK.
S_pneumoniae         274   RLKDLRQVTTAVGDGAIAGQEAYKFITEHS....
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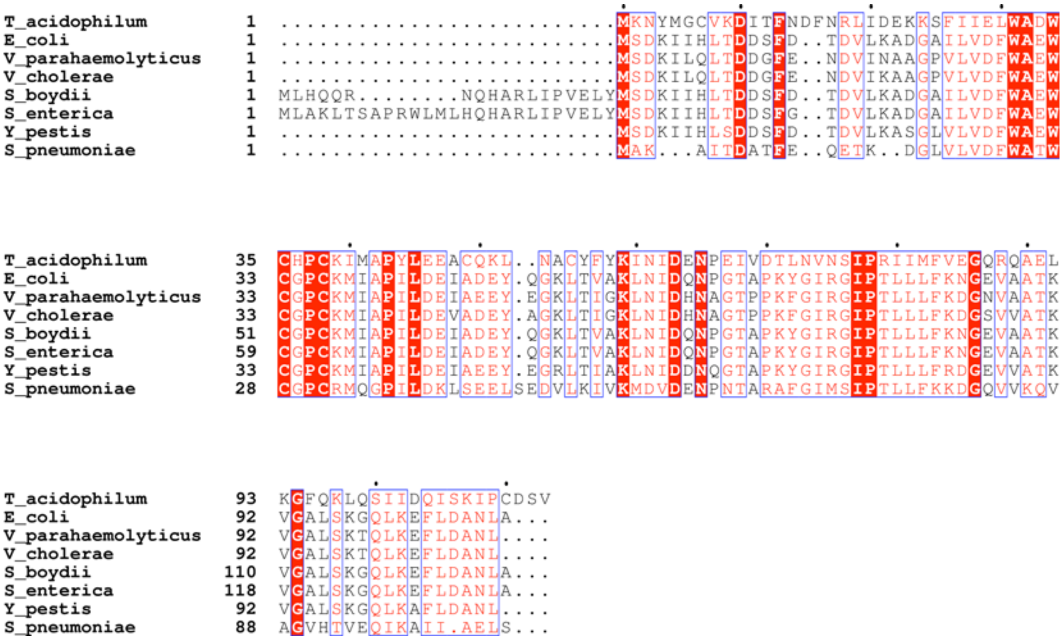
Figure S2. Alignment of selected Trx sequences from PSI-Blast (1). Sequences were aligned using the program ClustalW (2) and the output was rendered using the web interface ESPript 2.2 (3). Sequences are *E. coli* (gi:67005950), *V. parahaemolyticus* (gi:28899775), *V. cholerae* 0395 (gi:147675356), *S. boydii* BS512 (gi:82546135), *S. enterica* subsp. *enterica serovar Typhi* str. CT18 (gi:62182391), *Y. pestis* CO92 (gi:16124003), *S. pneumoniae* R6 (gi:15903644). White letters in red blocks are strictly conserved residues. Red letters in black boxes are more than 50% conserved residues.



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T_acidophilum          1  ..........................MKNYMGCVKDITFNDFNRLIDEKKSFIIELWADW
E_coli                 1  ..........................MSDKIIHLTDDSFD..TDVLKADGAILVDFWAEW
V_parahaemolyticus     1  ..........................MSDKILQLTDDGFE..NDVINAAGPVLVDFWAEW
V_cholerae             1  ..........................MSDKILQLTDDGFE..NDVIKAAGPVLVDFWAEW
S_boydii               1  MLHQQR........NQHARLIPVELYMSDKIIHLTDDSFD..TDVLKADGAILVDFWAEW
S_enterica             1  MLAKLTSAPRWLMLHQHARLIPVELYMSDKIIHLTDDSFG..TDVLKADGAILVDFWAEW
Y_pestis               1  ..........................MSDKIIHLSDDSFD..TDVLKASGLVLVDFWAEW
S_pneumoniae           1  ..........................MAK...AITDATFE..QETK..DGLVLVDFWATW

