SUPPLEMENTARY DATA

Figure S1. **Omit map for the bound ATP.** The final *Hl*AIPT/ATP/PO_4 complex model was subjected to 10 different cycles of simulated annealing with the ATP omitted. The resulting 10 models with R values of 21.6 – 21.9% and R_{free} of 26.6 – 27.5% were then combined to calculate a composite omit map, which had an overall FOM of 0.84. The model of ATP is shown as sticks superimposed on the omit map contoured as purple mesh at 3-σ level. The protein model is shown as a cartoon diagram in cyan and so are some side chains as rods.

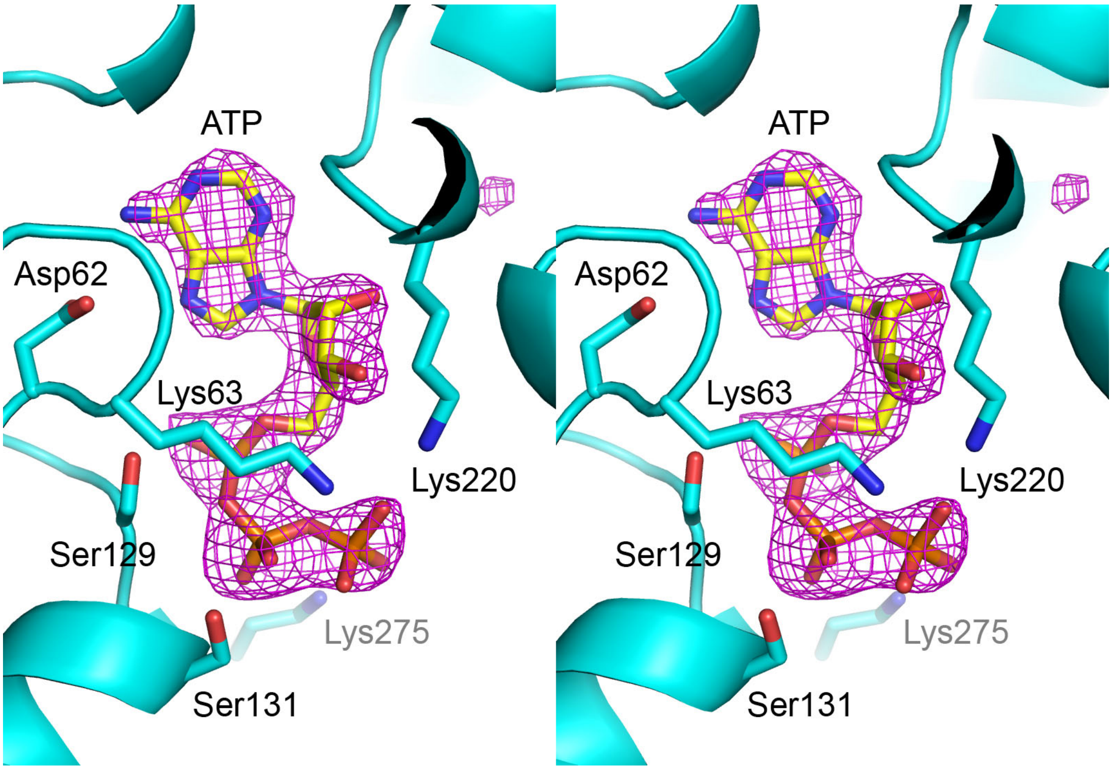
Figure S2. **Surface charge distribution in the nucleotide binding site.** The protein surfaces of three complex structures *Hl*AIPT/ATP, *Agrobacterium* AIPT/AMP and tRNA-IPT/tRNA (core domain, aa13-122 and aa197-340) reveal that the distribution of positively charged residues of *Hl*AIPT is more similar to that of tRNA-IPT than *Agrobacterium* AIPT.

Figure S3. **Multiple sequence alignment of AIPTs**. The sequence of *Hl*AIPT is compared with other plant AIPT (isozymes from *Arabidopsis*) and *Agrobacterium* AIPT (Tzs). The identical and similar amino acid residues are shaded in colors according to their types. The conserved phosphate binding motif, [G or A]-X_4-G-K-[S or T], is highlighted in a black box. The red circles indicate the interacting amino acids with the bound phosphate ion (in the DMAPP-binding site), and the blue asterisks indicate the interacting residues with the ATP molecule.

