

## Pre-Steady State Product Formation Rates for Mutant and Wild Type Toluene 4-monooxygenases

*Abstract: This study measures the pre-steady state product formation rate for toluene 4-monooxygenase. T4MO catalyzes the NADH and O<sub>2</sub> dependent hydroxylation of toluene to form p-cresol. By using alternative substrates with altered benzene ring substitution patterns, the hypothesis that the chemical mechanism of hydroxylation by T4MO is an electrophilic aromatic substitution will be tested. T4moH has been expressed and purified with active site mutants to investigate the role of specific amino acid side chains during catalysis. Rapid chemical-mix quench was used to obtain the product which was analyzed with gas chromatography. The percent turnover for nitrobenzene and chlorobenzene was well below 100% in both the wild-type and T201A mutant. The percent turnover of both toluene and methoxybenzene in the wild-type and T201A mutant was almost 100%. It was observed that each alcohol group was added to the para position of each substrate and that the rate of product formation was higher in T201A than the wild-type.*

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