# Reaction mechanisms of non-heme diiron hydroxylases characterized in whole cells

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Whole cells, expressing metalloenyzmes of interest (AlkB, T4MO), were used to probe reaction mechanisms of selected non-heme diiron hydroxylases. AlkB catalyzes the hydroxylation of the radical clock substrates bicyclo[4.1.0]heptane (norcarane), spirooctane and 1,1-diethylcyclopropane, and does not catalyze the hydroxylation of the radical clocks 1,1-dimethylcyclopropane or 1,1,2,2-tetramethylcyclopropane. The hydroxylation of norcarane yields a distribution of products consistent with an "oxygen-rebound" mechanism for the enzyme in both the wild type *P. putida* GPo1 and AlkB from *P. putida* GPo1 expressed in *E. coli*. Evidence for the presence of a substrate-based radical during the reaction mechanism is clear. With norcarane, the lifetime of that radical varies with experimental conditions. Experiments with higher substrate concentrations yield a shorter radical lifetime (approximately 1 nanosecond), while experiments with lower substrate concentrations yield a longer radical lifetime (approximately 15 nanoseconds). Consistent results were obtained using either wild type or AlkB-equipped host organism using either "resting cell" or "growing cell" approaches. Toluene 4-monooxygenase (T4MO) expressed in *E. coli* also catalyzes the hydroxylation of norcarane with a radical lifetime of approximately 0.07 nanoseconds. No radical lifetime dependence on substrate concentration was seen. Results from experiments with diethylcyclopropane, spirooctane, dimethylcyclopropane, and diethylcyclopropane are consistent with a restricted active site for AlkB.