

**P004** Conformational dynamics in the cytochrome P450 BM3/  
N-palmitoylglycine complex: a temperature-jump spectro-  
scopy study on the proposed proximal-distal transition  
**Sibylle Brenner, Sam Hay, Hazel M. Girvan,  
Andrew W. Munro, and Nigel S. Scrutton**  
*Manchester Interdisciplinary Biocentre and Faculty of Life  
Sciences, University of Manchester, 131 Princess Street,  
Manchester M1 7DN, United Kingdom*

The ferric spin state equilibrium of the heme iron was analysed in wild-type cytochrome P450 BM3 and its F87G mutant by using temperature (T)-jump relaxation spectroscopy and static equilibrium experiments. In the substrate-free enzyme no relaxation process was measurable indicating a relaxation process with a rate constant  $\gg 10\,000\text{ s}^{-1}$ . In contrast, a slow spin state transition process was observed in the *N*-palmitoylglycine (NPG)-bound enzyme species. This transition occurred with an observed rate constant (298 K) of  $\sim 800\text{ s}^{-1}$  in the wild-type, and  $\sim 2500\text{ s}^{-1}$  in the F87G mutant, which suggests a significant contribution of the phenylalanine side chain to a reaction step rate limiting the actual spin state transition. In accordance with results from X-ray crystallography, NMR studies, and molecular dynamics simulations, these findings are interpreted in terms of an equilibrium between different binding modes of the substrate, including a position 7.5 Å away from the heme iron (“distal”) and the catalytically relevant “proximal” binding site.