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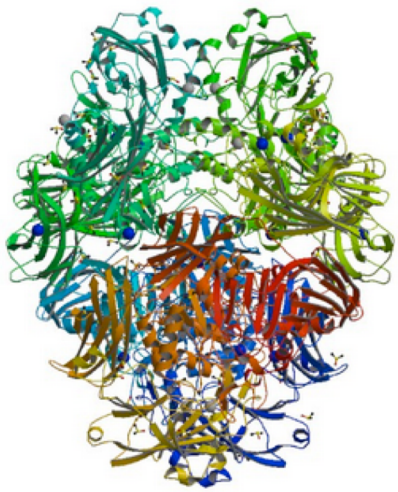
Optimizing metal acquisition by commercial metalloenzymes

"The BBSRC NIBB has brought me into contact with diverse researchers in the industrial sector, people who I would probably have never encountered through other channels." Kevin Waldron, Newcastle University

Kevin Waldron, Newcastle University
Stuart West, Biocatalysts Ltd

OUTCOMES: Three variants of magnesium-dependent galactosidase were selected for analysis. Because of the relative instability of complexes formed by magnesium compared to other transition metals, we hypothesised that it was likely that these enzymes could wrongly acquire a non-native, more stable complex-forming metal ion, either during heterologous expression or during sample preparation. Such association with the non-native metal cofactor is likely to lead to inhibition of the enzyme activity.
Our studies showed that small amounts of copper, zinc and nickel (but not iron) were present on the target enzyme in the preparations. The quantity of contaminating metals varied slightly between the longitudinal samples, and interestingly also varied between the three enzyme variants, suggesting possible differences in their metal-binding properties. Importantly, although the absolute quantity of these contaminating metals was low when expressed as a percentage of the total enzyme present (ranging from 3.8 — 12.8% occupancy), suggesting they would make only a minor diminution of the total enzyme activity of the enzyme preparations; a potential increase in enzyme activity of 4-12% would impact on profitability of commercial products.



Crystal structure of beta-galactosidase, PDB ID: 3SEP

INITIAL AIMS: Biocatalysts Ltd produce a number of metalloenzymes of commercial value through expression in bacteria and fungi. However, metal supply to these 'foreign' enzymes may not be optimal in these protein production hosts, so that a proportion of the commercial product is either bound to the 'wrong' metal, or lacks a bound metal ion altogether. Increasing the proportion of the enzyme that is correctly metal-loaded can directly increase profitability of metalloenzyme products. We will analyse the metal content of samples of the metalloenzymes produced by Biocatalysts.

- Although only a small amount of contaminating metals were detected, they reduce enzymatic activity – eliminating these contaminants would increase activity and thus profitability of these commercial enzyme preparations.