Structure, *19*

Supplemental Information

Classification of a *Haemophilus influenzae* ABC Transporter HI1470/71 through Its Cognate Molybdate Periplasmic Binding Protein, MolA

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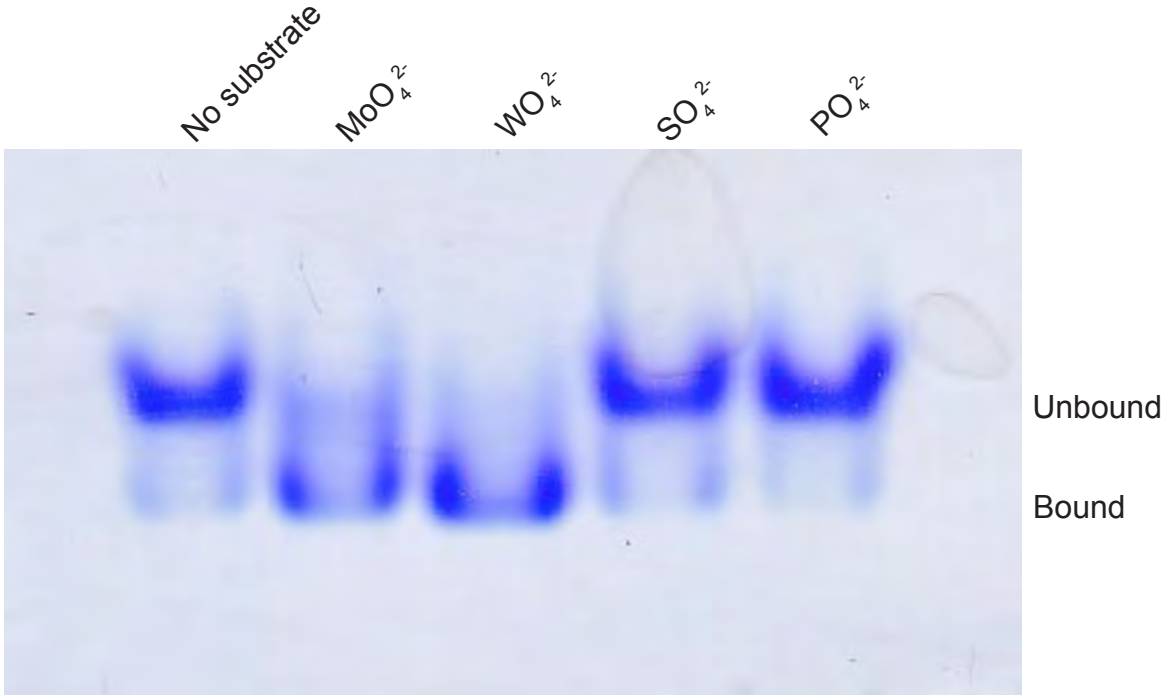
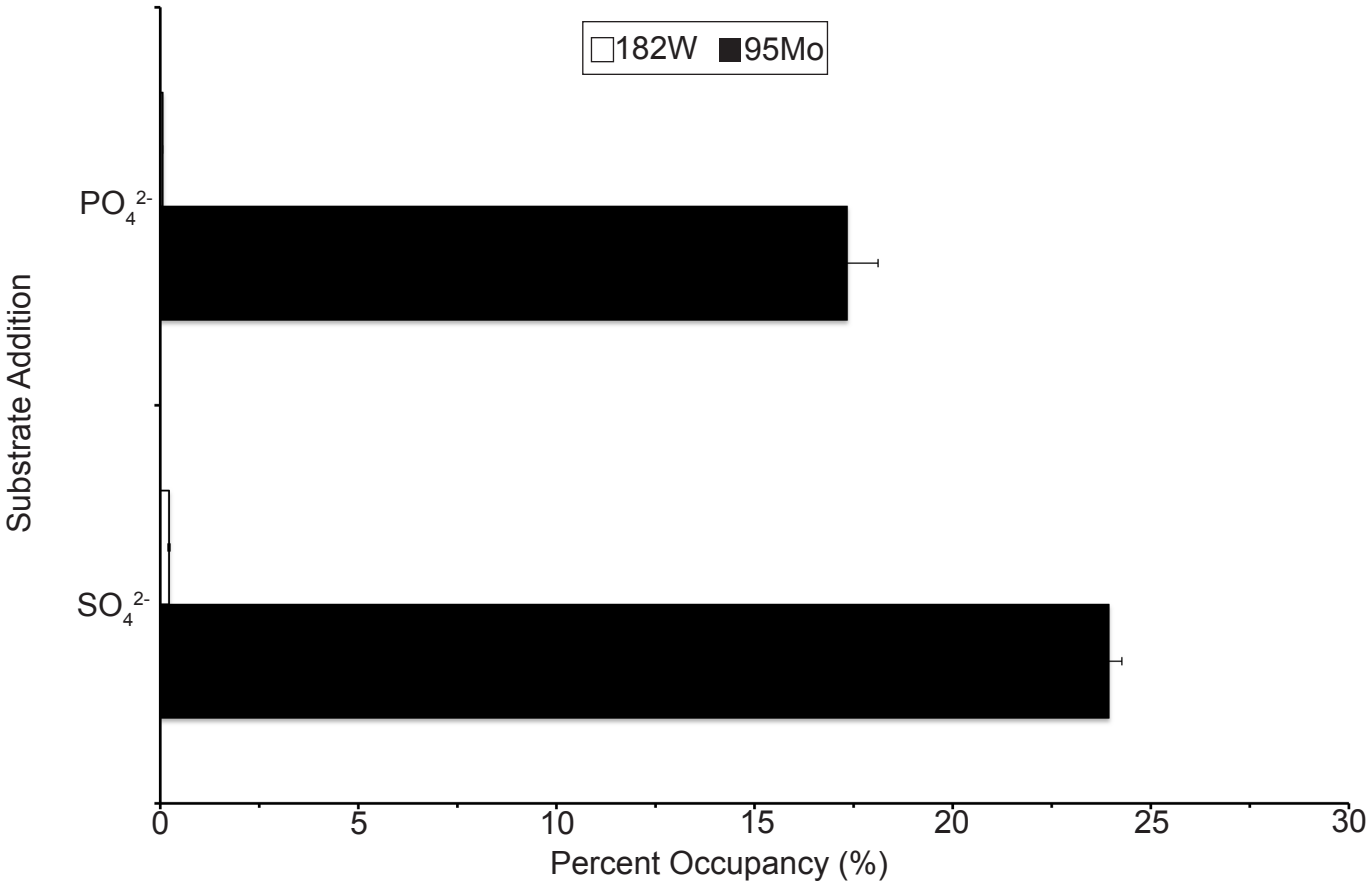
Inventory of Supplemental Information

