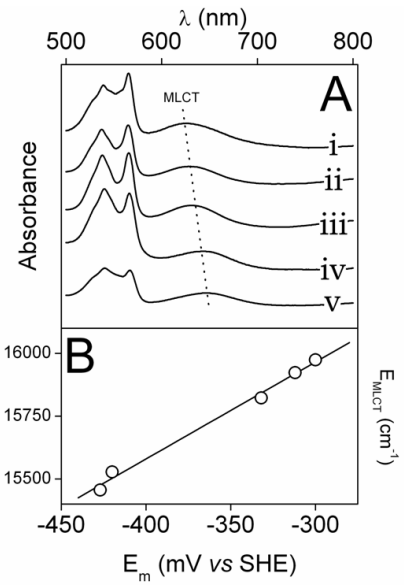
4-Cyanopyridine; a versatile probe for Cytochrome P450 BM3

J. L. Ross Anderson, Jonathan P. Clark, Tobias W. B. Ost, Lesley J. Yellowlees, Simon Daff, Stephen K. Chapman.

EaStCHEM, School of Chemistry, University of Edinburgh, Edinburgh, EH9 3JJ, UK.

Ubiquitous throughout the natural world, cytochromes P450 are a super-family of NAD(P)H-dependent, *b*-type heme-containing monooxygenases[1]. Catalytic regulation of these enzymes is critical in order to prevent the uncoupled consumption of NAD(P)H and it is known that for cytochrome P450 BM3, from *Bacillus megaterium*, the binding of substrate kinetically and thermodynamically controls electron transfer from NADPH to the heme. Two assays using the nitrogenous π-acceptor ligand, 4-cyanopyridine (4CNPY), have been developed in order to probe the thermodynamic parameters of this step[2].



4CNPY exhibits reversible ligation to reduced P450 BM3 *via* its pyridine ring nitrogen and is competitively displaced upon substrate binding. This competition allows a convenient route to the determination of substrate dissociation constants for ferrous P450 BM3 - previously unobtainable without the use of electrochemistry - highlighting an increase in P450 substrate affinity on heme reduction.

An unusual spectral feature in the red region of the visible spectrum of the reduced P450 BM3-4CNPY adduct is assigned as a metal-to-ligand charge transfer (MLCT). The energy of this MLCT band (E_{MLCT}) varies linearly with reduction potential (E_m) over a range of P450 BM3 mutants (E_{MLCT} = $(3.53E_m)$ + 17,500cm^{-1}) and allows a quick and accurate method for the prediction of heme reduction potentials without the need for redox potentiometry (*see figure*).

1. J. L. R. Anderson, S. K. Chapman, (2005), *Dalton trans*., **1**, 13.

2. T. W. B. Ost, J. P. Clark, J. L. R. Anderson, L. J. Yellowlees, S. Daff, S. K. Chapman, (2004), *J. Biol. Chem*., **47**, 48876.