# SUPPLEMENTARY DATA

## A structural and functional investigation of the periplasmic arsenate-binding protein, ArrX from *Chrysiogenes arsenatis*

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**Running Title**: Structure and function of the ArrX protein.

**Keywords:** arsenate, *Chrysiogenes arsenatis*, periplasmic binding protein (PBP), isothermal titration calorimetry (ITC), X-ray crystallography

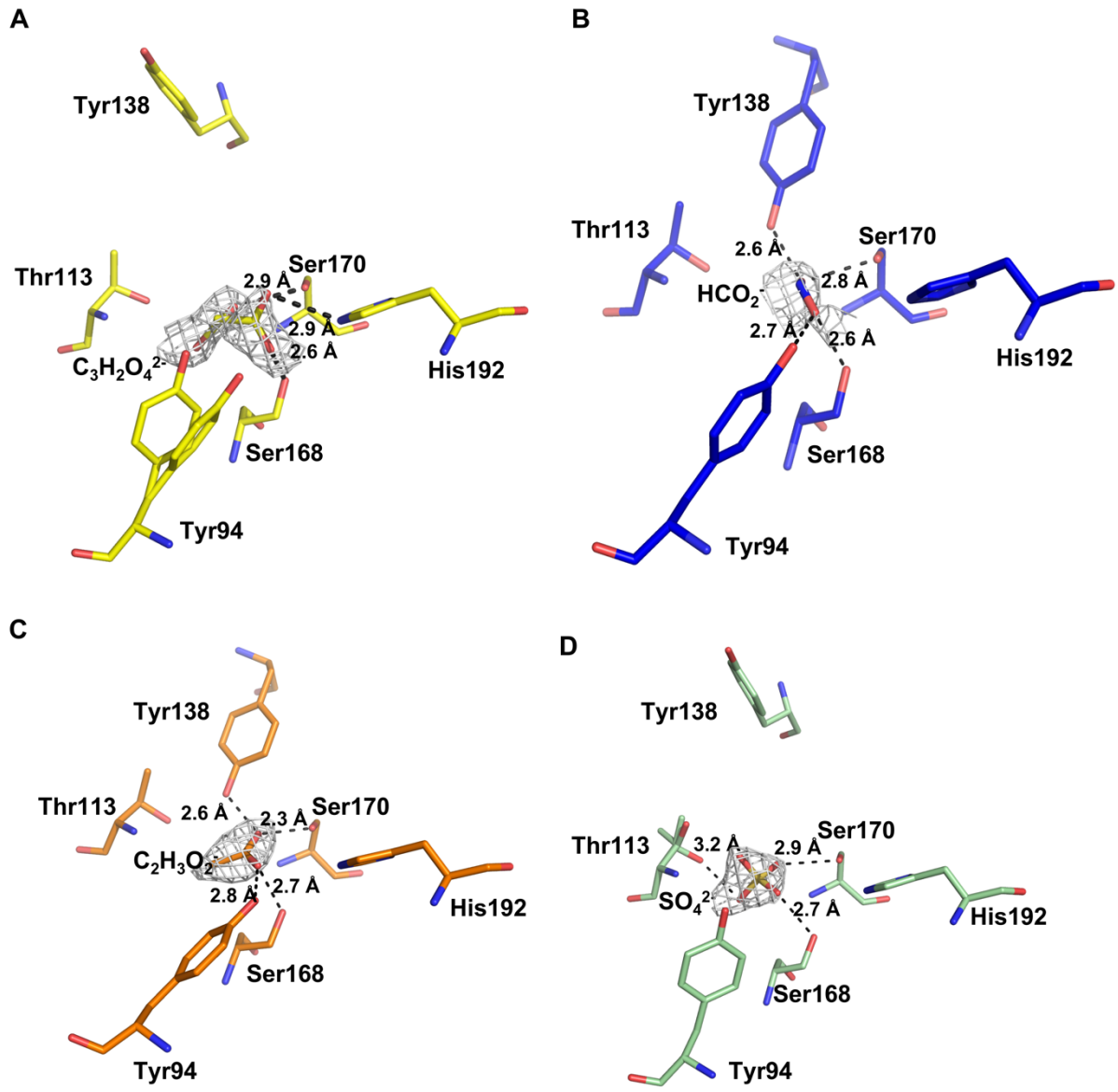


**Figure S1. Ligand binding site of His-ArrX with bound oxyanions.** $F_o$- $F_c$ difference Fourier electron density maps (grey) contoured at 3$\sigma$, prior to addition of the coordinates of the oxyanions to the model: **(A)** malonate-His-ArrX ($C_3H_2O_4^{2-}$, yellow sticks); **(B)** formate-His-ArrX ($HCO_2^-$, blue sticks); **(C)** acetate-His-ArrX ($C_2H_3O_2^-$, orange sticks); **(D)** chloride-soak-His-ArrX (sulfate ($SO_4^{2-}$), yellow sticks, remains bound in the binding site). All hydrogen-bonding interactions between the bound oxyanions and surrounding residues are indicated as black, dashed lines.