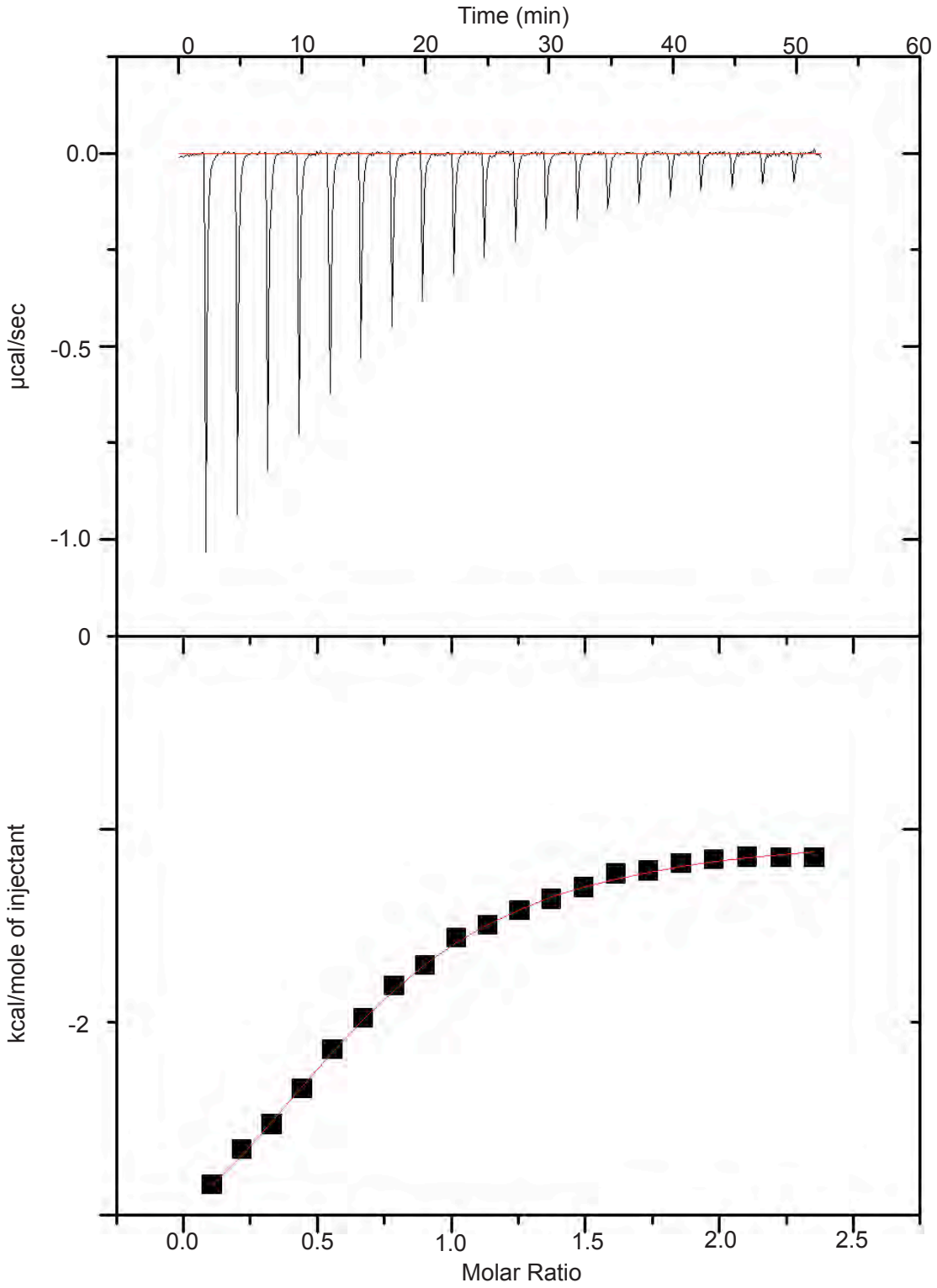
S.1.