T_acidophilum         35  CHPCKIMAPYLEEACQKL..NACYFYKINIDENPEIVDTLNVNSIPRIIMFVEGQRQAEL
E_coli                33  CGPCKMIAPILDEIADEY.QGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATK
V_parahaemolyticus    33  CGPCKMIAPILDEIAEEY.EGKLTIGKLNIDHNAGTPPKFGIRGIPTLLLFKDGNVAATK
V_cholerae            33  CGPCKMIAPILDEVADEY.AGKLTIGKLNIDHNAGTPPKFGIRGIPTLLLFKDGSVVATK
S_boydii              51  CGPCKMIAPILDEIADEY.QGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATK
S_enterica            59  CGPCKMIAPILDEIADEY.QGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATK
Y_pestis              33  CGPCKMIAPILDEIAEEY.EGRLTIAKLNIDDNQGTAPKYGIRGIPTLLLFRDGEVVATK
S_pneumoniae          28  CGPCRMQGPILDKLSEELSEDVLKIVKMDVDENPNTARAFGIMSIPTLLFKKDGQVVKQV

T_acidophilum         93  KGFQKLQSIIDQISKIPCDSV
E_coli                92  VGALSKGQLKEFLDANLA...
V_parahaemolyticus    92  VGALSKTQLKEFLDANL....
V_cholerae            92  VGALSKTQLKEFLDANL....
S_boydii             110  VGALSKGQLKEFLDANLA...
S_enterica           118  VGALSKGQLKEFLDANLA...
Y_pestis              92  VGALSKGQLKAFLDANL....
S_pneumoniae          88  AGVHTVEQIKAII.AELS...
```

Figure S3. DNA and corresponding amino acid sequence of ORF Ta0984 from *T. acidophilum* encoding for a putative TrxR. DNA sequence was confirmed by the MIT Biopolymers Laboratory.

```
atggaatttaacctgcatgcagtatcatcagaggaaaaagaaagagattttgatgttgtc  60
 M  E  F  N  L  H  A  V  S  S  E  E  K  E  R  D  F  D  V  V
atcgtcggcgccggtgctgccggttttcagcggcagtctatgctgcaaggtcaggattc 120
 I  V  G  A  G  A  A  G  F  S  A  A  V  Y  A  A  R  S  G  F
agcgttgcaattctcgacaaggccgtagctggaggccttactgcagaggcaccactggta 180
 S  V  A  I  L  D  K  A  V  A  G  G  L  T  A  E  A  P  L  V
gagaattatcttggtttcaagagcattgtcggatcagagcttgcgaaacttttcgccgac 240
 E  N  Y  L  G  F  K  S  I  V  G  S  E  L  A  K  L  F  A  D
catgcagcgaactatgccaagataagggagggcgtagaggtaagatccataaagaaaacg 300
 H  A  A  N  Y  A  K  I  R  E  G  V  E  V  R  S  I  K  K  T
caaggcggctttgacattgagacaaatgacgatacgtaccatgccaaatatgtgataata 360
 Q  G  G  F  D  I  E  T  N  D  D  T  Y  H  A  K  Y  V  I  I
acgactggaacaacgcataagcatttaggtgtcaaaggcgaatccgaatactttgggaag 420
 T  T  G  T  T  H  K  H  L  G  V  K  G  E  S  E  Y  F  G  K
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 G  T  S  Y  C  S  T  C  D  G  Y  L  F  K  G  K  R  V  V  T
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 I  G  G  G  N  S  G  A  I  A  A  I  S  M  S  E  Y  V  K  N
gtcaccataattgaatacatgcctaagtacatgtgcgaaaacgcctacgtacaggagatc 600
 V  T  I  I  E  Y  M  P  K  Y  M  C  E  N  A  Y  V  Q  E  I
aagaaaagaaacataccctacataatgaacgctcaggtaaccgagatcgttggggatgga 660
 K  K  R  N  I  P  Y  I  M  N  A  Q  V  T  E  I  V  G  D  G
aagaaggtcaccggagttaaatacaaagacaggacaacgggtgaagaaaaactcatagaa 720
 K  K  V  T  G  V  K  Y  K  D  R  T  T  G  E  E  K  L  I  E
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 T  D  G  V  F  I  Y  V  G  L  I  P  Q  T  S  F  L  K  D  S
ggcgtaaaactggatgagagagggtacatagtggtggattcaaggcagagaactagcgtt 840
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ccaggggtatatgcagcaggagacgttacatccggcaacttcgcacagattgcctctgcc 900
 P  G  V  Y  A  A  G  D  V  T  S  G  N  F  A  Q  I  A  S  A
gttggagatggctgcaaggcagcgctttccttgtattctgattctatatccaaaaaa    957
 V  G  D  G  C  K  A  A  L  S  L  Y  S  D  S  I  S  K  K
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Figure S4. DNA and corresponding amino acid sequence of ORF Ta0866 from *T. acidophilum* encoding for a putative Trx. DNA sequence was confirmed by the MIT Biopolymers Laboratory.

```
atgaagaattatatgggctgcgttaaagatataacattcaatgatttcaatcgtctcata  60
 M  K  N  Y  M  G  C  V  K  D  I  T  F  N  D  F  N  R  L  I
gatgagaaaaaaagcttcataattgagttatgggctgattggtgccatccctgcaagatc 120
 D  E  K  K  S  F  I  I  E  L  W  A  D  W  C  H  P  C  K  I
atggcgccatacctggaggaggcgtgtcagaagttgaacgcctgctactttttacaagata 180
 M  A  P  Y  L  E  E  A  C  Q  K  L  N  A  C  Y  F  Y  K  I
aatatcgatgagaatcctgagatcgttgacacgcttaatgtgaacagcataccaaggata 240
 N  I  D  E  N  P  E  I  V  D  T  L  N  V  N  S  I  P  R  I
ataatgtttgttgaaggccagcgccaggccgagcttaagggatttcagaagcttcaatca 300
 I  M  F  V  E  G  Q  R  Q  A  E  L  K  G  F  Q  K  L  Q  S
attattgaccagatttcaaaaattccctgcgattccgtc                      339
 I  I  D  Q  I  S  K  I  P  C  D  S  V
```