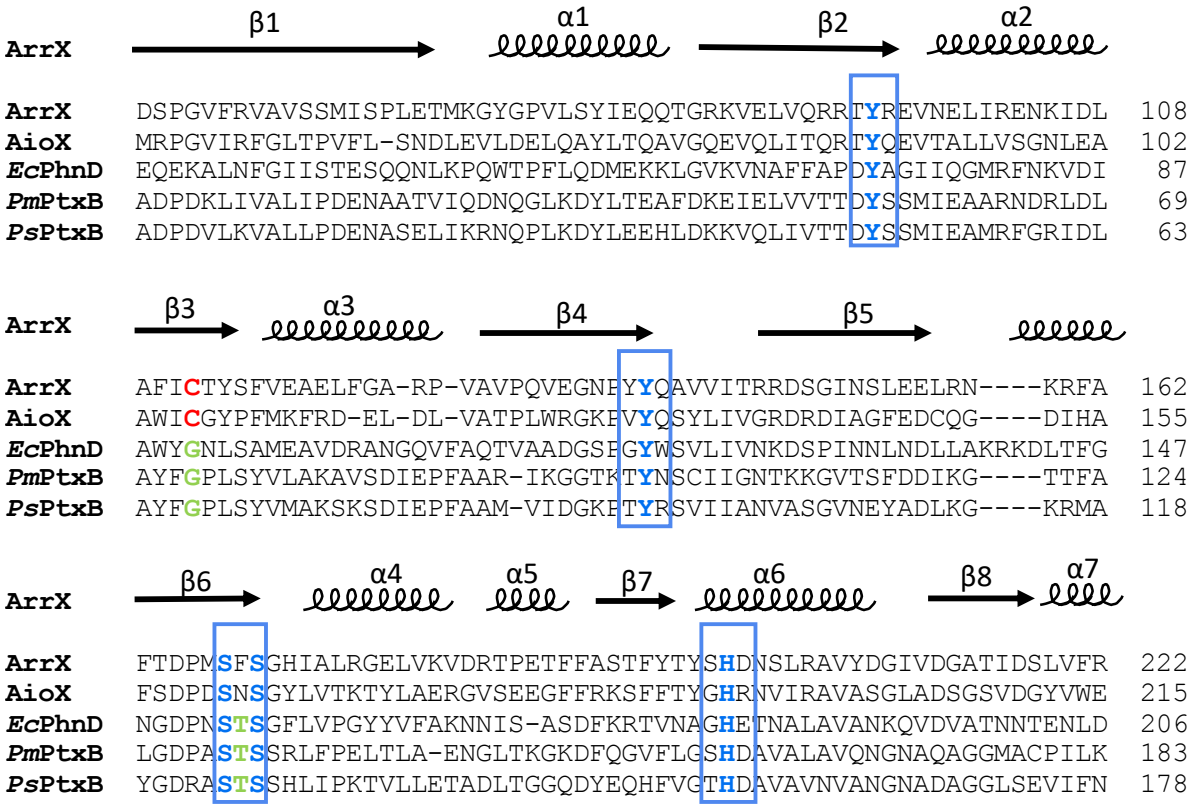


**Figure S2. Secondary structure-based sequence alignment of the ArrX, AioX, PhnD and PtxB proteins**. The ArrX structure was used as the template structure. Highlighted residues are the conserved residues in the ligand binding pockets of the PBPs (blue). Residues in the binding pocket, which are only conserved in ArrX and AioX are shown in red. Residues in the binding pocket, which are only conserved in *Ec*PhnD, *Pm*PtxB and *Ps*PtxB are shown in green. The alignment was generated with Clustal Omega [60] and secondary structure annotation with ESPript 3.0 [61].

