Structure and function of fumarate-adding glycyl radical enzymes: biochemistry, modelling and application (*FAEREACTION*)

A remarkable biochemical reaction discovered in anaerobic bacterial hydrocarbon degradation pathways is the enzymatic formation of a new C-C bond between the hydrocarbon and a fumarate cosubstrate to yield a stereochemically defined hydrocarbon-succinate adduct. The first reported example of this unusual reaction was addition of toluene and fumarate, which is catalyzed by the glycyl-radical-enzyme benzylsuccinate synthase (BSS). This reaction represents a new biochemical principle, and in recent years BSS has become a model for many other similar enzymes catalyzing analogous radical-type fumarate additions in anaerobic metabolic pathways of other hydrocarbons, including alkanes.

In the *FAEREACTION* project will investigate the biochemistry and reaction mechanisms of the whole class of these new bacterial enzymes, which are called fumarate-adding enzymes (FAE). The FAE are highly oxygen-sensitive and need to be activated to the radical state by special activating enzymes. Moreover, many bacteria producing them for their metabolic needs grow very slowly and produce very small amounts of these enzymes. Because of all these factors, FAEs are very difficult objects to study which to date significantly hindered research progress, despite their high importance in bioremediation of polluted environments and their general contribution to the global carbon cycle. We undertake this project now, based on our recent development of a working system for recombinant production of BSS which can be adapted to produce other FAE in their activated state and allows access to studying their biochemistry for the first time.

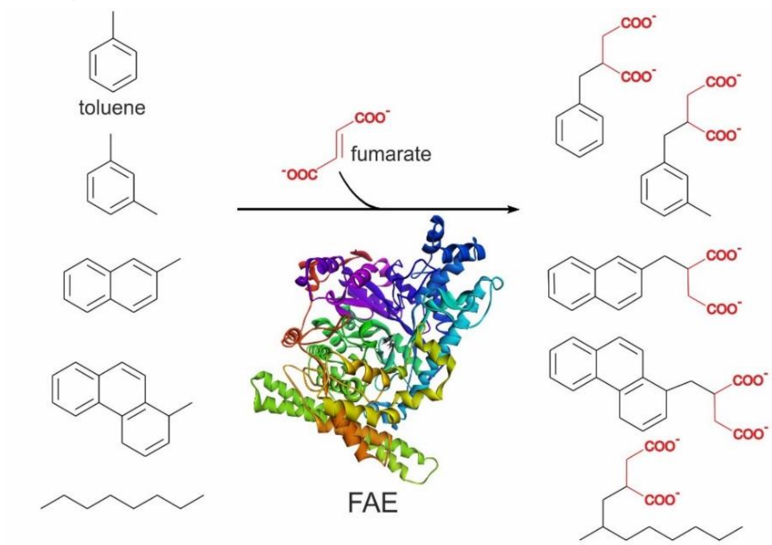

Fig. 1 The reactions catalyzed by different FAE

In the proposed project, two closely collaborating groups of scientists from Germany and Poland will employ directed mutagenesis to identify the functions of particular amino acid residues in the enzymatic active sites and extend the available product spectra of FAE. The project will allow characterization of novel FAE reacting with different substrates and provide opportunities for their structural characterization by means of X-ray crystallography. It will also enable *in-vitro* studies of the FAE activation processes by activating enzymes. Investigations of the reaction mechanism will be conducted with multiscale high-level computational techniques (simulations of dynamics and QM:MM calculations) backed up with experimental input. Finally, we will aim at the development of biotechnological production methods of potential novel high-value compounds synthesized by using FAE as biocatalysts in recombinant whole-cell systems.

We expect that recombinant bacteria will be able to produce chiral chemicals that may find future applications in the synthesis of pharmaceuticals, as well as monomers for the production of biodegradable bioplastics. The results of our study will also improve our current understanding of bioremediation processes of environmental contaminations caused by fossil fuel and chemical industries and will deliver fundamental biochemical data which may improve the use of microorganisms in bioremediation of hydrocarbons. Finally, the elucidation of mechanisms for the whole class of FAE will provide new insights into a novel biochemical reaction principle exploited by these enzymes.