No substrate
MoO_4^{2-}
WO_4^{2-}
SO_4^{2-}
PO_4^{2-}
Unbound
Bound

S.2.

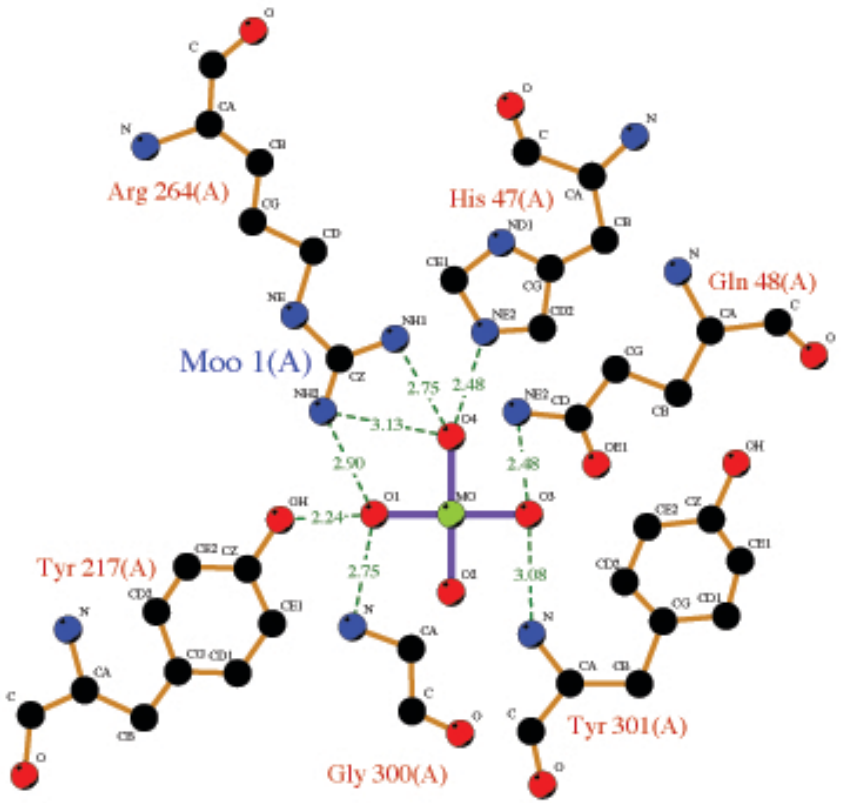

□182W ■95Mo
PO_4^{2-}
SO_4^{2-}
Substrate Addition
0
5
10
15
20
25
30
Percent Occupancy (%)

S.3.

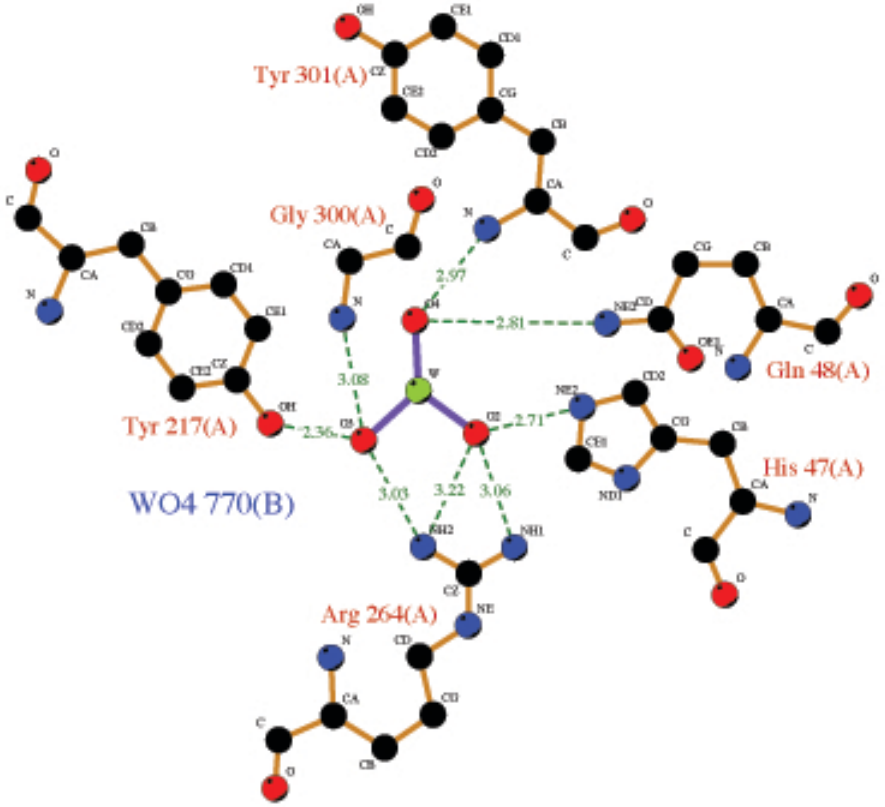

Time (min)
0
10
20
30
40
50
60
0.0
-0.5
-1.0
µcal/sec
0
-2
kcal/mole of injectant
0.0
0.5
1.0
1.5
2.0
2.5
Molar Ratio