Figure S4. **Typical ITC trace for the interaction between AIPT and nucleotides.** Titration data obtained from AIPT and nucleotides as described in Materials and Methods (upper panel). The binding isotherms for the interaction between *Hl*AIPT and ATP, ADP, dATP, GTP, CTP or UTP were obtained by isothermal titration calorimetry (bottom panel), which consisted of integrated heats (black squares) and the heat expected from the fitted model (black line). Results of ATP in the presence of 2.5 mM Mg^{2+} are also included. The heat of dilution of nucleotides into buffer has been subtracted.

Figure S5. **Comparison of binding site surrounding the hydrophobic end of the isopentenyl group**. Equivalent residues of *H. lupulus* and *Agrobacterium* AIPT are shown in gray and cyan. The surfaces of the isopentenyl group binding cavity are shown as mesh in magenta and cyan, respectively.

S1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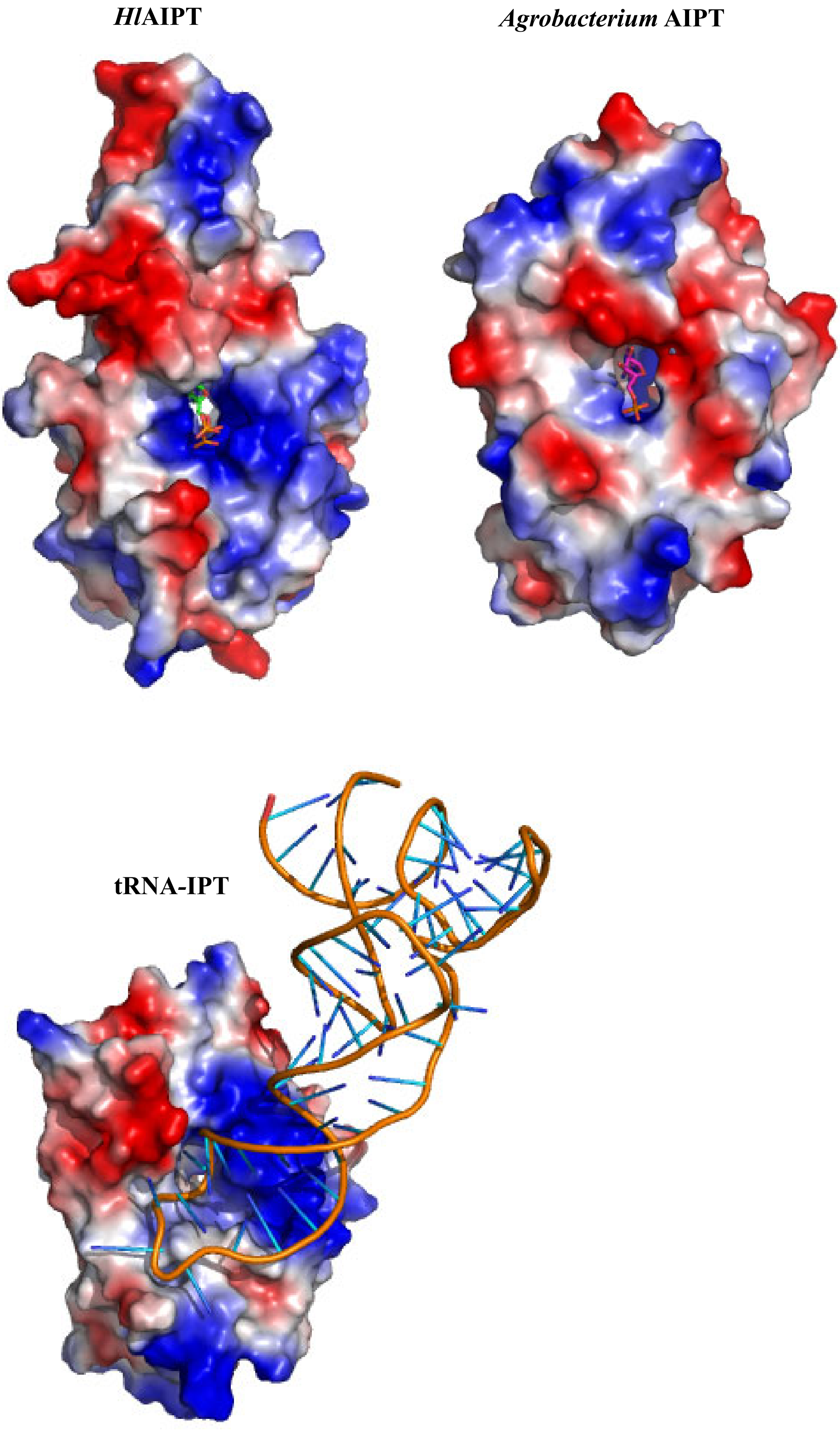
S2

