Characterisation of the solute-binding protein ModA from *Pseudomonas aeruginosa* and its role in molybdenum homeostasis

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For the clinically significant and opportunistic Gram-negative bacterial pathogen *Pseudomonas aeruginosa*, the redox-active trace metal molybdenum is essential for energy production and respiration under anaerobic conditions. Acquisition of molybdenum occurs via the high-affinity ATP-binding cassette permease ModABC. Because solute-binding proteins (SBPs) are only present in bacteria, this makes them attractive drug targets. Therefore, this study aims to characterise the periplasmic SBP ModA from *P. aeruginosa* PAO1 and its role in molybdenum homeostasis.

Ligand-dependent protein mobility shift gel electrophoresis and nano-differential scanning fluorimetry suggest that ModA binds specifically to group 6 metal oxyanions (chromate, molybdate, tungstate). Despite their similar sizes, binding affinity for chromate is ~ 900-fold lower than molybdate and tungstate. The X-ray crystal structure of ModA shows a non-contiguous dual-hinged bilobal structure, with the ligand-binding pocket located at the interface between the domains. Each domain has five -helices surrounding a central five-stranded mixed -sheet. Upon ligand binding, ModA employs a “Venus’ fly-trap” mechanism, resulting in a relative rotation of 22° of the domains and occluding the pocket from the bulk solvent, with the oxyanion coordinated by four residues contributing six hydrogen bonds. Phylogenetic analysis of 485 *Pseudomonas* ModA sequences shows that the ligand-binding residues and -sheet structural elements are highly conserved. Comparison with orthologous bacterial ModA structures shows that ModA is highly conserved within the cluster D-IIIa SBPs. Despite chromate exposure causing dysregulation of molybdenum homeostasis, deletion of *modA* had no impact on chromate sensitivity and accumulation. Interestingly, this is the first study to directly compare ligand-free and ligand-bound ModA from the same organism and to show a unique oxyanion-binding chemistry with one less coordinating residue and hydrogen bond. Given the stability of ModA and high binding affinity for the environmentally toxic chromate oxyanion, future research would include protein engineering for bioremediation, besides development as a potential drug target.

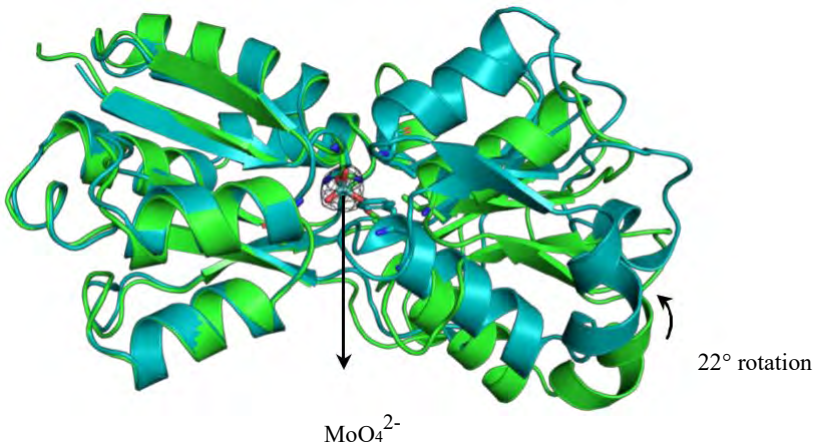

Figure 1. Structural superposition of the crystal structures of ligand-free ModA (green) and molybdate-bound ModA (teal). The polder (OMIT) electron density map of the molybdate ligand is coloured in black and contoured at 4.0 σ, within a 2.0 Å radius around the ligand. The coordinating residues are shown in stick representation. The nitrogen and oxygen atoms are coloured blue and red, respectively.

Reference: Maunders, E. A., Ngu, D. H. Y., Ganio, K., Hossain, S. I., Lim, B. Y. J., Leeming, M. G., Luo, Z., Tan, A., Deplazes, E., Kobe., B. and McDevitt., C. A. (2022). The Impact of Chromate on *Pseudomonas aeruginosa* Molybdenum Homeostasis. Frontiers in Microbiology, **13**:903146. doi: 10.3389/fmicb.2022.903146