

Bacterium *Aromatoleum aromaticum* is an extremely versatile microorganism, able to live both in aerobic and anaerobic conditions. Genetic research has demonstrated that this bacteria can „eat” (i.e., use as a source of carbon and energy) a wide range of quite exotic and toxic for most organisms substances, such as toluene, ethylbenzene, p-cresol, acetone, benzoic acid, phenol or aromatic amino acids. While under the aerobic conditions, *A. aromaticum* uses typical enzymes known from other aerobic organisms, under anaerobic conditions it has to manage in a much more original way. Most methods of hydrocarbons metabolism involve their oxidation with O₂. In such reactions, the energy necessary for life is generated as well as the valuable chemical building blocks (such as acetyl-CoA) are formed, which are crucial for the synthesis of important cell components (such as fatty acids, amino acids, nucleic basis, sugars, cofactors). Although the oxygen molecule itself is not very reactive, there are special enzymes that can activate it and carry out the oxidation reaction in a very effective way. Such enzymes, often using an iron atom, a heme or flavin cofactor, are widespread in all life forms (from bacteria to animals and humans).

Under anaerobic conditions, the situation is much more complicated, because one cannot use the commonly available oxidant on our planet - O₂. That is why the bacteria that live in such conditions have developed completely different catalytic strategies (or they have retained the old good ways from the times when completely different conditions existed on Earth). Such enzymes also catalyse oxidation reactions of organic compounds, providing bacteria with energy and building blocks, but often contain special cofactors in their active centre capable of retrieving an oxygen atom from water and thanks to redox reactions (electron transfer) they are able to transfer it to a hydrocarbon molecule.

One of such solutions enabling such reactions to be carried out are so-called molybdenum or tungsten metallopterin cofactors. These are quite complicated complexes of the Mo or W atom, placed inside the protein, whose chemical properties facilitate the two-electrons redox reaction needed to convert the oxygen atom from the H₂O molecule into the much more reactive ligand Mo=O or W=O. As a result these cofactors are able to activate the hydrocarbon and transfer oxygen atom to it under anoxic conditions.

The subject of the project is a tungsten-containing aldehyde oxidoreductase (AOR) - an enzyme oxidizing aldehydes to carboxylic acids under anaerobic conditions. In the redox reaction, oxygen atom comes from the water molecule, and electrons, extracted from the aldehyde substrate, are transported along ‘molecular wire’ (composed of iron-sulphur centres) to a special protein used to carry electrons – ferredoxin. The tungsten atom is the heaviest element found in living organisms and little is known about the biochemistry of tungsten cofactor, despite the fact that its structure was examined more than 20 years ago. Also in the case of the AOR enzyme, its physiological role in *A. aromatoleum*, the mechanism of catalytic reaction or even exact structure of the enzyme and its cofactor are not very clear. In the project I intend to take a closer look at the AOR enzyme, check which substrates at which rate are oxidized, examine in detail the kinetics of the reaction in function of the temperature, pH, concentration of substrates and enzyme reoxidants (which in the laboratory replace natural ferredoxin). I will also try to obtain the AOR crystal and solve the exact structure with the X-ray diffraction methods, so that ultimately it will be possible to employ the most modern computational methods and capacity of the Polish supercomputer network PL-Grid to explore the catalytic secrets of this enzyme.

Exploring these secrets is extremely important for our understanding of bacteria metabolism, which to some extent clean up after our civilization, meticulously metabolizing oil spill under gas stations and refineries, cleaning the riverbeds and lakeshores from toxic industrial products, or cleansing urban sewage. What is more, atypical catalytic strategies used in anaerobic conditions sometimes turn out to be extremely interesting for the biotechnology industry, in particular in the synthesis of expensive compounds for the production of drugs. But first of all, exploring these strange enzymes is a very interesting challenge - in a sense, it gives a opportunity to study ‘alien life forms’ – microbes like not from this planet, without the need for a space flight to other stars.