

P006 Reduction of diflavin enzymes by photoexcitable thiouredopyrene-3,6,8-trisulfonate (TUPS)
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Cytochrome P450 reductase (CPR) and nitric oxide synthase (NOS) are related to a number of diflavin reductases including methionine synthase reductase and the cancer-associated protein novel reductase 1. Previously, the mechanism of electron transfer in these two enzymes has been studied in detail by using stopped-flow kinetic and potentiometric methods, although direct identification of individual steps in the electron transfer mechanism is compromised by the multiple kinetic phases observed in stopped-flow studies. Hence, new methodologies are now required to facilitate the direct observation of interflavin electron transfer rates in these diflavin related enzymes. In the present work we have used laser flash photolysis methods to reduce the flavin molecules in CPR and NOS by using the efficient photoexcitable electron donor thiouredopyrene-3,6,8-trisulfonate (TUPS). The rapid formation of the neutral semiquinone species was monitored at 630 nm following laser irradiation at 355 nm for both enzymes. A subsequent decay in absorbance at 630 nm revealed that slower kinetic processes could be observed although further confirmation of the assignment of these steps is now required. Hence, we have now used the TUPS compound to develop a potentially new method to study the rate of interflavin electron transfer in the diflavin enzymes.