Effect of Iron charge state on P450 BM3 stability

P450s are a large family of heme bound monoxygenases that are capable of performing a variety of reactions such as hydroxylation, epoxidation, and carbon-hydrogen activation. These enzymes are well known for their role in human health, specifically steroid synthesis and xenobiotic metabolism. Despite the fact that P450s belong to a large and diverse family of enzymes, they all share a similar fold and utilize the same catalytic cycle. P450 BM3, a bacterial protein that hydroxylates medium to long chain fatty acids, can be used as a model system in order to study the stability of distinct phases of the catalytic cycle. Utilizing site directed mutagenesis we have engineered a BM3 pentuple mutant to act as model system for the more promiscuous human P450s. Biophysical techniques, such as pulse proteolysis and urea titrations monitored by absorbance and circular dichroism, were used. These techniques probe various aspects of the denaturation process, which is supported by results showing their differences in sensitivity to urea. Additionally, using these techniques, we are able to compare selectivity, promiscuity, and determine biophysical differences that arise due to the oxidation state and identity of the substrate or product. Initial results show substantial changes in P450 stability due to differences of oxidation state and substrates/products bound to the protein.