

# Enzyme Engineering for Biodegradation of Chlorinated Organic Pollutants

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## 1.0 OBJECTIVES

The focus of our program is the development of tailor-made biological catalysts for modifying or degrading halogenated compounds and other environmental pollutants. Our approach takes advantage of the tremendous chemical diversity of antibodies. Recently, this diversity and specificity have been merged with our understanding of chemical reactivity to generate a new class of antibody molecules-catalytic antibodies. This new technology makes possible the generation of antibody molecules that not only selectively *bind* but that *also chemically transform* virtually any molecule of interest. Because antibodies can be elicited to a huge array of biopolymers, natural products, or synthetic molecules, catalytic antibodies offer a unique approach for generating tailor-made enzyme-like catalysts with a desired selectivity. At the same time, the characterization of catalytic antibodies provides fundamental state stabilization, proximity effects, general acid and base catalysis, and electrophilic and nucleophilic catalysis and strain.

## 2.0 APPROACH

Our initial goal is to develop powerful new methods for screening or selecting catalytic antibodies from large antibody libraries. We are using as a model system the catalytic antibody 43C9, which hydrolyzes aryl amides. We have cloned and expressed this antibody under the arabinose promoter as a chimeric Fab. Induction leads to the expression of 10 to 100 mM levels of antibody in the periplasm. An amide derivative of p-aminobenzoic acid (PABA) has been synthesized. Hydrolysis of the amide bond by antibody 43C9 will lead to the release of PABA that will complement an aro C *E. coli* auxotroph. Mutants of 43C9 generated by *in vitro* mutagenesis methods can then be selected based on this growth selection. The requisite aro C mutant has been generated and background growth rates on PABA and the amide derivative have been measured. We are currently generating libraries of variable region mutants by DNA shuffling. We are also synthesizing an amide derivative of the substrate that when hydrolyzed will release indigo, providing a chromogenic assay of catalytic activity that can be used in a plate screen of libraries of mutants.

If these experiments are successful they will provide a general strategy for evolving protein catalysts with a broad range of specificities and activities. We then will directly apply these approaches to the generation of antibodies that catalyze the hydrolysis of halogenated aromatics. In this case we will use an antibody that binds halogenated nitrobenzene but that currently lacks hydrolytic activity (such an antibody is in hand). We will generate libraries of variable region mutants and select or screen for those with hydrolytic activity using the approaches described above.