SUPPORTING INFORMATION

Structure, Kinetic, and Chemical Mechanism of Isocitrate Dehydrogenase-1 from *Mycobacterium tuberculosis*

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TABLE S1: "The Effect of Different Divalent Cations on the Rate of ICDH-1."

Metal [5 mM]	V/E sec^{-1} Replicate 1	V/E sec^{-1} Replicate 2	V/E sec^{-1} Average
None	0.08	0.09	0.085
$MnCl_2$	16.18	15.86	16.02
$MgCl_2$	14.94	13.76	14.35
$CaCl_2$	0.18	0	0.09
$ZnCl_2$	0.18	0.25	0.215

Assays were performed with 100 mM HEPES pH 7.0, 600 μM isocitrate, 300 μM $NADP^+$, and 5 mM of the metal tested. Note that the maximum rate is decreased from other experiments since subsequent experiments were performed with ICDH-1 in which 5 mM $MnCl_2$ had been added to the gel filtration and storage buffers.

SCHEME S1: “Synthesis of [2*R*-^{2}H]isocitrate.”

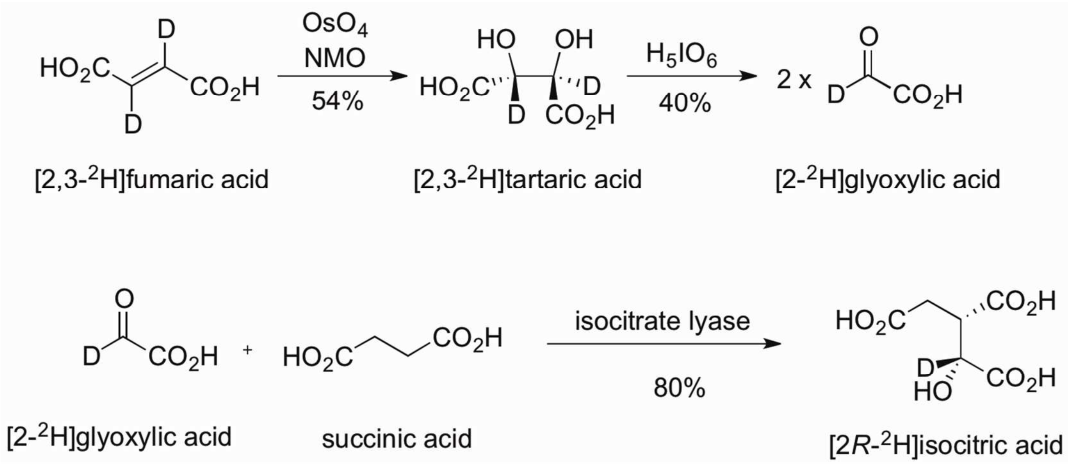


FIGURE S1: "Transfer of ^{2}H from [2*R*-^{2}H]isocitrate to [4*R*-4-^{2}H]NADPH." (A) ^{1}H NMR of [4*R*-4-^{2}H]NADPH. (B) ^{1}H NMR of [4*R*-4-^{1}H]NADPH.