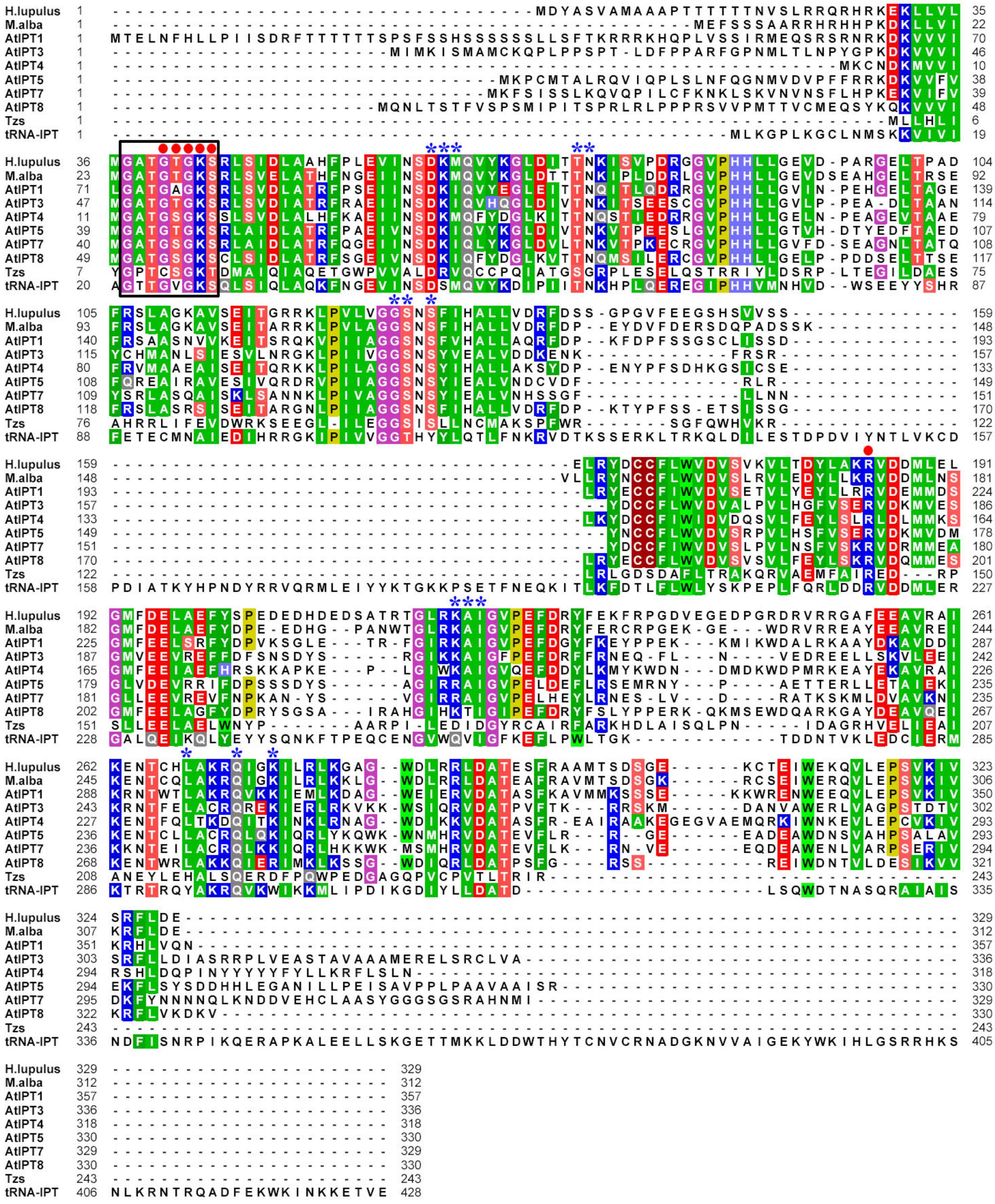


*Hl*AIPT

Agrobacterium AIPT

tRNA-IPT

S3



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M.alba     1   ------------------------------------------------MEFSSSAARRHRHHPKDKLLVI   22
AtIPT1     1   MTELNFHLLPIISDRFTTTTTTSPSFSSHSSSSSSLLSFTKRRRKHQPLVSSIRMEQSRSRNRKDKVVVI   70
AtIPT3     1   -----------------------MIMKISMAMCKQPLPPSPT-LDFPPARFGPNMLTLNPYGPKDKVVVI   46
AtIPT4     1   ------------------------------------------------------------MKCNDKMVVI   10
AtIPT5     1   --------------------------------MKPCMTALRQVIQPLSLNFQGNMVDVPFFRRKDKVVFV   38
AtIPT7     1   -------------------------------MKFSISSLKQVQPILCFKNKLSKVNVNSFLHPKEKVIFV   39
AtIPT8     1   ----------------------MQNLTSTFVSPSMIPITSPRLRLPPPRSVVPMTTVCMEQSYKQKVVVI   48
Tzs        1   ----------------------------------------------------------------MLLHLI   6
tRNA-IPT   1   ---------------------------------------------------MLKGPLKGCLNMSKKVIVI   19