Figure S5. SDS-PAGE [4-20% (w/v)] gel of the isolated **(A)** *ta*TrxR and (**B**) *ta*Trx from the overexpression *E. coli* Rosetta™ (DE3) pLysS strain.

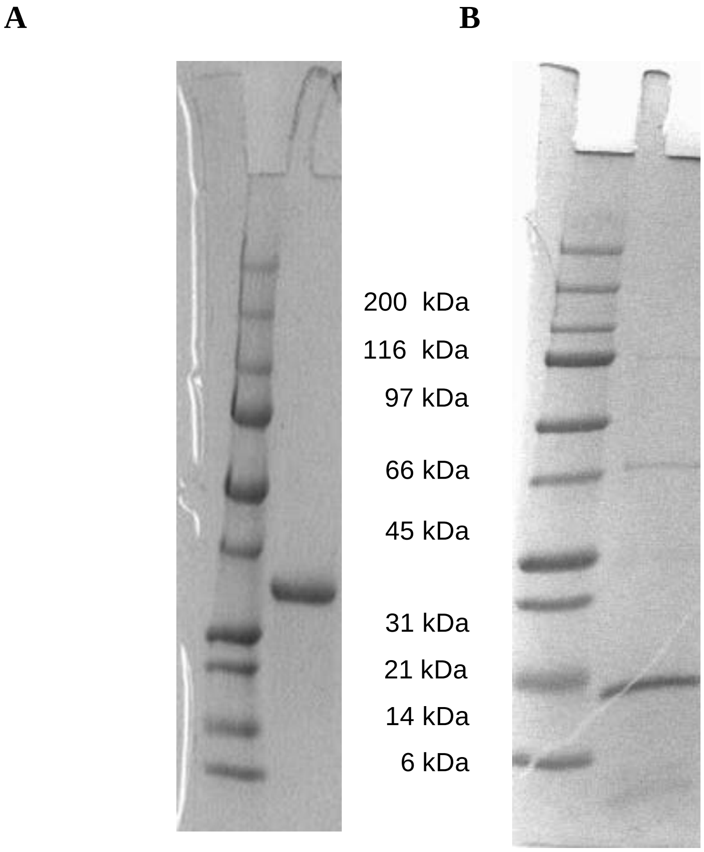


Figure S6. Spectral recordings of temperature dependent degradation of NADPH and NADH measured at 340 nm. **(A)** NADPH at 20º C (solid line), NADPH at 80º C (dashed line), and heat treated NADPH cooled down to 20º C (dotted line). (**B**) Spectra of temperature dependent degradation of NADH. NADH at 20º C (solid line), NADH at 80º C (dashed line), and heat treated NADH cooled down to 20º C (dotted line). **(C)** Time dependent degradation of NADPH at 20º C (square), 60º C (circle), and 80º C (triangle). **(D)** Time dependent degradation of NADH at 20º C (square), 60º C (circle), and 80º C (triangle).

