Prof. Ioulia Smonou

Biocatalysis-Biotransformations in Organic Synthesis
Asymmetric synthesis of bioactive compounds using biocatalytic methods.

Research Interests

The focus in our research is on the applicability of biocatalysts in organic synthesis. Moreover, our group has been interested in mechanisms of enzymatic reactions, reactive intermediates as well as stereoselective reactions.

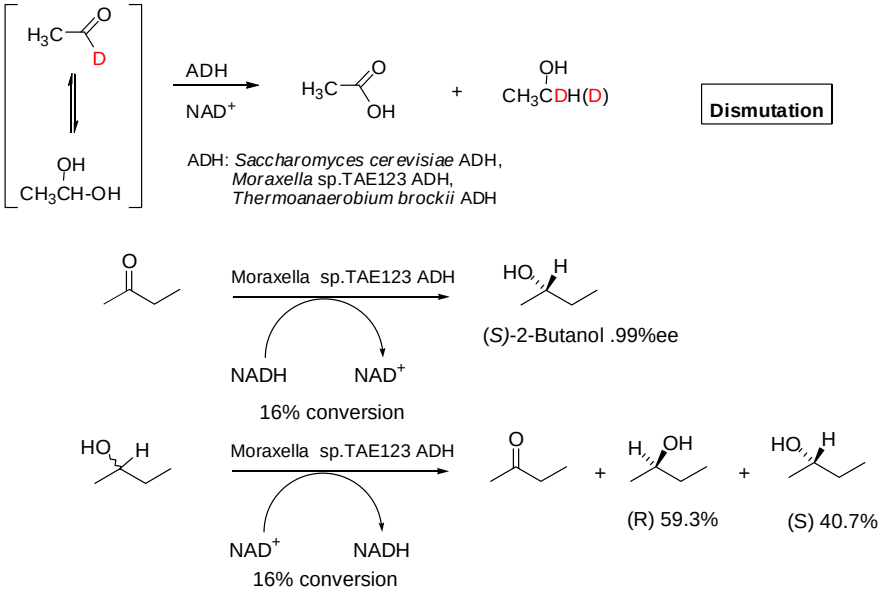
Based on biocatalytic methods, we design chemoenzymatic syntheses of natural products and other fine chemicals. Biocatalysts have unique characteristics when compared with chemical (homogeneous and heterogeneous) catalysts. The most special features that distinguish biocatalysts from chemical catalysts are: *Selectivity, Safety of the reaction, Natural catalysts, Catalyst preparation, Large scale synthesis and space-time yield.*

Using isolated enzymes as catalysts for organic reactions is becoming a more standardized and practical tool in the hands of synthetic chemists, mainly because of the increasing need for environmentally benign methods, the commercial availability and low price of purified enzymes.

1) Earlier work in our group has been devoted in the determination of the limits of stereoselectivity of **alcohol dehydrogenases** in order to extend their synthetic applications as well as to understand better their mechanisms. In our comparative studies we were using enzymes from mesophiles, thermophiles and psychrophiles. In collaboration with the Institute of Molecular Biology and Biotechnology, University of Crete, we have purified and characterized the first NAD^+-dependent alcohol dehydrogenase from Antarctic psychrophilic bacterium, which shows quite attractive substrate specificity.

Investigation of the stereochemistry of hydride transfer in reactions catalyzed by this enzyme- Moraxella sp. TAE 123-, showed that it catalyzes stereospecifically the transfer of the pro-R hydrogen from the pyridine 4-position of the reduced coenzyme