H.lupulus  36  MGATGTGKSRLSIDLAAHFPLEVINSDKMQVYKGLDITTNKISVPDRGGVPHHLLGEVD-PARGELTPAD  104
M.alba     23  MGATGTGKSRLSVELATHFNGEIINSDKMQVYKGLDTTTNKIPLDDRLGVPHHLLGEVDSEAHGELTRSE  92
AtIPT1     71  LGATGAGKSRLSVDLATRFPSEIINSDKIQVYEGLEITTNQITLQDRRGVPHHLLGVIN-PEHGELTAGE  139
AtIPT3     47  MGATGTGKSRLSVDIATRFRAEIINSDKIQVHQGLDIVTNKITSEESCGVPHHLLGVLP-PEA-DLTAAN  114
AtIPT4     11  MGATGSGKSSLSVDLALHFKAEIINSDKMQFYDGLKITTNQSTIEDRRGVPHHLLGELN-PEAGEVTAAE  79
AtIPT5     39  MGATGTGKSRLAIDLATRFPAEIVNSDKIQVYKGLDIVTNKVTPEESLGVPHHLLGTVH-DTYEDFTAED  107
AtIPT7     40  MGATGSGKSRLAIDLATRFQGEIINSDKIQLYKGLDVLTNKVTPKECRGVPHHLLGVFD-SEAGNLTATQ  108
AtIPT8     49  MGATGSGKSCLSIDLATRFSGEIVNSDKIQFYDGLKVTTNQMSILERCGVPHHLLGELP-PDDSELTTSE  117
Tzs        7   YGPTCSGKTDMAIQIAQETGWPVVALDRVQCCPQIATGSGRPLESELQSTRRIYLDSRP-LTEGILDAES  75
tRNA-IPT   20  AGTTGVGKSQLSIQLAQKFNGEVINSDSMQVYKDIPIITNKHPLQEREGIPHHVMNHVD--WSEEYYSHR  87