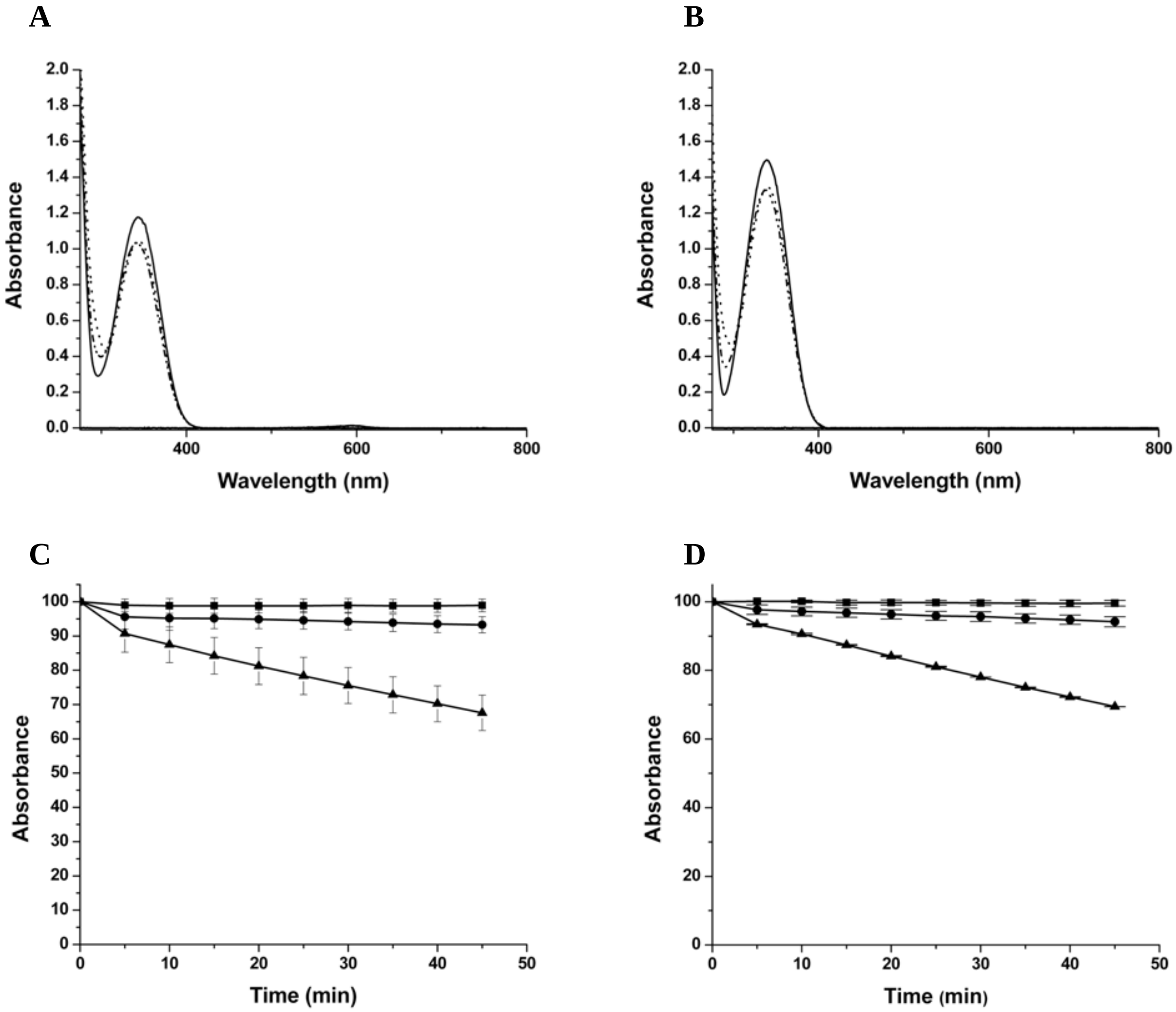


Figure S7. Reduction of insulin catalyzed by *ta*Trx. The DTT dependent reduction of bovine insulin was carried out as described in Methods. The increase in turbidity at 650 nm is plotted against the reaction time. Solid line is 70 μM *ta*Trx, dashed line is 66 μM *ta*Trx, and dotted line is 43 μM *ta*Trx.