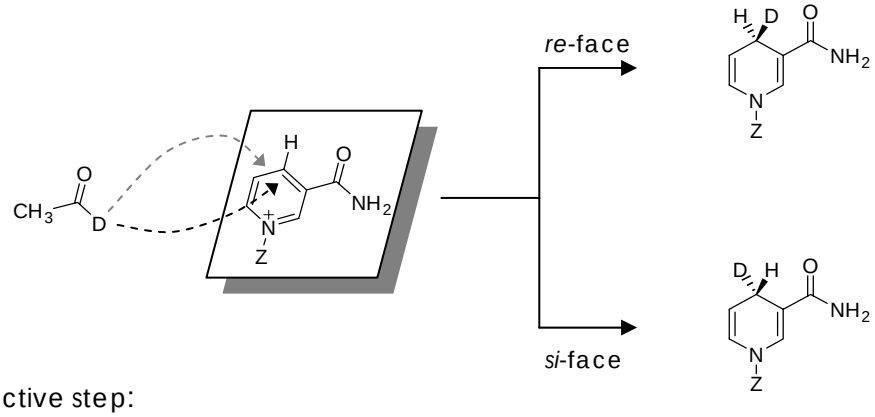
to the re-face of the carbonyl substrate, i.e., it is a type-A dehydrogenase,



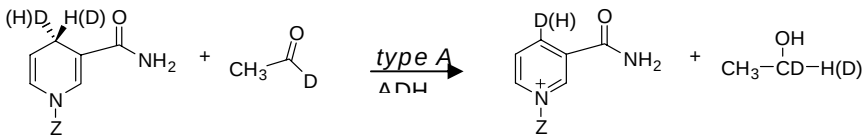
and produces the (S)-alcohol with high enantiospecificity 99%ee.

We have also studied the dismutation reaction of aldehydes catalyzed by three different ADHs (YADH, TBADH and Moraxella spTAE 123) which belong to different ADH families and exhibit different substrate specificity and kinetic behavior. We were interested on the stereospecificity of hydride transfer during the dismutation of acetaldehyde catalyzed by these ADHs .

(a): Oxidative step:



(b): Reductive step:

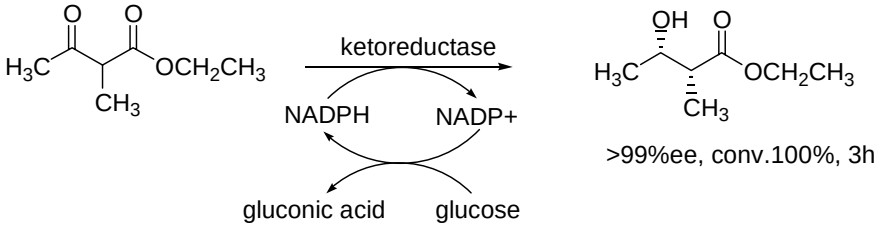


(a) Possible stereochemical outcome of hydride transfer to the re- or si- face of NAD+ during the oxidative step of the ADH catalyzed dismutation of acetaldehyde-1-d1. (b) Type A ADH stereoselectivity expected on the reductive step of the dismutation.

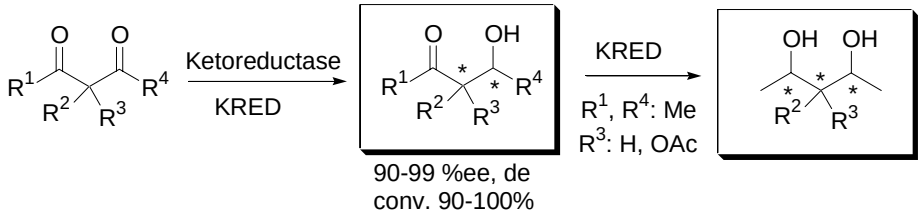
The stereochemistry of the oxidation of aldehydes to acids was studied with alcohol dehydrogenases with respect to the selectivity towards the cofactor.

2) **Hydrolases** have become key parts in the growing area of industrial biotechnology covering the major part in the modern synthetic applications. Our extensive studies on the specificity and stereoselectivity of Ferulic acid esterase from Humicola insolens (FAE) have shown that its is a suitable biocatalyst for the kinetic resolution of various secondary alcohols. FAE was found to catalyze transesterifications of secondary alcohols with high enantioselectivity. In all cases the enzyme showed R enantiopreference. Our research with lipases and esterases includes mechanistic as well as synthetic studies. We are investigating the use of these enzymes in asymmetric synthesis of optically active carbonyl compounds, which are useful precursors in synthesis of many interesting natural products. It is our intention to investigate further new areas of applications of hydrolytic enzymes focusing in the formation of new carbon-carbon bonds.