H.lupulus  105 FRSLAGKAVSEITGRRKLPVLVGGSNSFIHALLVDRFDSS-GPGVFEEGSHSVVSS--------------  159
M.alba     93  FRSLAGKAVSEITARRKLPVLAGGSNSFIHALLVDRFDP--EYDVFDERSDQPADSSK------------  148
AtIPT1     140 FRSAASNVVKEITSRQKVPIIAGGSNSFVHALLAQRFDP--KFDPFSSGSCLISSD--------------  193
AtIPT3     115 YCHMANLSIESVLNRGKLPIIVGGSNSYVEALVDDKENK------------FRSR---------------  157
AtIPT4     80  FRVMAAEAISEITQRKKLPIILAGGSNSYIHALLAKSYDP--ENYPFSDHKGSICSE--------------  133
AtIPT5     108 FQREAIRAVESIVQRDRVPIIAGGSNSYIEALVNDCVDF--------------RLR--------------  149
AtIPT7     109 YSRLASQAISKLSANNKLPIVAGGSNSYIEALVNHSSGF-------------LLNN--------------  151
AtIPT8     118 FRSLASRSISEITARGNLPIIAGGSNSFIHALLVDRFDP--KTYPFSS-ETSISSG--------------  170
Tzs        76  AHRRLIFEVDWRKSEEGL-ILEGGSISLLNCMAKSPFWR-------SGFQWHVKR---------------  122
tRNA-IPT   88  FETECMNAIEDIHRRGKIPIVVGGTHYYLQTLFNKRVDTKSSERKLTRKQLDILESTDPDVIYNTLVKCD  157

H.lupulus  159 --------------------------------------ELRYDCCFLWVDVSVKVLTDYLAKRVDDMLEL  191
M.alba     148 -------------------------------------VLLRYNCCFLWVDVSLRVLEDYLLKRVDDMLNS  181
AtIPT1     193 ---------------------------------------LRYECCFIWVDVSETVLYEYLLRRVDEMMDS  224
AtIPT3     157 -----------------------------------------YDCCFLWVDVALPVLHGFVSERVDKMVES  186
AtIPT4     133 ---------------------------------------LKYDCCFIWIDVDQSVLFEYLSLRLDLMMKS  164
AtIPT5     149 -----------------------------------------YNCCFLWVDVSRPVLHSFVSERVDKMVDM  178
AtIPT7     151 -----------------------------------------YDCCFIWVDVSLPVLNSFVSKRVDRMMEA  180
AtIPT8     170 ---------------------------------------LRYECCFLWVDVSVSVLFEYLSKRVDQMMES  201
Tzs        122 ---------------------------------------LRLGDSDAFLTRAKQRVAEMFAIRED---RP  150
tRNA-IPT   158 PDIATKYHPNDYRRVQRMLEIYYKTGKKPSETFNEQKITLKFDTLFLWLYSKPEPLFQRLDDRVDDMLER  227

H.lupulus  192 GMFDELAEFYSPEDEDHDEDSATRTGLRKAIGVPEFDRYFEKFRPGDVEGEDPGRDRVRRGAFEEAVRAI  261
M.alba     182 GMFDELAEFYDPE-EDHG--PANWTGLRKAIGVPEFDRYFERCRPGEK-GE---WDRVRREAYEEAVREI  244
AtIPT1     225 GMFEELSRFYDPVKSGLE---TR-FGIRKAIGVPEFDGYFKEYPPEK---KMIKWDALRKAAYDKAVDDI  287
AtIPT3     187 GMVEEVREFFDFSNSDYS------RGIKKAIGFPEFDRFFRNEQ-FL---N----VEDREELLSKVLEEI  242
AtIPT4     165 GMFEEIAEFHRSKKAPKE---P--LGIWKAIGVQEFDDYLKMYKWDN---DMDKWDPMRKEAYEKAVRAI  226
AtIPT5     179 GLVDEVRRIFDPSSSDYS------AGIRRAIGVPELDEFLRSEMRNY---P----AETTERLLETAIEKI  235
AtIPT7     181 GLLEEVREVFNPKAN-YS------VGIRRAIGVPELHEYLRNES-LV---D----RATKSKMLDVAVKNI  235
AtIPT8     202 GMFEELAGFYDPRYSGSA---IRAHGIHKTIGIPEFDRYFSLYPPERK-QKMSEWDQARKGAYDEAVQEI  267
Tzs        151 SLLEELAELWNYP-------AARPI-LEDIDGYRCAIRFARKHDLAISQLPN-----IDAGRHVELIEAI  207
tRNA-IPT   228 GALQEIKQLYEYYSQNKFTPEQCENGVWQVIGFKEFLPWLTGK------------TDDNTVKLEDCIERM  285