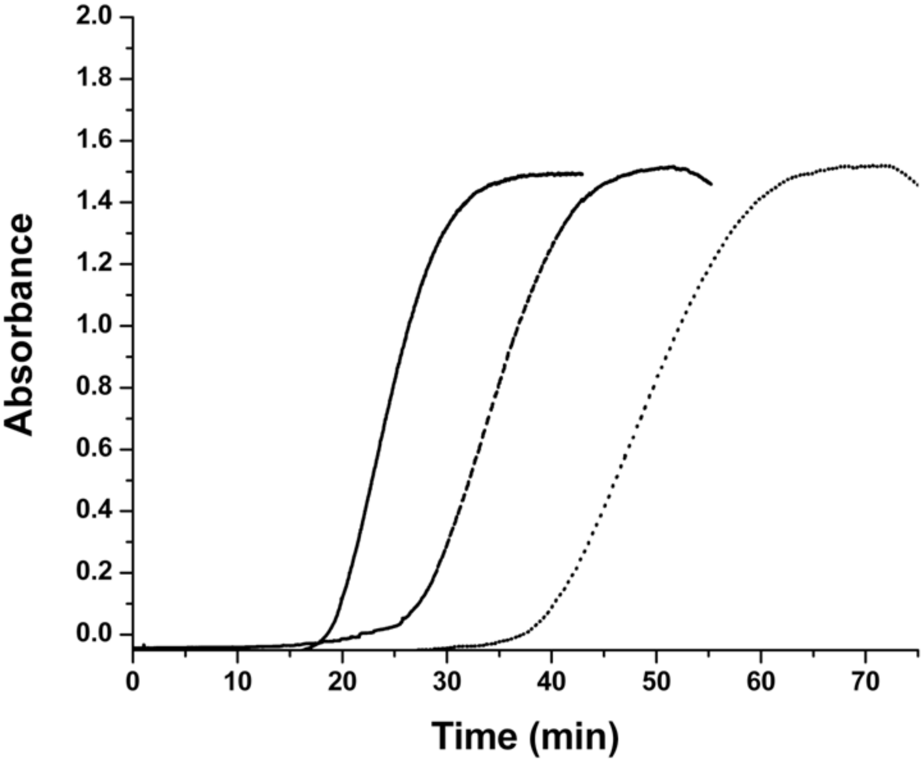


Figure S8. Trx activity in thioredoxin system using *ta*Trx and *ec*TrxR. The assays were performed using 3.8 μM *ec*TrxR and varying concentrations of *ta*Trx. Assays were performed as described in Methods.

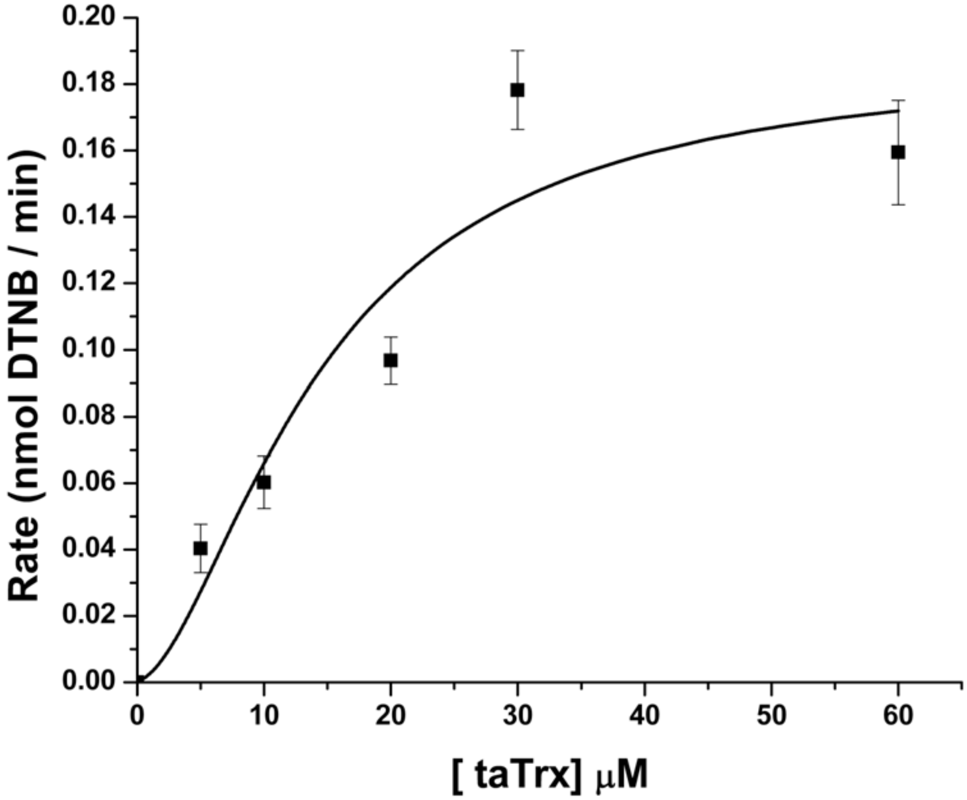


Figure S9. View of the FAD binding pocket of *ta*TrxR. The FAD molecule is shown in composite omit map density contoured at 1 σ. FAD atoms are colored in CPK format (C in light grey, N in blue, O in red, P in orange).