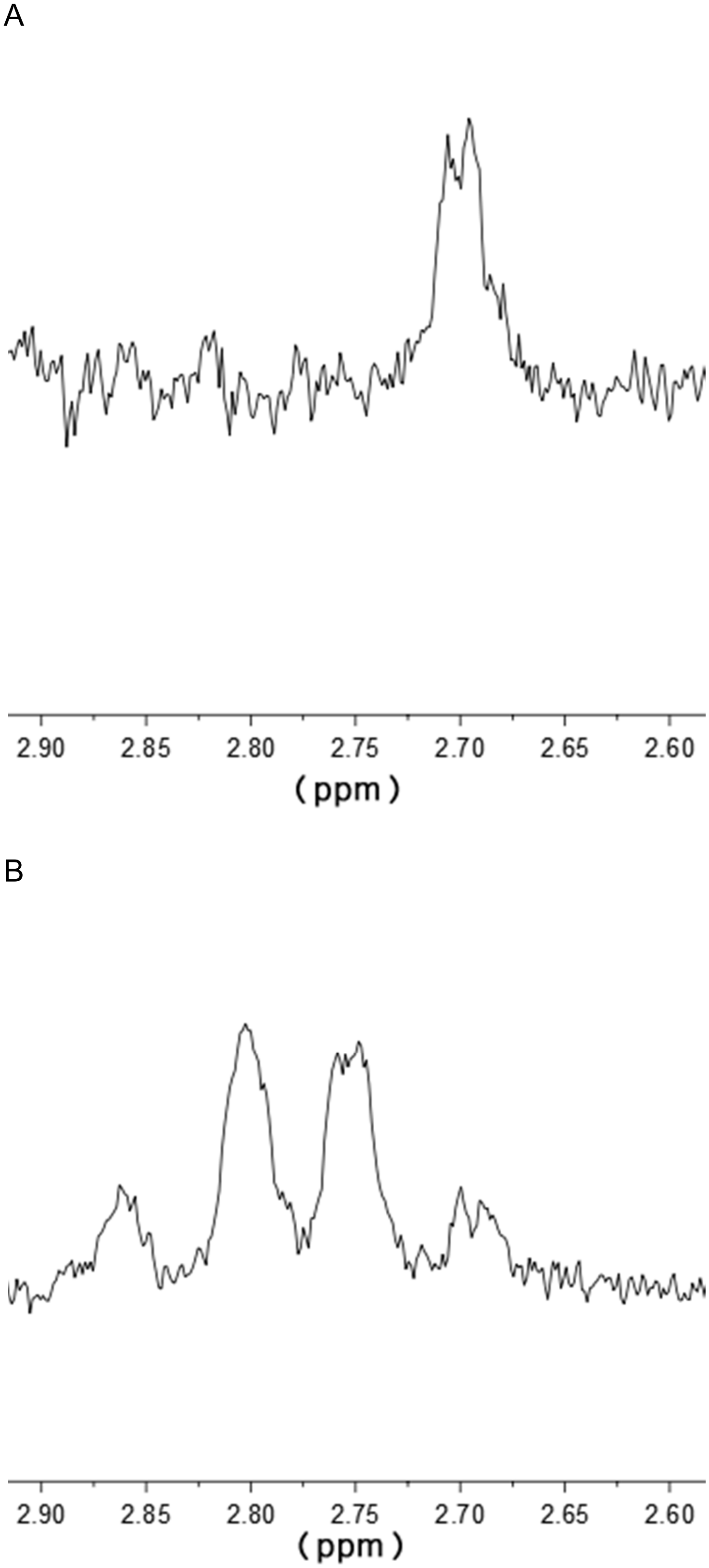
FIGURE S2: "Confirmation of αHG by Mass Spectrometry." Mass spectrum of secondary reaction product, αHG, $m/z = 147.0301$ in negative mode. The peak to the left $m/z = 147.0187$ is αKG in negative mode A + 2. Data were collected on a 12 Tesla Fourier transform ion cyclotron resonance mass spectrometer (Agilent).

FIGURE S3: "Preliminary Kinetics of Secondary Reaction." (A) The rate of the secondary ICDH-1 reaction was measured with saturating NADPH (100 μM) and varying αKG (1-50 mM). Fitting to eq 1 $k_{\mathrm{cat}} = 0.12 \pm 0.02$ sec^{-1}, $K_{\alpha\mathrm{KG}} = 8.3 \pm 1.2$ mM. (B) The rate of the secondary ICDH-1 reaction was measured with constant αKG (40 mM) and varying NADPH (1-50 μM). Fitting to eq 1 $k_{\mathrm{cat}} = 0.10 \pm 0.02$ sec^{-1}, $K_{\mathrm{NADPH}} = 0.9 \pm 0.8$ μM. (C) A replicate of panel (B). Fitting to eq 1 $k_{\mathrm{cat}} = 0.17 \pm 0.02$ sec^{-1}, $K_{\mathrm{NADPH}} = 2.3 \pm 1.0$ μM.

FIGURE S4: "Sequence Alignment of Relevant ICDHs." ClustalO sequence alignment using EMBL-EBI server of the top three BLAST hits in the PDB against Mtb ICDH-1, plus *E. coli*. UniProt identifiers are on the right. Orange highlights are Lys 215 and Tyr 142 in the proposed chemical mechanism. Yellow highlighted residues are NADPH contacts, purple are Mn^{2+} contacts, blue are residues referenced in the discussion of regulation, and green is the analogous Hc R132 mutation location.