H.lupulus  262 KENTCHLAKRQIGKILRLKGAG--WDLRRLDATESFRAAMTSDSGE------KCTEIWEKQVLEPSVKIV  323
M.alba     245 KENTCQLAKRQIGKILRLKKLG--WDLRRLDATEAFRAVMTSDSGK------RCSEIWERQVLEPSVKIV  306
AtIPT1     288 KRNTWTLAKRQVKKIEMLKDAG--WEIERVDATASFKAVMMKSSSE-----KKWRENWEEQVLEPSVKIV  350
AtIPT3     243 KRNTFELACRQREKIERLRKVKK-WSIQRVDATPVFTK---RRSKM------DANVAWERLVAGPSTDTV  302
AtIPT4     227 KENTFQLTKDQITKINKLRNAG--WDIKKVDATASFR-EAIRAAKEGEGVAEMQRKIWNKEVLEPCVKIV  293
AtIPT5     236 KENTCLLACRQLQKIQRLYKQWK-WNMHRVDATEVFLR---R--GE------EADEAWDNSVAHPSALAV  293
AtIPT7     236 KKNTEILACRQLKKIQRLHKKWK-MSMHRVDATEVFLK---RN-VE------EQDEAWENLVARPSERIV  294
AtIPT8     268 KENTWRLAKKQIERIMKLKSSG--WDIQRLDATPSFG----RSS----------REIWDNTVLDESIKVV  321
Tzs        208 ANEYLEHALSQERDFPQWPEDGAGQPVCPVTLTRIR----------------------------------  243
tRNA-IPT   286 KTRTRQYAKRQVKWIKKMLIPDIKGDIYLLDATD--------------------LSQWDTNASQRAIAIS  335

H.lupulus  324 SRFLDE----------------------------------------------------------------  329
M.alba     307 KRFLDE----------------------------------------------------------------  312
AtIPT1     351 KRHLVQN---------------------------------------------------------------  357
AtIPT3     303 SRFLLDIASRRPLVEASTAVAAAMERELSRCLVA------------------------------------  336
AtIPT4     294 RSHLDQPINYYYYYFYLLKRFLSLN---------------------------------------------  318
AtIPT5     294 EKFLSYSDDHHLEGANILLPEISAVPPLPAAVAAISR---------------------------------  330
AtIPT7     295 DKFYNNNNQLKNDDVEHCLAASYGGGSGSRAHNMI-----------------------------------  329
AtIPT8     322 KRFLVKDKV-------------------------------------------------------------  330
Tzs        243 ----------------------------------------------------------------------  243
tRNA-IPT   336 NDFISNRPIKQERAPKALEELLSKGETTMKKLDDWTHYTCNVCRNADGKNVVAIGEKYWKIHLGSRRHKS  405

H.lupulus  329 -----------------------  329
M.alba     312 -----------------------  312
AtIPT1     357 -----------------------  357
AtIPT3     336 -----------------------  336
AtIPT4     318 -----------------------  318
AtIPT5     330 -----------------------  330
AtIPT7     329 -----------------------  329
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Tzs        243 -----------------------  243
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S4

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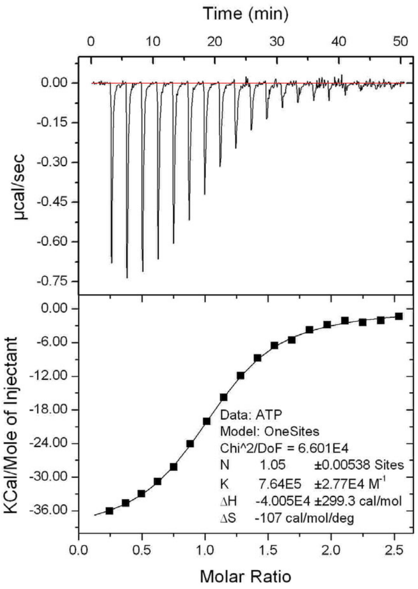