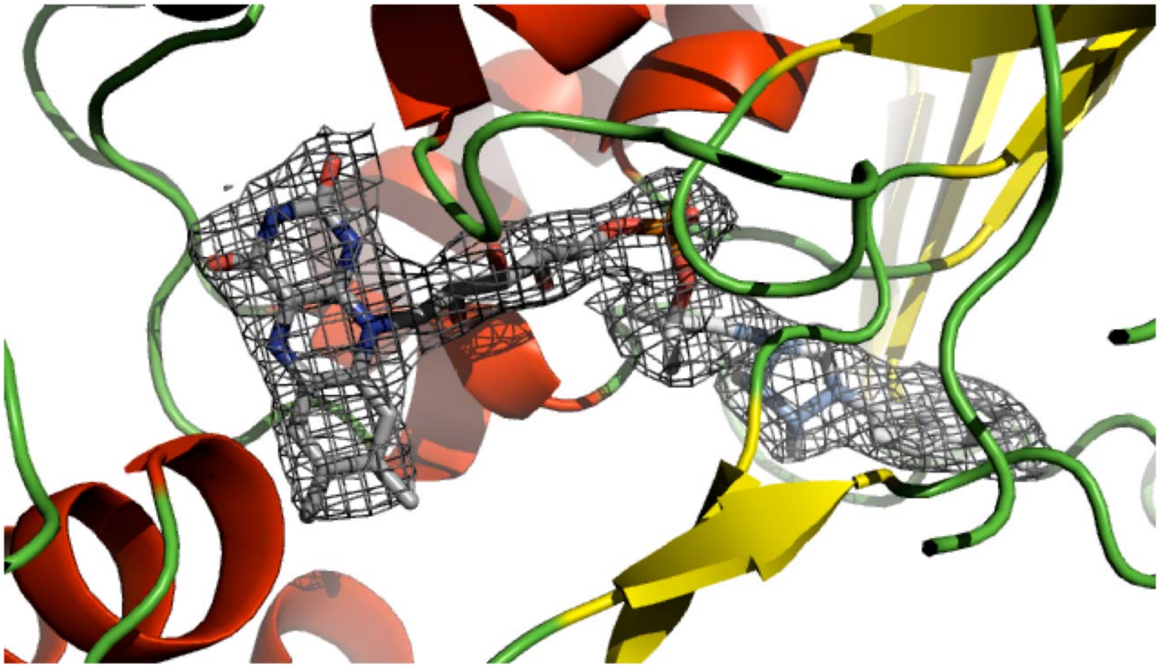


Figure S10. Schematic drawing of the FAD molecule in *ta*TrxR. Polar atoms of residues of molecule A that are within 4 Å of atoms of FAD are listed. Distances are in Å.