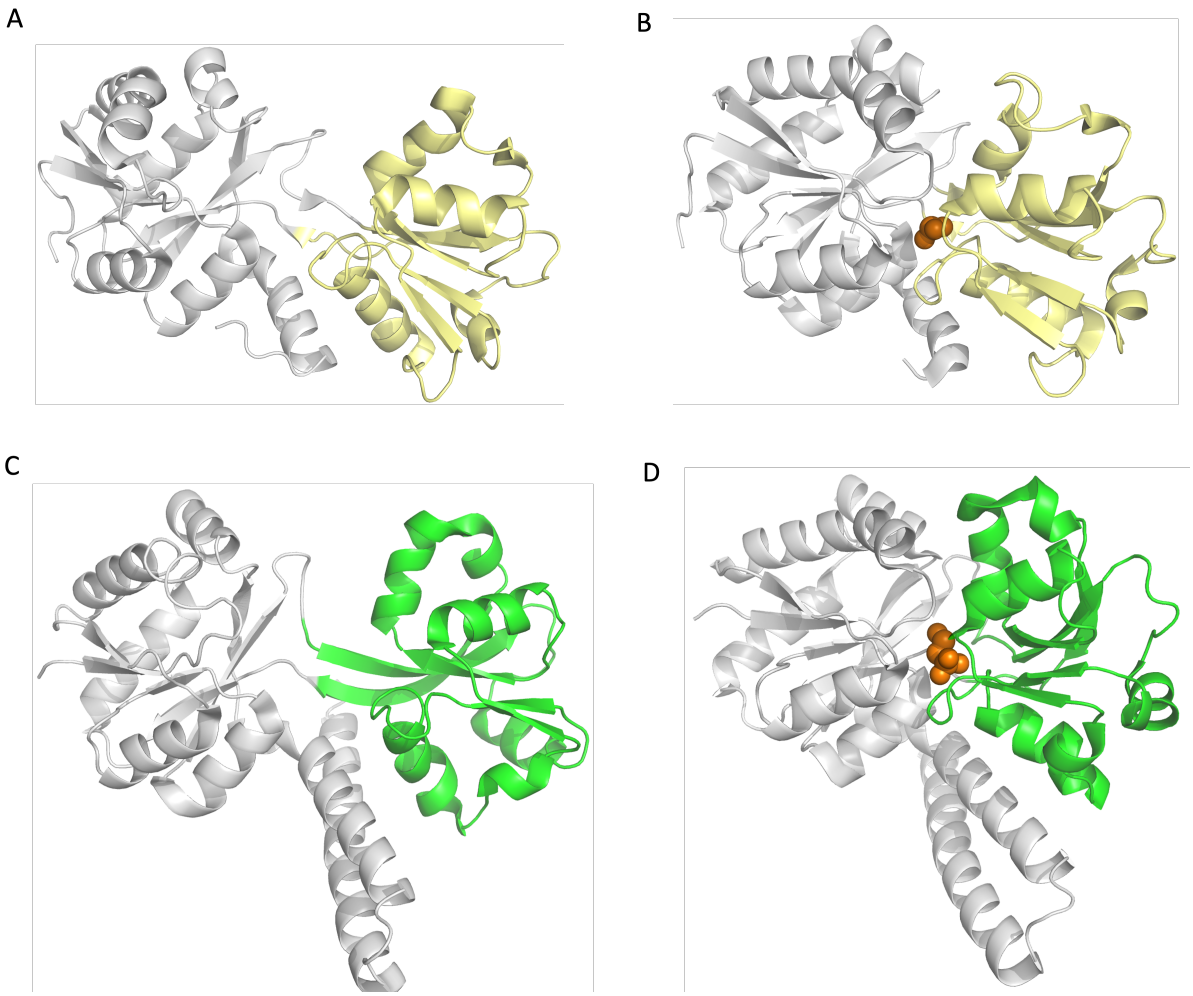


**Figure S3. Substrate binding among PBPs. (A)** *apo-Ps*PtxB (PDB 5O2K) [59]. **(B)** phosphite-*Ps*PtxB (PDB 5O2J [59]; the phosphite molecule is shown as orange spheres). In both A and B, the C-terminal domain is shown in pale yellow. **(C)** *apo-Ec*PhnD (PDB 3S4U) [36]. **(D)** 2AEP-*Ec*PhnD (PDB 3P7I [36]; the 2AEP molecule is shown as orange spheres). In both C and D, the C-terminal domain is shown in bright green.