FIGURE S5: “Contacts between NADPH and Mtb ICDH-1.” A LigPlot(1) generated figure where red spheres represent oxygen, blue spheres represent nitrogen, black spheres represent carbon. The gold lines are the backbone of Mtb ICDH-1, and the purple lines are the backbone of NADPH. The red spokes are hydrophobic interactions, and the green numbers are distances in angstroms.

Figure S1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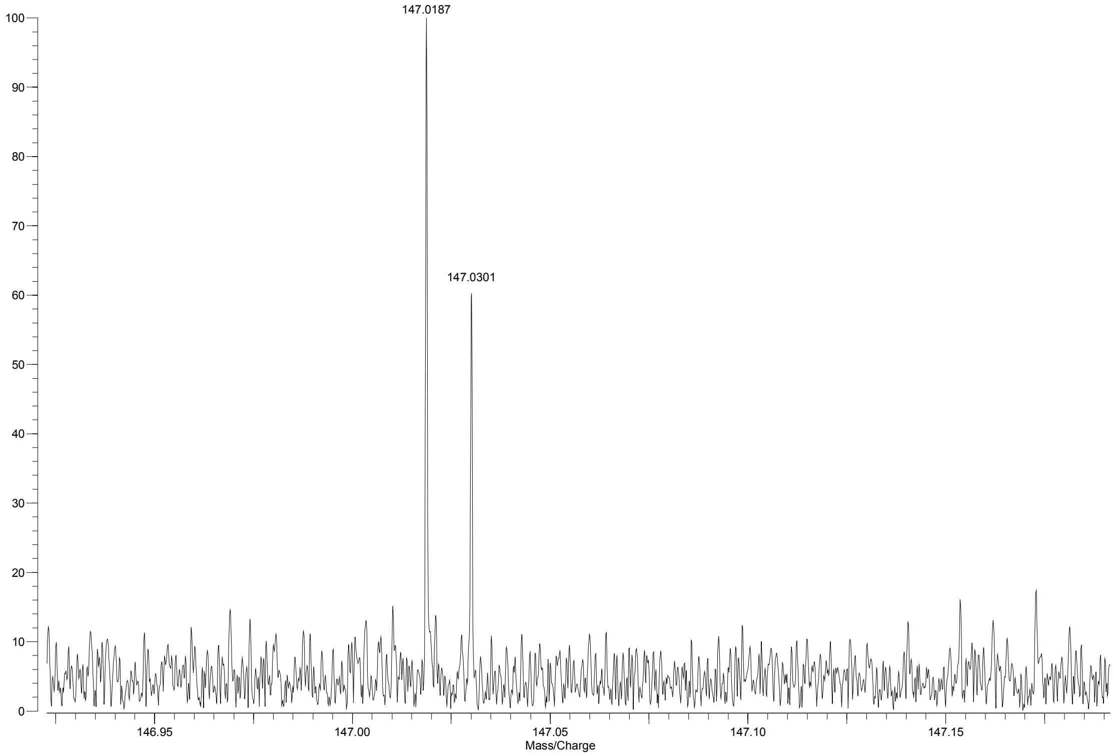
2.90
2.85
2.80
2.75
2.70
2.65
2.60
(ppm)
B
2.90
2.85
2.80
2.75
2.70
2.65
2.60
(ppm)

Figure S2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147.0301
100
90
80
70
60
50
40
30
20
10
0
146.95
147.00
147.05
147.10
147.15
Mass/Charge