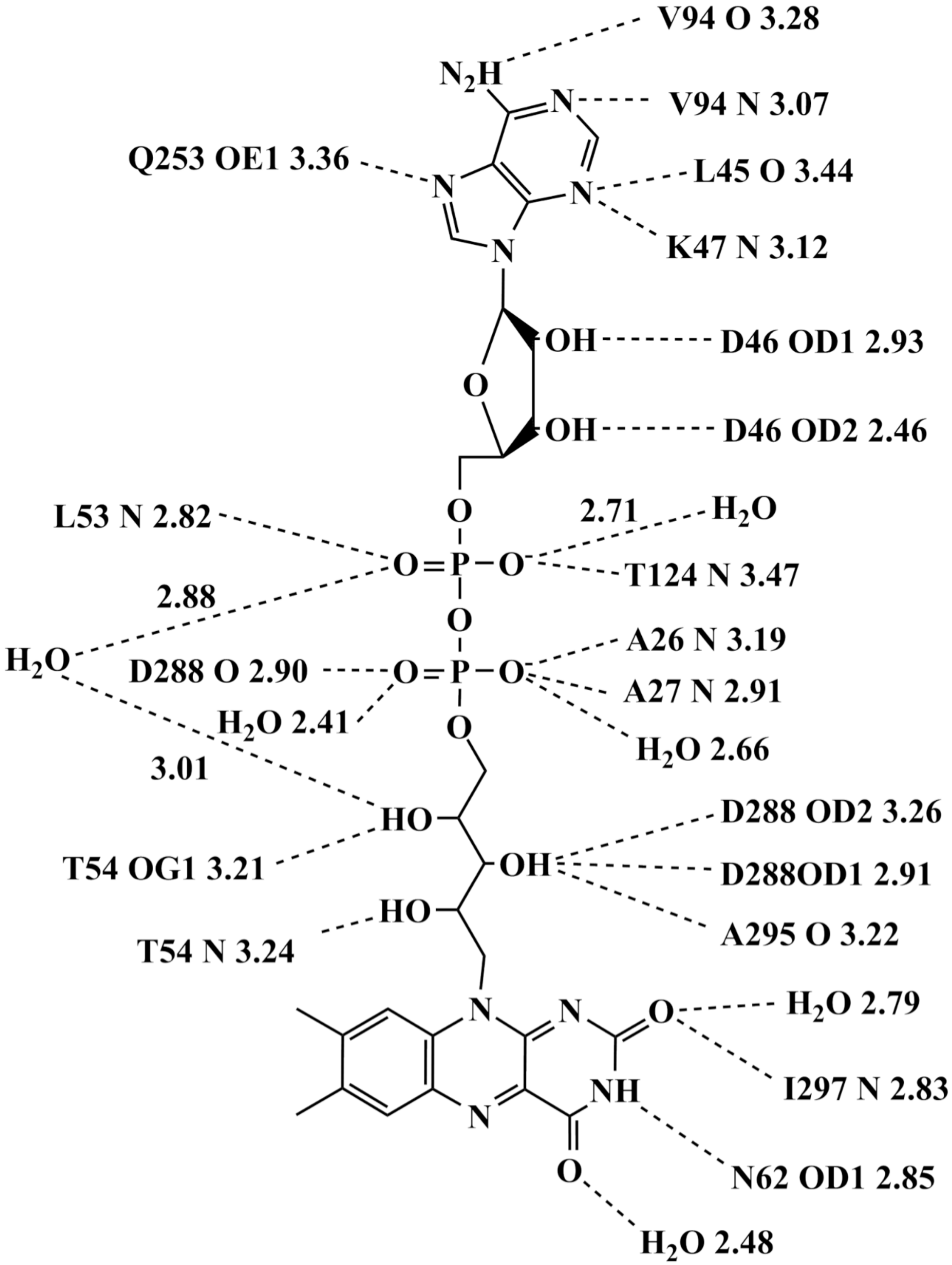


Figure S11. Space filling surface model of **(A)** *ec*TrxR and **(B)** *ta*TrxR NADPH binding domain. The positively charged residues are represented in blue and the negatively charged residues are represented in red. Neutral residues are represented as white. Surface calculations were made using the APBS module in Pymol (4).

