Poster Presentation

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Rubrerythrin from Trichomonas vaginalis- structural insights into its mechanism of action

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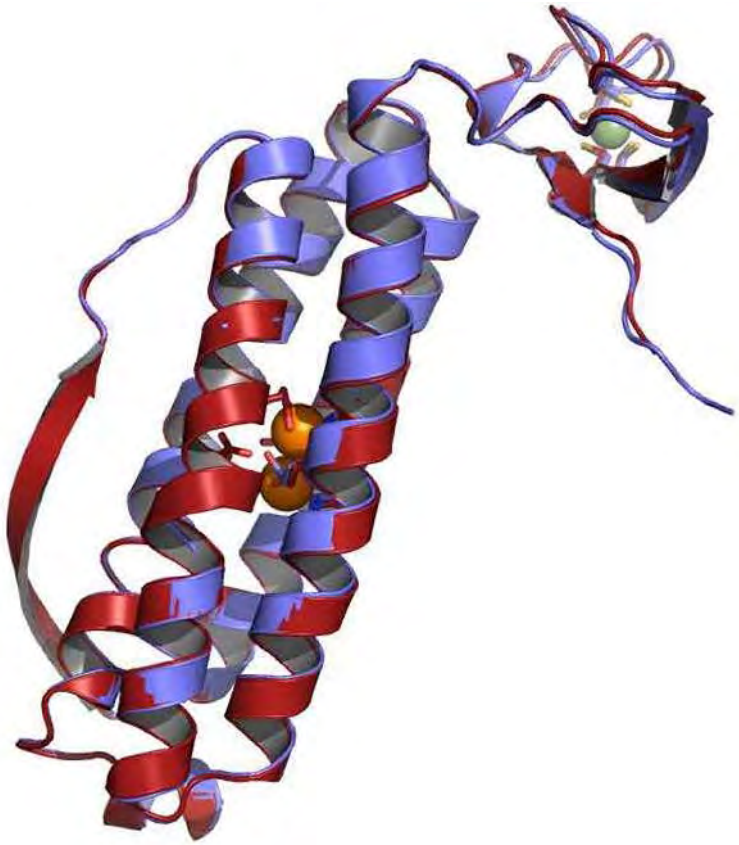
Trichomoniasis is a common STD with an estimated prevalence of 4 million infections in the United States. The organism that causes trichomoniasis, Trichomonas vaginalis, is essentially anaerobic and contains proteins such as peroxidases that help detoxify reactive oxygen species in its environment. A unique non-heme peroxidase called rubrerythrin, found in this organism, is homologous to bacterial peroxidases, and is markedly upregulated during oxidative stress. It could represent a target for therapeutic intervention given the importance of an anaerobic environment for this organism's survival. Using protein crystallography, we have determined the three-dimensional structure of two forms of this enzyme, the wild-type rubrerythrin which is reddish in colour and a mutant protein, T48A, which is purple. The genes were cloned and expressed in E. coli and purified using liquid chromatography. Crystallization was carried out using vapor diffusion methods. Following optimization, data sets were collected to 2.2Å at the Australian Synchrotron in Melbourne. The phase problem was solved using molecular replacement. Subsequent refinement in Space Group P21212 has yielded a structure with an Rwork/Rfree of 20.6 and 27.3 respectively. The resulting rubrerythrin structure is a dimer of four-helix bundles each containing two metal centers in a geometry and fold very similar to bacterial orthologs. Kinetic studies indicate that it can function as an efficient peroxidase, but only if all sites are occupied by iron. The key to the purple color of the T48A mutant rubrerythrin appears to involve the serendipitous formation of a charge-transfer complex involving the diiron site and a tyrosine, which is facilitated by this mutation. This is the first structure reported of a eukaryotic non-haem iron peroxidase. It is a potentially important virulence factor in T. vaginalis and will serve as a basis for further work to characterize its function within the organism.



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