Figure S3

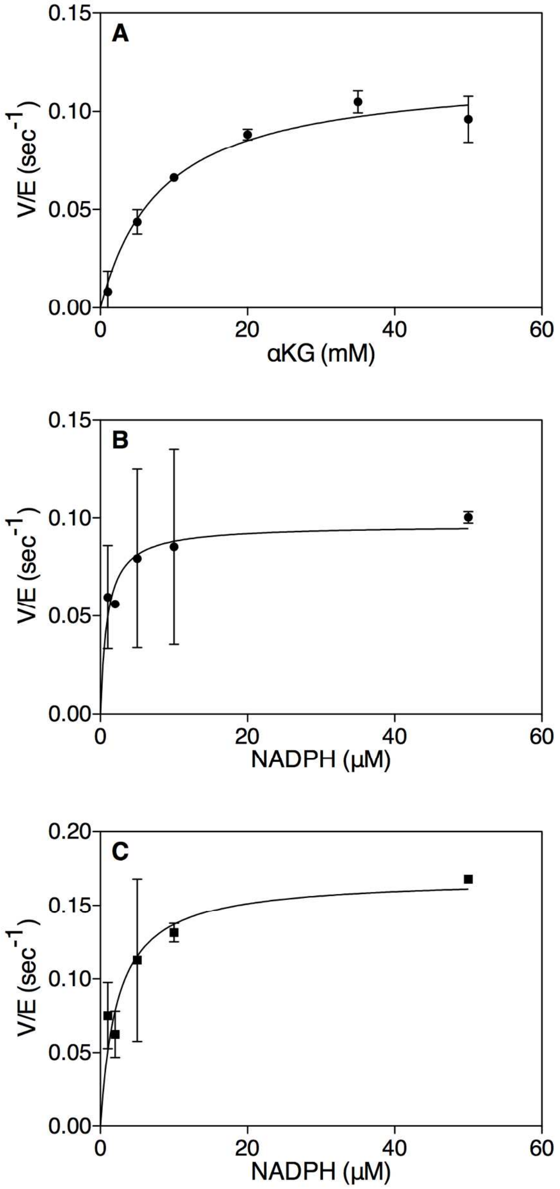
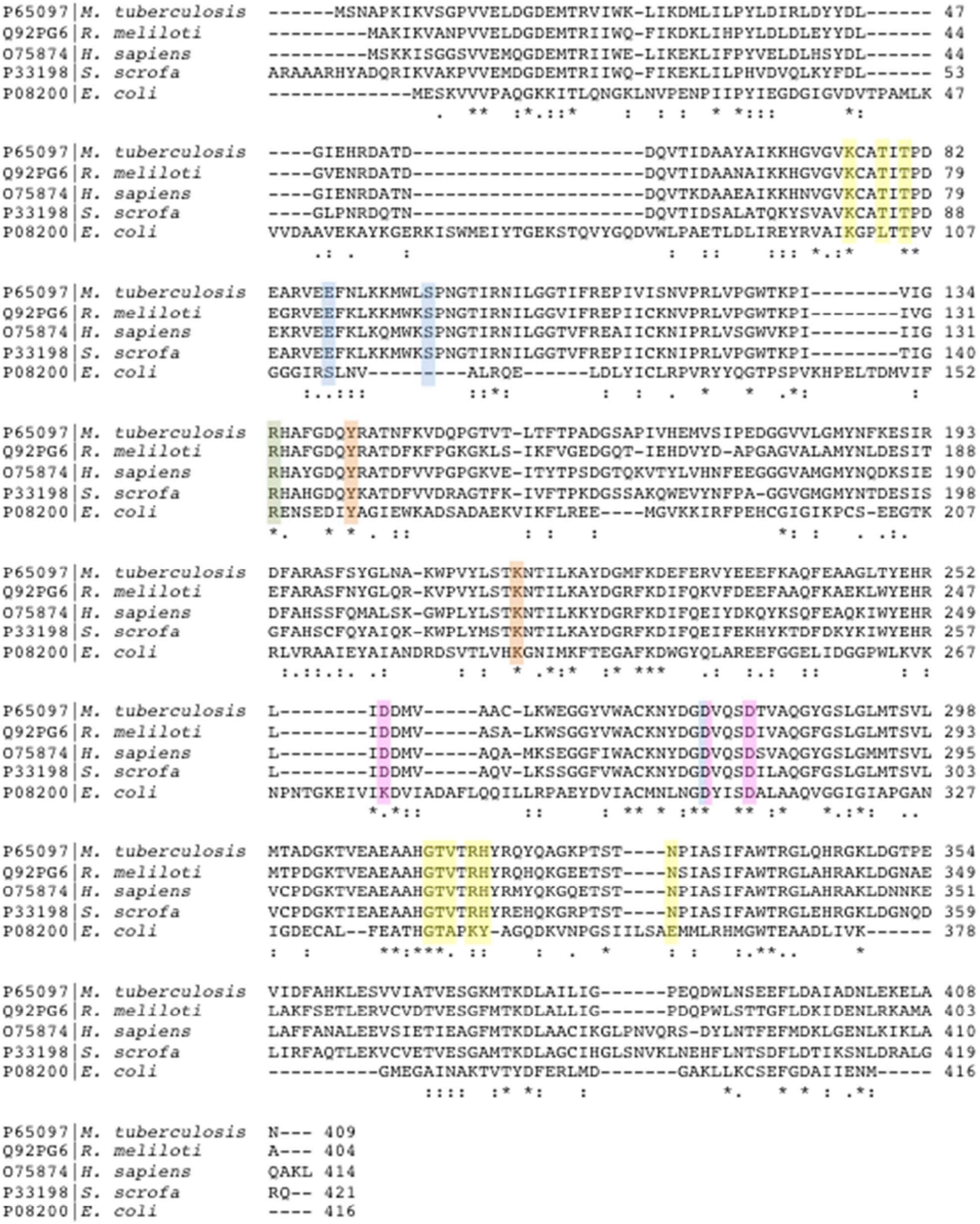

