Enzymatic reaction mechanism of arsenite oxidase

Filipa S. S. Engrola[1*]; Márcia, Correia[1]; Joanne M. Santini[2]; Cameron Watson[2]; Maria João Romão[1]; Teresa Santos-Silva[1].

[1]UCIBIO, NOVA School of Science & Technology, 2829-516 Caparica, Portugal; [2]Institute of Structural and Molecular Biology, University College London, WC1E 6BT London, United Kingdom.

*f.engrola@campus.fct.unl.pt

Arsenic contamination: a global problem

Arsenic (As) is a **toxic** metalloid that, due to anthropogenic and natural causes, **pollutes waters worldwide** (Figure 1).

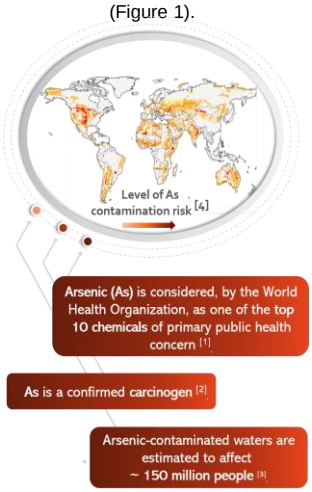

Figure 1. Global arsenic contamination in groundwater[1-4].

Two different Aio structures, from *Alcaligenes faecalis* (*A.f.*) and *Rhizobium* sp. NT-26 (NT-26), respectively, present **different reaction intermediates bound to the active site** (Figure 4).

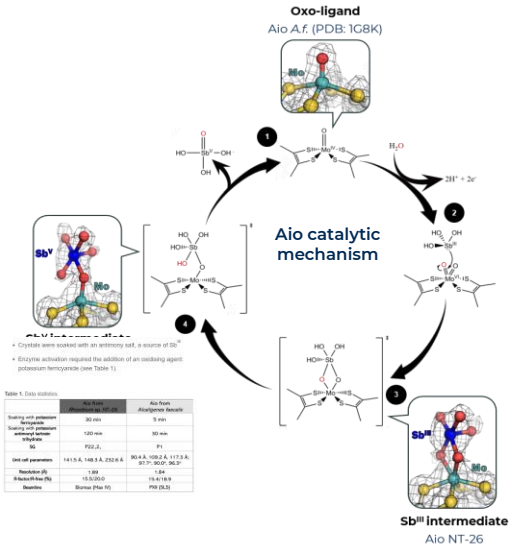

Figure 4. Proposed reaction mechanism at the Aio active site in which the Mocofactor oxidises Sb^{III} to Sb^{V}. Inset plot: 1) Aio reduced active site - Mo^{VI} – as observed in X-ray structures (PDB: 1G8K, 4AAY, 5NQD[6,7,8]); 2) NT-26 Aio bound to an Sb^{III} oxyanion intermediate; 3) *Af* Aio bound to an Sb^{V} oxyanion intermediate

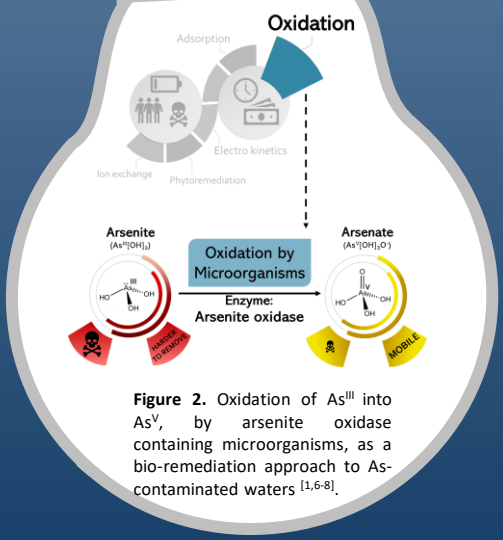

Figure 2. Oxidation of As^{III} into As^{V}, by arsenite oxidase containing microorganisms, as a bio-remediation approach to As-contaminated waters [1,6-8].

Towards the solution: Aio

Arsenite oxidase - Aio (Figure 3), is capable of functioning as **biosensor** and as a **bioremediation** system for arsenic.

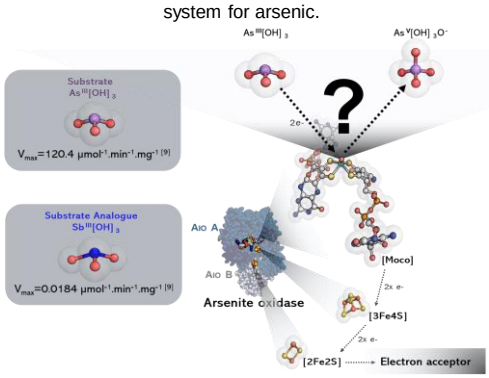

Figure 3. Aio electron transfer pathway between cofactors, from the substrate and substrate analogue.

Antimonite (**$Sb^{III}[OH_3]$**), works as a **substrate analogue** at a much **slower** rate than **arsenite** (**$As^{III}[OH_3]$**)[9].

Take home message

❑ Detailed structural information on **two Aio complexes**, bearing **two different Sb reaction intermediates bound to the active site** were obtained.

❑ This work contributed to the **clarification of the enzymatic reaction mechanism of the oxidation of As^{III} to the less toxic As^{V}**, showing, at the molecular level, transient protein complexes.

❑ The elucidation of this mechanism is considered **vital in the design of new and improved enzymes** to deal with the **worrying environmental crisis of As^{III} and Sb^{III} water pollution**.

References

[1] WHO. Chemical Aspects. Guidelines for Drinking-Water Quality; 4 th Edition; World Health Organization: Geneva, 2011, 178.
[2] Shi, H, *et al.* Oxidative Mechanism of Arsenic Toxicity and Carcinogenesis. Mol. Cell. Biochem. 2004, 255 (1–2), 67–78.
[3] P. Ravenscroft, *et al.* Arsenic Pollution: A Global Synthesis (John Wiley & Sons, 2009), vol. 28.
[4] Schwarzenbach, R. P., *et al.* Global Water Pollution and Human Health. Annu. Rev. Environ. Resour. 2010, 35 (1), 109–136.
[5] Singh, R.; *et al.* Arsenic Contamination, Consequences and Remediation Techniques: A Review. Ecotoxicol. Environ. Saf. 2015, 112, 247–270.
[6] Ellis, P. J., *et al* Crystal Structure of the 100 kDa Arsenite Oxidase from Alcaligenes faecalis in Two Crystal Forms at 1.64 Å and 2.03 Å. Structure. 2001, 9 (2), 125-132.
[7] Warelow, T. P, *et al.* The Respiratory Arsenite Oxidase: Structure and the Role of Residues Surrounding the Rieske Cluster. PLoS One 2013, 8 (8), e72535.
[8] Watson, C.; *et al.* Electron Transfer through Arsenite Oxidase: Insights into Rieske Interaction with Cytochrome C. Biochim. Biophys. Acta - Bioenerg. 2017, 1858 (10), 865–872
[9] Wang, Q., *et al.* Arsenite Oxidase Also Functions as an Antimonite Oxidase. Appl. Environ. Microbiol. 2015, 81 (6), 1959–1965.

Acknowledgements

- This work was supported by the PTDC/BBB-BEP-PRO/1185/2014 and UID/Multi/04378/2019 from Fundação para a Ciência e a Tecnologia (Portugal).
- The authors thank Swiss Light Source and the Max IV synchrotron Facility.