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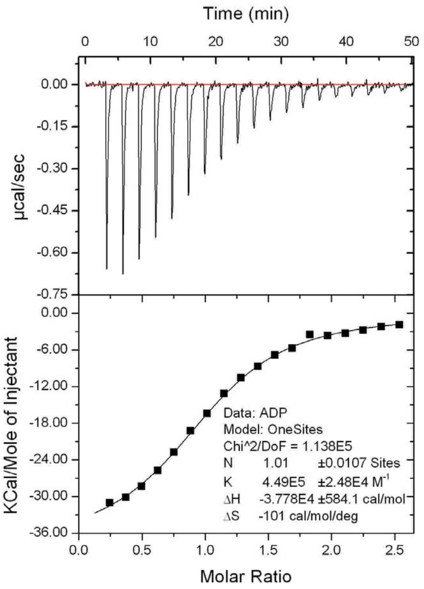

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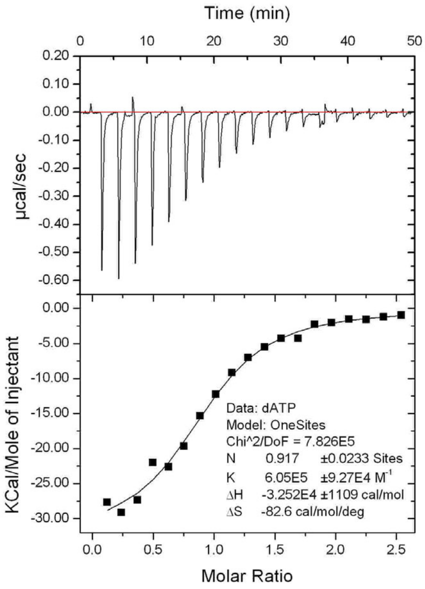
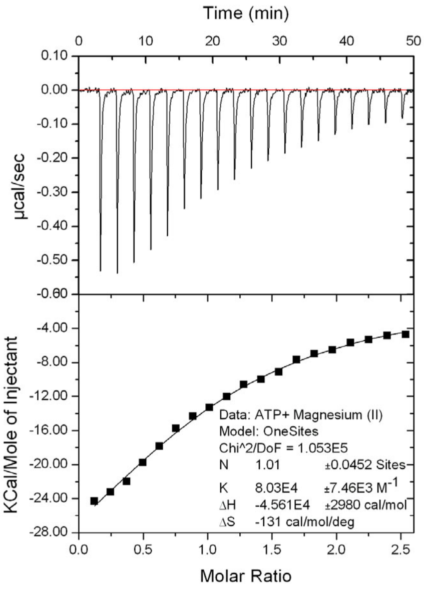
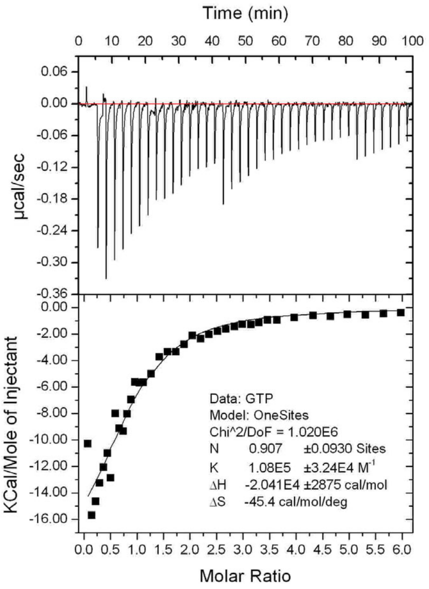


ATP/Mg^{2+}



continued

GTP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0.00 -2.00 -4.00 -6.00 -8.00 -10.00 -12.00 -14.00 -16.00
Data: GTP
Model: OneSites
Chi^2/DoF = 1.020E6
N 0.907 ±0.0930 Sites
K 1.08E5 ±3.24E4 M-1
ΔH -2.041E4 ±2875 cal/mol
ΔS -45.4 cal/mol/deg
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Molar Ratio