A
V/E (sec^{-1})
0.15
0.10
0.05
0.00
0
20
40
60
αKG (mM)
B
V/E (sec^{-1})
0.15
0.10
0.05
0.00
0
20
40
60
NADPH (μM)
C
V/E (sec^{-1})
0.20
0.15
0.10
0.05
0.00
0
20
40
60
NADPH (μM)

Figure S4



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Q92PG6|R. meliloti         ---------MAKIKVANPVVELDGDEMTRIIWQ-FIKDKLIHPYLDLDLEYYDL------ 44
O75874|H. sapiens          ---------MSKKISGGSVVEMQGDEMTRIIWE-LIKEKLIFPYVELDLHSYDL------ 44
P33198|S. scrofa           ARAAARHYADQRIKVAKPVVEMDGDEMTRIIWQ-FIKEKLILPHVDVQLKYFDL------ 53
P08200|E. coli             -------------MESKVVVPAQGKKITLQNGKLNVPENPIIPYIEGDGIGVDVTPAMLK 47
                                          .  **  :*.::*    :  :  :  *  *:::      *:

P65097|M. tuberculosis     ----GIEHRDATD---------------------DQVTIDAAYAIKKHGVGVKCATITPD 82
Q92PG6|R. meliloti         ----GVENRDATD---------------------DQVTIDAANAIKKHGVGVKCATITPD 79
O75874|H. sapiens          ----GIENRDATN---------------------DQVTKDAAEAIKKHNVGVKCATITPD 79
P33198|S. scrofa           ----GLPNRDQTN---------------------DQVTIDSALATQKYSVAVKCATITPD 88
P08200|E. coli             VVDAAVEKAYKGERKISWMEIYTGEKSTQVYGQDVWLPAETLDLIREYRVAIKGPLTTPV 107
                               .: .    :                       :  ::    ::: *.:*    **

P65097|M. tuberculosis     EARVEEFNLKKMWLSPNGTIRNILGGTIFREPIVISNVPRLVPGWTKPI--------VIG 134
Q92PG6|R. meliloti         EGRVEEFKLKKMWKSPNGTIRNILGGVIFREPIICKNVPRLVPGWTKPI--------IVG 131
O75874|H. sapiens          EKRVEEFKLKQMWKSPNGTIRNILGGTVFREAIICKNIPRLVSGWVKPI--------IIG 131
P33198|S. scrofa           EARVEEFKLKKMWKSPNGTIRNILGGTVFREPIICKNIPRLVPGWTKPI--------TIG 140
P08200|E. coli             GGGIRSLNV---------ALRQE------LDLYICLRPVRYYQGTPSPVKHPELTDMVIF 152
                              :..:::         ::*:        :  :  .  *   *  .*:         :

P65097|M. tuberculosis     RHAFGDQYRATNFKVDQPGTVT-LTFTPADGSAPIVHEMVSIPEDGGVVLGMYNFKESIR 193
Q92PG6|R. meliloti         RHAFGDQYRATDFKFPGKGKLS-IKFVGEDGQT-IEHDVYD-APGAGVALAMYNLDESIT 188
O75874|H. sapiens          RHAYGDQYRATDFVVPGPGKVE-ITYTPSDGTQKVTYLVHNFEEGGGVAMGMYNQDKSIE 190
P33198|S. scrofa           RHAHGDQYKATDFVVDRAGTFK-IVFTPKDGSSAKQWEVYNFPA-GGVGMGMYNTDESIS 198
P08200|E. coli             RENSEDIYAGIEWKADSADAEKVIKFLREE----MGVKKIRFPEHCGIGIKPCS-EEGTK 207
                           *.   * * . ::          : :   :                *: :   . .:.

