

P017 Initial characterisation of a temperature-adapted old yellow enzyme homologue

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An Old Yellow Enzyme homologue from a temperature adapted bacteria, *Thermoanaerobacter pseudethanolicus*, was found through a bioinformatic search. The gene codons were optimised for *E. coli* expression and synthesised commercially incorporating a His6-tag, thus allowing one step purification. The crystal structure of the Temperature-adapted Old Yellow Enzyme (TOYE) has been solved, at 1.6 Å resolution, showing both resemblance to other OYE homologues and characteristics of temperature adaptation. TOYE is reduced by both NADPH and NADH, with 2-cyclohexenone as an oxidising substrate. Circular dichroism (CD) studies indicate that the secondary structure is retained at temperatures up to 90°C. Due to this stability TOYE has great potential in biocatalysis, and studies of enzymatic H-tunneling.