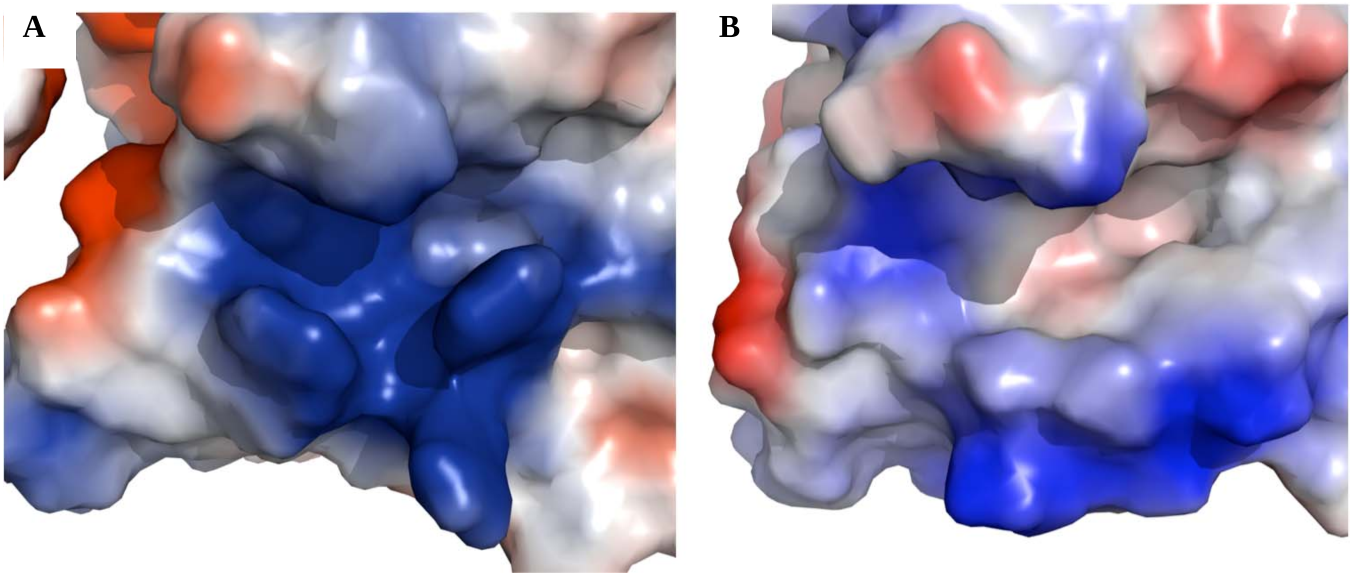
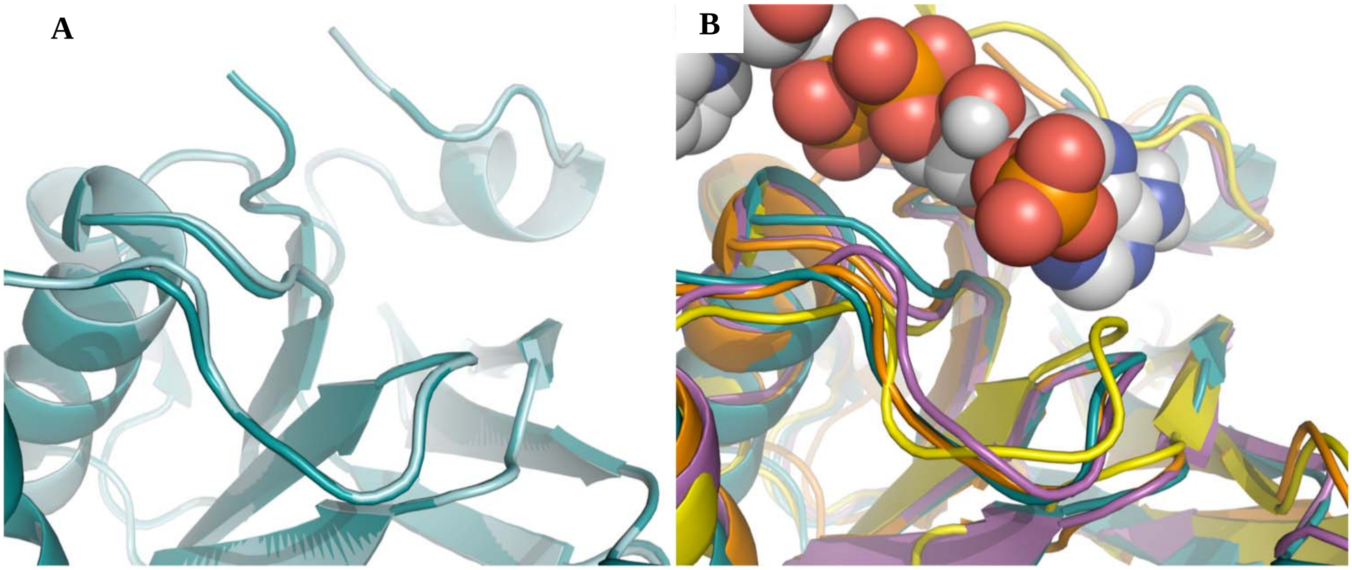


Figure S12. Structural superposition of TrxR NADPH binding domains. **(A)** The *ec*TrxR NADPH binding domains crystallized with NADPH analogue AADP (1TDF) is in teal and without NADPH (1TDE) is in cyan. **(B)** The *ec*TrxR (1TDF) co-crystallized with AADP, an NADPH analogue, is in teal, *A. thaliana* TrxR (1VDC) structure is in orange, *M. tuberculosis* TrxR (2A87) co-crystallized with NADP is in magenta, and *ta*TrxR is in yellow.



1. Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25, 3389-3402.
2. Thompson J.D., Higgins D.G., Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*22, 4673-4680.
3. Gouet, P., Courcelle, E., Stuart, D. I., Metoz, F. (1999) ESPript: analysis of multiple sequence alignments in PostScript. *Bioinformatics* 15, 305-308.
4. Baker, N. A., Sept, D., Joseph, S., Holst, M. J., McCammon, J. A. (2001) Electrostatics of nanosystems: application to microtubules and the ribosome. *Proc Natl Acad Sci U S A* 98, 10037-10041.