3) Our most recent work has been devoted in the synthesis of optically active keto alcohols or hydroxy esters by using **ketoreductases** in a **Dynamic Kinetic Resolution** scheme.



The biocatalytic reduction of α-alkyl-1,3-diketones and α-alkyl-β-keto esters employing 1 of 20 different isolated NADPH-dependent ketoreductases proved to be a highly efficient method for the preparation of optically pure keto-alcohols or hydroxy esters.



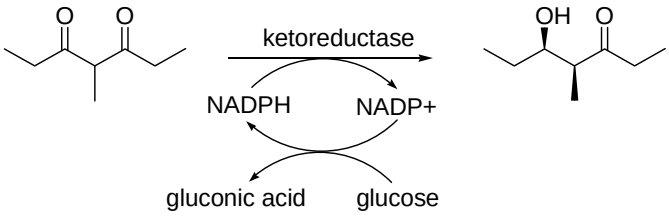
Regio and stereoselective reductions of α-substituted 1,3-diketones to the corresponding β-keto alcohols or 1,3-diols by using commercially available ketoreductases (KREDs) has been succeeded. A number of alpha mono- or di-alkyl substituted symmetrical as well as non-symmetrical diketones were reduced in high optical purities and chemical yields, in one or two enzymatic reduction steps. In most cases, two or even three out of the four possible diastereomers of α-alkyl β-keto alcohols were synthesized by using different enzymes. These enzymatic reactions provide a simple, highly stereoselective and quantitative method for the synthesis of

different diastereomers of valuable chiral synthons from non-chiral, easily accessible 1,3-diketones.

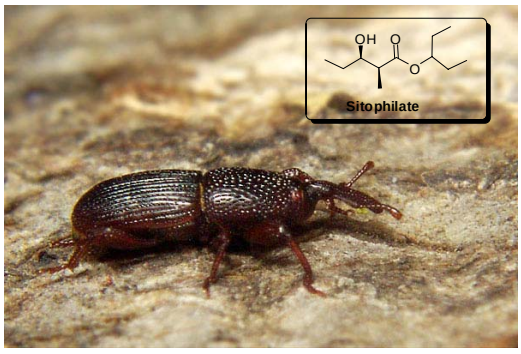
Application of Biocatalysis in the Synthesis of Insect Pheromones

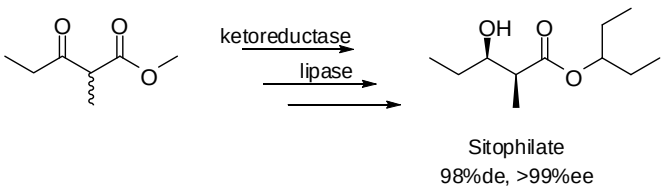
Another major area of investigation is to develop useful biocatalytic transformations for applications in organic synthesis.

1) Isolated, NADPH-dependent ketoreductases were used for the synthesis of the aggregation pheromone of the pests rice weevil (*Sitophilus oryzae* L.) and maize weevil (*Sitophilus zeamais* M.). This is the easiest and most straight forward synthesis of pheromone (+)-Sitophilure in two steps and overall yield 81%, starting from commercially available 3,5-heptanedione.



2) Two stereoselective enzymatic reactions, a ketone reduction followed by an ester hydrolysis, were the key steps for the synthesis of Sitophilate in good isolated yield and excellent chemical and optical purity. An isolated NADPH dependent ketoreductase (KRED-EXP-A1B) and an isolated hydrolase (ICR-112) were used for the synthesis of Sitophilate, the aggregation pheromone of the granary weevil *Sitophilus granarius,* in high optical (98% de, >99% ee), and chemical purity (>99%), with overall yield 63%. The starting materials for this synthesis involved non-chiral and readily commercially available chemicals, such as methyl 3-oxopentanoate. Every reaction in this synthesis can be easily scaled to larger amounts, thus an efficient method for the synthesis of this natural pheromone is provided.





Sitophilate
98%de, >99%ee