S.4


β1 β2 β3 α1 β4 α2 R1 α3 β5 R2 β6
MolA 22 DRIITDQLDRKVTIPDHINRAVVLQHQTLNIAVQLDATKQIVGVLSNWKKQLGKNYVRLAPELENMAMPGDLNSVN 97
α4 β7 R3 α5 β8 R4 α6
MolA 98 IESLLALKPDVVFVTNYAPSEMIKQISDVNIPVVAISLRTGEVGEKGKLNPTLTDEDKAYNDGLKQGIELIAEVFEK 174
α7 β9 β10 α8 β11 β12 α9
MolA 175 KQQGDELVKAAFANRKLLADRLGDVSADKRVRTYMANPDLGTYGSGKYTGLMMEHAGAYNVAAATIKGFKQVSLENVLEW
β13 α10 α11 β14 R5 α11
MolA 255 NPAVILVQDRYPDVVPQILNDQGWANIQALKDKKVFLMPEYAKAWGYPMPEALALGEVWLAKALYPQRFQDVD 327
α12
MolA 328 LDKMVNDYYQKFYRTSYKPD 347
β1-2 α1 β1-1 R1/R2 α2 β1-3 R3 α3
TroA 32 GKPLVVTTIGMIADAVKNIAQGDVHLKGLMGPGVDPHLYTATAGDVEWLGNADLILYNGLHLETKMGEVFSKLRGSR 108
β1-4 β2-1 R4 β2-2 α4 α5
TroA 109 LVVAVSETIPVSQRLSLEEAEFDPHVWFDVKLWSYSVKAVYESLCKLLPGKTREFTQRYQAYQQQLDKLDAYVRRKAQSLPA 189
β3-2 α6 β3-1 α7 β3-3 α8
TroA 190 AERRVLVTAHDAFGYFSRAYGFEVKGLQGVSTASEASAHDMQELAAFIAQRKLPAIFIESSIPHKNVEALRDAVQARGHVV 270
β3-4 R5 α9
TroA 271 GIGGELFSDAMGDAGTSEGTYVGMVTHNIDTIVAALAR 308
β1 α1 β2 R1 α2 β3 R2 α3 β4 R3 α4
BtuF 44 AAPRVITLSPANTELAFAAGITPVGVSSYSDYPPQAQKIEQVSTWQGMNLERIVALKPDLVIAWRGGNAERQVDQLASLGIK 102
β5 R4 α5 α6 β6 α7
BtuF 103 KVMWVDATSIEQIANALRQLAPWSPQPDKAEQAAQSLLDQYAQLKAQYADKPKKRVFLQFGINPPFTSGKESIQNQVLEVCG 184
β7 α8 β8 α9 β9 α10 R5 α11
BtuF 185 GENIFKDSRVPWPQVSREQVLARSPQAIVITGGPDQIPKIKQYWGEQLKIPVIPLTSDWFERASPRIILAAQQLCNALSQVD 266



MolA-molybdate



MolA-tungstate

Key

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length

Figure S1 related to Figure 3A. Ligand-dependent mobility shift of HI1472. Protein was incubated with a 4000-fold molar excess of various oxyanions prior to running on a 12% native polyacrylamide gel. Molybdate and tungstate were the only oxoanions capable of causing a mobility shift.

Figure S2 related to Figure 3A. Metal content analysis of HI1472. ICP-MS quantitation of molybdenum and tungsten content of HI1472. Protein was expressed in sodium sulfate (SO) and sodium phosphate (PO). The molybdate and tungstate occupancy do not decrease significantly from that of HI1472 grown in LB alone indicating that these oxoanions are not competing with the contaminating molybdate and tungstate available in the media.

Figure S3 related to Figure 3B. Tungstate binding affinity to HI1472**.** ITC of tungstate into HI1472. Sodium tungstate at 4.5 mM was titrated into 0.3 mM HI1472 to give a final molar ratio of 2.25:1. At the given concentrations, the estimated K_d was 84 uM. The Origin Software determined that a HI1472 concentration of 4 mM would be required to get an optimal binding curve.

Figure S4 related to Figure 6. Sequence and structural schematic of MolA, and structural homologs TroA and BtuF. Green arrows represent β-strands; α-helices and 3_{10} helices are represented by red and blue cylinders respectively (classification by DSSP)(1). The asterisks (*) indicate residues that form bonds with the substrate. Blue dashed lines denote the loop regions in each structure that make up the inner cavity of the binding pocket of MolA, TroA and BtuF.

Figure S5 related to Figure 2. Schematic representations of the molybdate/tungstate-binding pockets from MolA-molybdate and MolA-tungstate produced using the program LIGPLOT(2). The hydrogen bonds are indicated by dashed lines and their lengths are shown in angstroms. Atoms are colored as follows: blue, nitrogen; red, oxygen; black, carbon; green, tungsten/molybdenum.

References:

1. Kabsch W, Sander C. How good are predictions of protein secondary structure? FEBS Lett. 1983 May 8;155(2):179-82.
2. Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng. 1995 Feb;8(2):127-34.