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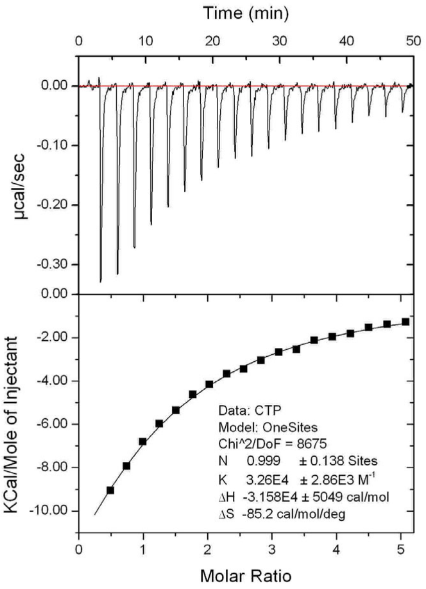
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KCal/Mole of Injectant
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Data: CTP
Model: OneSites
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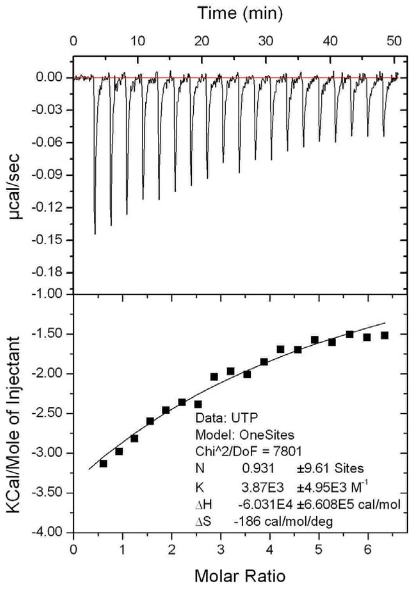
N 0.999 ± 0.138 Sites
K 3.26E4 ± 2.86E3 M-1
ΔH -3.158E4 ± 5049 cal/mol
ΔS -85.2 cal/mol/deg
0 1 2 3 4 5
Molar Ratio

UTP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µcal/sec
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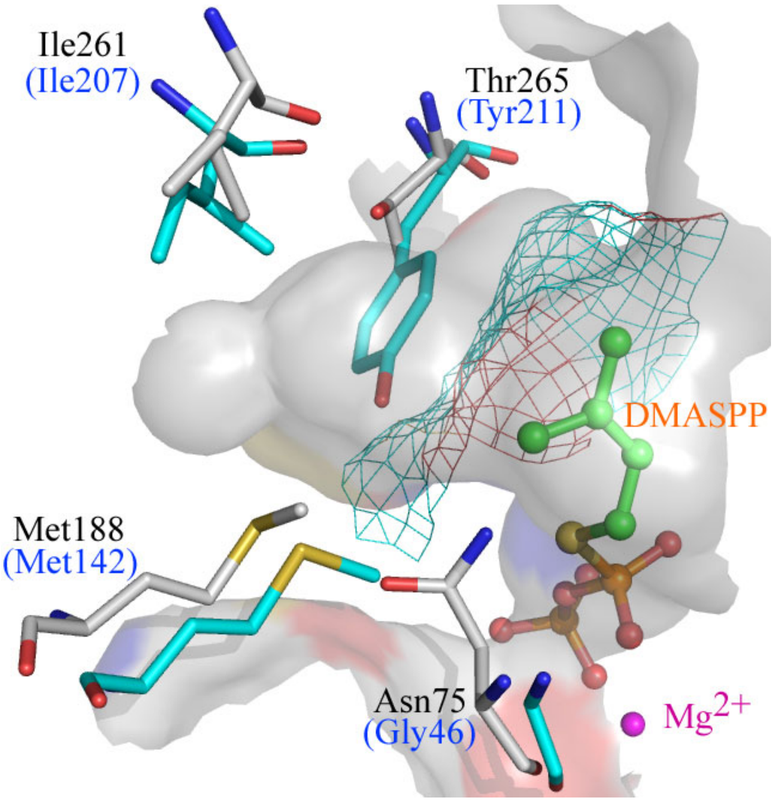
KCal/Mole of Injectant
-1.00 -1.50 -2.00 -2.50 -3.00 -3.50 -4.00
Data: UTP
Model: OneSites
Chi^2/DoF = 7801
N 0.931 ±9.61 Sites
K 3.87E3 ±4.95E3 M-1
ΔH -6.031E4 ±6.608E5 cal/mol
ΔS -186 cal/mol/deg
0 1 2 3 4 5 6
Molar Ratio

S5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