P65097|M. tuberculosis     DFARASFSYGLNA-KWPVYLSTKNTILKAYDGMFKDEFERVYEEEFKAQFEAAGLTYEHR 252
Q92PG6|R. meliloti         EFARASFNYGLQR-KVPVYLSTKNTILKAYDGRFKDIFQKVFDEEFAAQFKAEKLWYEHR 247
O75874|H. sapiens          DFAHSSFQMALSK-GWPLYLSTKNTILKKYDGRFKDIFQEIYDKQYKSQFEAQKIWYEHR 249
P33198|S. scrofa           GFAHSCFQYAIQK-KWPLYMSTKNTILKAYDGRFKDIFQEIFEKHYKTDFDKYKIWYEHR 257
P08200|E. coli             RLVRAAIEYAIANDRDSVTLVHKGNIMKFTEGAFKDWGYQLAREEFGGELIDGGPWLKVK 267
                            :.::.:. .:      : :  * .*:*  :* ***   .:  :.:  ::       : :

P65097|M. tuberculosis     L--------IDDMV-----AAC-LKWEGGYVWACKNYDGDVQSDTVAQGYGSLGLMTSVL 298
Q92PG6|R. meliloti         L--------IDDMV-----ASA-LKWSGGYVWACKNYDGDVQSDIVAQGFGSLGLMTSVL 293
O75874|H. sapiens          L--------IDDMV-----AQA-MKSEGGFIWACKNYDGDVQSDSVAQGYGSLGMMTSVL 295
P33198|S. scrofa           L--------IDDMV-----AQV-LKSSGGFVWACKNYDGDVQSDILAQGFGSLGLMTSVL 303
P08200|E. coli             NPNTGKEIVIKDVIADAFLQQILLRPAEYDVIACMNLNGDYISDALAAQVGGIGIAPGAN 327
                                    *.*::         ::     : ** * :**  ** :*   *.:*:  ..

P65097|M. tuberculosis     MTADGKTVEAEAAHGTVTRHYRQYQAGKPTST----NPIASIFAWTRGLQHRGKLDGTPE 354
Q92PG6|R. meliloti         MTPDGKTVEAEAAHGTVTRHYRQHQKGEETST----NSIASIFAWTRGLAHRAKLDGNAE 349
O75874|H. sapiens          VCPDGKTVEAEAAHGTVTRHYRMYQKGQETST----NPIASIFAWTRGLAHRAKLDNNKE 351
P33198|S. scrofa           VCPDGKTIEAEAAHGTVTRHYREHQKGRPTST----NPIASIFAWTRGLEHRGKLDGNQD 359
P08200|E. coli             IGDECAL--FEATHGTAPKY-AGQDKVNPGSIILSAEMMLRHMGWTEAADLIVK------ 378
                           :  :      **:***. ::    :  .  *     : :   :.**..     *

P65097|M. tuberculosis     VIDFAHKLESVVIATVESGKMTKDLAILIG------PEQDWLNSEEFLDAIADNLEKELA 408
Q92PG6|R. meliloti         LAKFSETLERVCVDTVESGFMTKDLALLIG------PDQPWLSTTGFLDKIDENLRKAMA 403
O75874|H. sapiens          LAFFANALEEVSIETIEAGFMTKDLAACIKGLPNVQRS-DYLNTFEFMDKLGENLKIKLA 410
P33198|S. scrofa           LIRFAQTLEKVCVETVESGAMTKDLAGCIHGLSNVKLNEHFLNTSDFLDTIKSNLDRALG 419
P08200|E. coli             ----------GMEGAINAKTVTYDFERLMD-------GAKLLKCSEFGDAIIENM----- 416
                                         ::::  :* *:   :            *.   * * : .*:

