Oral presentation

Structures of bacillithiol and mycothiol disulfide reductases of bacilli and mycobacteria

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Bacilli and mycobacteria have both unique low-molecular-weight (LMW) thiols that play critical roles in the defense system to buffer the intracellular redox environment and counteract oxidative stress encountered by human neutrophils during infections. Bacilli species like the pathogen *Staphylococcus aureus* use the LMW thiol bacillithiol (BSH), while mycobacteria like *Mycobacterium tuberculosis* use the LMW thiol mycothiol (MSH) to buffer the redox balance. During this defense the BSH and MSH become oxidised to bacillithiol disulfide (BSSB) and mycothiol disulfide (MSSM), respectively. It is crucial to maintain the reduced pool of BSH/MSH and cellular redox balance, therefore these organisms use the NADPH-dependent FAD-containing oxidoreductase bacillithiol disulfide reductase (Bdr) to reduce BSSB to BSH and mycothiol disulfide reductase (Mtr) to reduce MSSM to MSH, respectively. We have solved the first structures of these enzymes (Fig. 1), the Bdrs from *Staphylococcus aureus* and *Bacillus cereus* [1], and the Mtrs from *Rhodococcus erythropolis* and *Mycobacterium smegmatis* [2]. Both have the low-molecular weight thioredoxin reductase fold [3], but Bdr are tetrameric instead of dimeric and the Mtr have the additional interface domain of *e.g.* gluthathione reductases. For Bdr the absence of a redox active cysteine in the vicinity of the FAD isoalloxazine ring implies a new direct disulfide reduction mechanism, which is backed by the presence of a potentially gated channel, serving as a putative binding site for BSSB in the proximity of the FAD cofactor. For Mtr structural analyses and docking calculations provide insight into the nature of Mtrs, with regard to the binding and reduction of the MSSM substrate. The putative binding site for MSSM suggests a similar binding to that described for the homologous glutathione reductase, but with distinct structural differences shaped to fit the bulkier MSSM substrate. As MSH and BSH have been acknowledged as attractive antibacterial targets, the structural findings presented in this work may contribute towards future antimicrobial drug development.

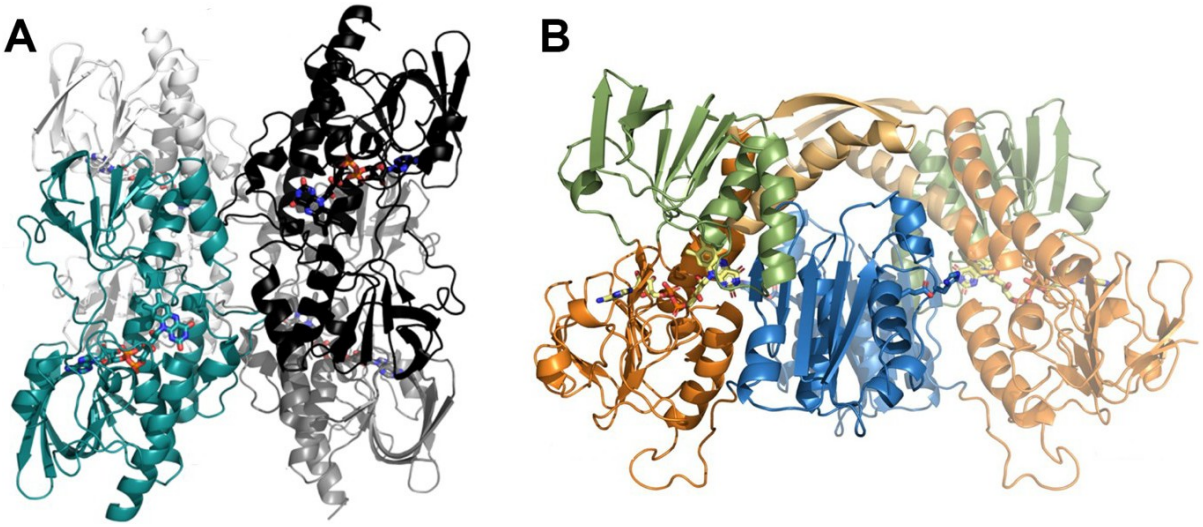

Figure 1. Structures of the Bdr tetramer (A) and the Mtr dimer (B).

[1] Hammerstad, M., Gudim, I. & Hersleth, H.-P. (2020) *Biochemistry* **59**, 4793-4798.

[2] Gutiérrez-Fernández, J., Hersleth, H.-P. & Hammerstad, M. (2024) *Acta Cryst*. D**80**, 181-193.

[3] Hammerstad, M & Hersleth, H.-P. (2021) *Arch. Biochem. Biophys.* **702**, 108826.