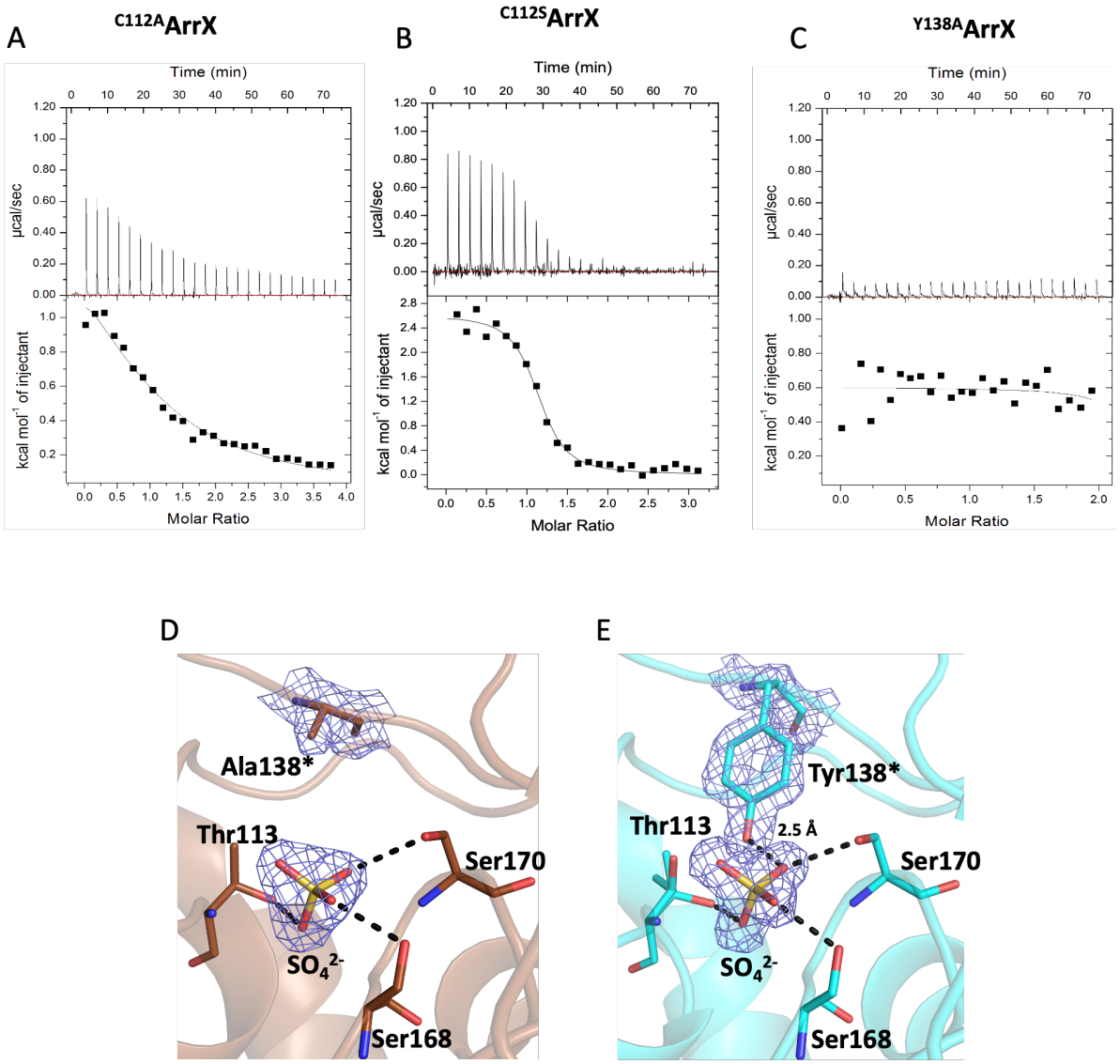


**Figure S4. Representative ITC measurements and structure of mutant variants of ArrX. (A)** $^{C112A}$ArrX with arsenate. **(B)** $^{C112S}$ArrX with arsenate**. (C)** $^{Y138A}$ArrX with arsenate. Sodium arsenate (2 mM) was titrated to purified protein samples (200 µM) at 25ºC. Curves were fitted to single site (N=1) model and the values for $K_D$ were calculated from replicate experiments (n=3, ± SEM). **(D)** The $^{Y138A}$ArrX structure (brown): with binding site residues represented as sticks. **(E)** The His-ArrX structure (cyan): with binding site residues represented as sticks. In both D and E, $2F_o$-$F_c$ electron density maps (grey) contoured at 1.5$\sigma$ is shown for bound sulfate ions (in stick model, yellow) and residue 138 is marked as *. Hydrogen-bonding interactions are indicated as black, dashed line.

**Table S1. Primers used for site-directed mutagenesis for the generation of ArrX and AioX variants**

| Protein | Primer | Primer sequence | Annealing Temperature (°C) |
