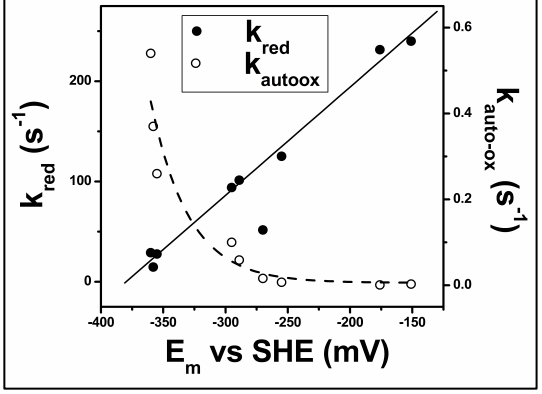
The active site of P450 BM3 – thermodynamic control, substrate recognition and catalytic coupling

Jonathan P. Clark*, Tobias W. B. Ost*, Caroline S. Miles[¥], Chris G. Mowat*, Simon N. Daff*, Greame A. Reid[¥], Stephen K. Chapman*

**EaStCHEM*, School of Chemistry, University of Edinburgh.
[¥]Institute of Structural and Molecular Biology, University of Edinburgh.

Figure 1. E_m vs. k_{red} and $k_{auto\text{-}ox}$



Cytochrome P450 BM3 from the soil bacterium *Bacillus megaterium* is one of the most commonly studied P450 enzymes. Multiple substitutions of two highly conserved active-site residues (Phe 393 and Thr 268) have been made.

Phe 393 exerts thermodynamic control over the heme, heavily influencing the heme reduction potentials. A series of substitutions have provided active enzyme exhibiting E_m values across a 200 mV range. Mutation of the phenylalanine to a histidine results in a positive shift (+ 140 mV) in the heme reduction potential in both the substrate free and substrate saturated state[1]. This modulation of the heme reduction potential has resulted in an increase in the rate of the first electron transfer (2-fold) and acts to stabilise the oxy-ferrous complex (50-fold). Thr 268, whilst not massively influencing heme reduction potential, does interfere with the substrate-induced spin-state shift and concomitantly with the customary substrate induced potential shift (150 mV). Substitution of the threonine with an asparagine leads to a spin-state shift of less than 10% and a ΔE_m of 10 mV. Analysis of the turnover products for Thr 268 substitutions reveals a substantial increase in uncoupling to peroxide highlighting its importance in maintaining an efficiently functioning enzyme. Comparison of the kinetic and thermodynamic parameters indicates a strong correlation between reduction potential and both k_{red} and $k_{auto\text{-}ox}$ (Fig. 1). More positive reduction potentials lead to enhanced rate constants for heme reduction and a more stable oxy ferrous species[1].

[1] Ost, T.W.B., *et. al.* (2003*) J. Am. Chem. Soc.* **125**, 15010