P65097|M. tuberculosis     N--- 409
Q92PG6|R. meliloti         A--- 404
O75874|H. sapiens          QAKL 414
P33198|S. scrofa           RQ-- 421
P08200|E. coli             ---- 416
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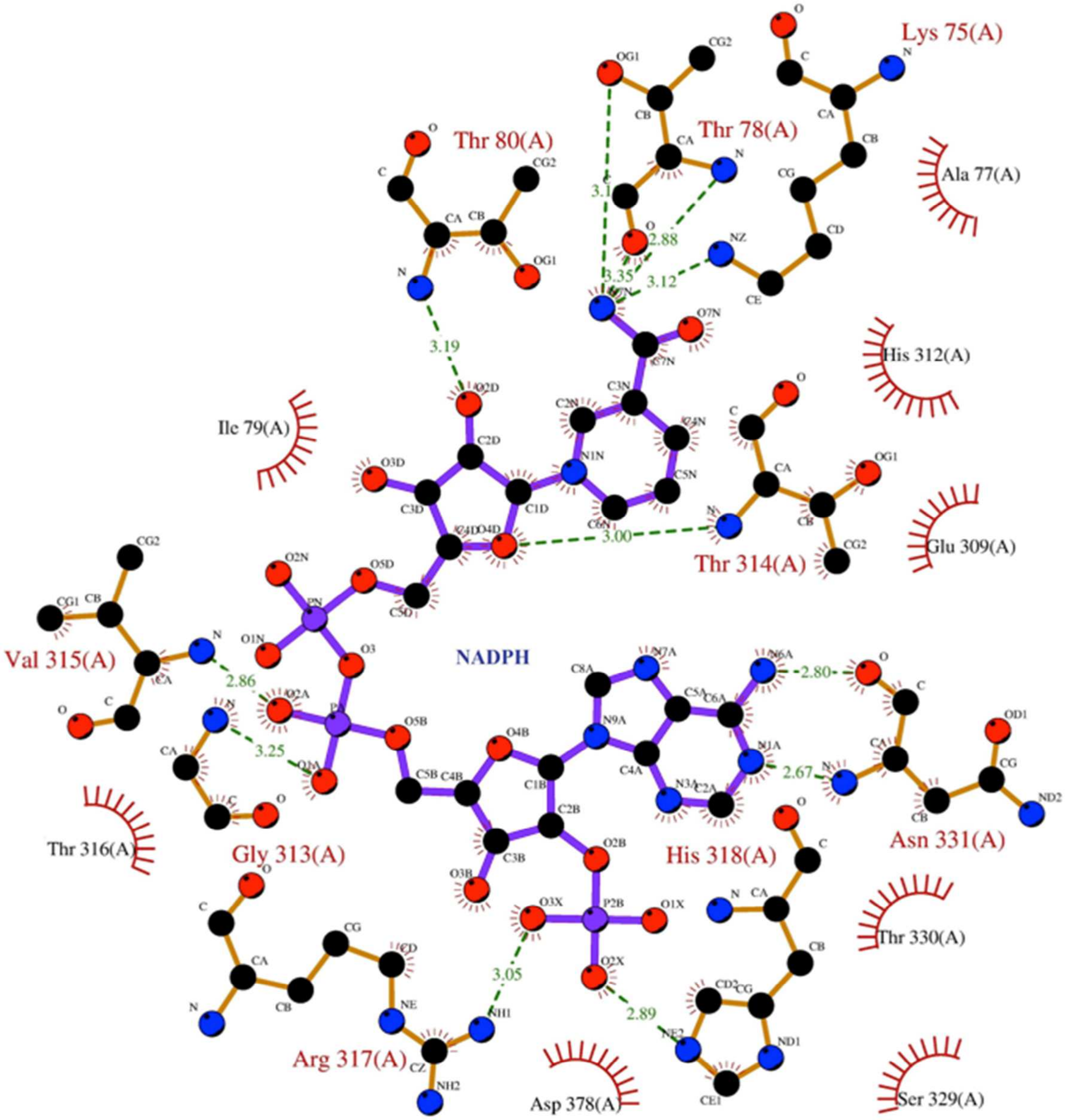
Figure S5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Thr 314(A)
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Gly 313(A)
Thr 316(A)
His 318(A)
Asn 331(A)
Thr 330(A)
Arg 317(A)
Asp 378(A)
Ser 329(A)
3.1
2.88
3.35
3.12
3.19
3.00
2.86
3.25
2.80
2.67
3.05
2.89

1. Wallace, A. C., Laskowski, R. A., and Thornton, J. M. (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, *Protein engineering 8*, 127-134.