|---|---|---|---|
| $^{WT}$AioX *46* | Forward[a] | GC**GGATCC**ACTGTCGGGCTTACCGCATTG | - |
| | Reverse[a] | GC**GAATTC**CTCATCCCAGCCTCCGCACGCG | |
| $^{WT}$ArrX *46* | Forward[a] | GC**GGATCC**TCGGTAAAACCTATTCCGGTT | - |
| | Reverse[a] | GC**AAGCTT**CTACTCAACCACCTCTATTTT | |
| $^{C112A}$ArrX | Forward* | GGCATTTATCGCCACCTATTCATTTGTAG | 58°C |
| | Reverse* | AAATCAATTTTATTTTCGCGG | |
| $^{C112S}$ArrX | Forward* | GGCATTTATCTCCACCTATTCATTTG | 58°C |
| | Reverse* | AAATCAATTTTATTTTCGCGG | |
| $^{Y138A}$ArrX | Forward* | AAACCCTTACGCTCAGGCGGTGGTG | 64°C |
| | Reverse* | CCCTCAACTTGCGGGACA | |
| $^{C106A}$AioX | Forward* | CGCCTGGATCGCCGGCTATCCCTTC | 66°C |
| | Reverse* | GCCTCGAGATTGCCCGAT | |
| $^{C106S}$AioX | Forward* | CGCCTGGATCTCCGGCTATCCCT | 69°C |
| | Reverse* | GCCTCGAGATTGCCCGATAC | |
| $^{Y131A}$AioX | Forward* | CAAGCCCGTTGCCCAGTCCTACCTCATC | 68°C |
| | Reverse* | CCACGCCAGAGTGGCGTG | |

[a]Bold and underlined bases are restriction sites. *Bam*HI was used in conjunction with *Eco*RI and *Hin*dIII to clone *aioX* and *arrX,* respectively.

*Underlined bases correspond to the substituted codons of the mutant variants of ArrX and AioX proteins.

67 **Table S2 Optimized crystallization conditions for the His-ArrX protein**

| Crystallization conditions | Protein in Buffer | Protein concentration |
|---|---|---|
| 0.2 M lithium sulphate, 0.1 M Tris - HCl pH 8.5, 25% (w/v) PEG 3350 | 50 mM Tris-HCl, pH 8.0 | 10 mg/mL |
| 0.2 M ammonium acetate, 0.1 M Tris- HCl pH 7.5, 20% (w/v) PEG 3350 | 50 mM Tris-HCl, pH 8.0 | 10 mg/mL |
| 0.1 M sodium malonate pH 6.7, 18% (w/v) PEG 3350 | 20 mM Tricine, pH 7.3 | 10 mg/mL |
| 0.15 M magnesium formate dihydrate, 18% (w/v) PEG 3350 | 50 mM Tris-HCl, pH 8.0 | 15 mg/mL |

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1 **Table S3 Comparison of ArrX structures**

| **Structure** | **His-ArrX** | | **Arsenate-His-ArrX** | | **Malonate-His-ArrX** | | **Chloride-soak-His-ArrX** | | **Formate-His-ArrX** | | **Acetate-His-ArrX** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r.m.s.d. (Å) | No. Cα | r.m.s.d. (Å) | No. Cα | r.m.s.d. (Å) | No. Cα | r.m.s.d. (Å) | No. Cα | r.m.s.d. (Å) | No. Cα | r.m.s.d. (Å) | No. Cα |
| **His-ArrX** | - | - | 0.48 | 248 | 0.74 | 242 | 0.50 | 257 | 1.54 | 247 | 1.58 | 244 |
| **Arsenate-His-ArrX** | 0.48 | 248 | - | - | 0.43 | 244 | 0.18 | 250 | 1.67 | 240 | 1.73 | 237 |
| **Malonate-His-ArrX** | 0.74 | 242 | 0.43 | 244 | - | - | 0.35 | 241 | 1.82 | 237 | 1.84 | 234 |
| **Chloride-soak-His-ArrX** | 0.50 | 257 | 0.18 | 250 | 0.35 | 241 | - | - | 1.75 | 240 | 1.77 | 240 |
| **Formate-His-ArrX** | 1.54 | 247 | 1.67 | 240 | 1.82 | 237 | 1.75 | 240 | - | - | 0.22 | 255 |
| **Acetate-His-ArrX** | 1.58 | 244 | 1.73 | 237 | 1.84 | 234 | 1.77 | 240 | 0.22 | 255 | - | - |

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**Table S4 Sequence and structural similarities of ArrX with other PBPs**

| Protein | r.m.s.d. (Å) | Sequence identity (%) | No. Cα | PDB ID |
|---|---|---|---|---|
| Arsenite-AioX | 2.04 | 30 | 242 | 6EU7 [46] |
| Phosphite-*Pm*PtxB | 1.92 | 23 | 158 | 5LV1 [59] |
| Phosphite-*Ps*PtxB | 1.62 | 25 | 184 | 5O2J [59] |
| 2AEP-*Ec*PhnD | 1.70 | 27 | 136 | 3P7I [36] |