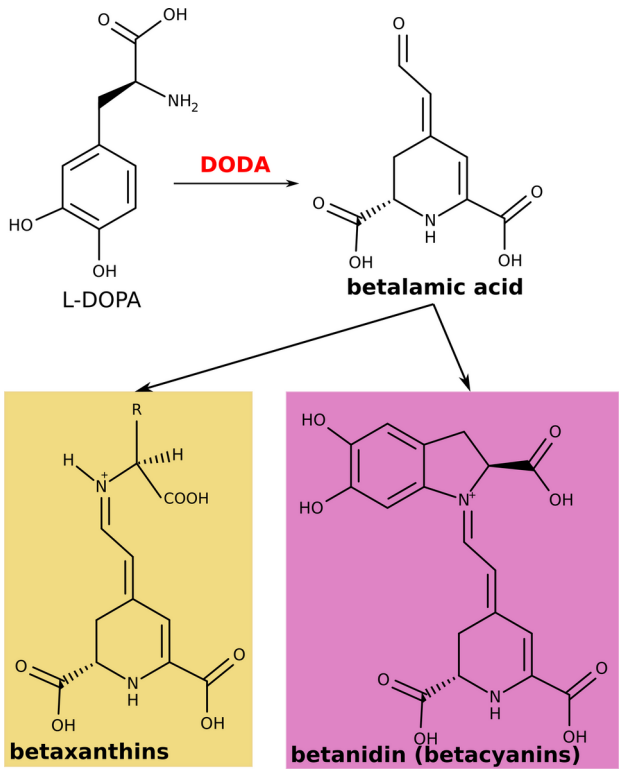
Mechanisms of cyclization reactions leading to biologically active compounds catalyzed by iron dependent enzymes.



Cyclic natural products are very common in Nature and many of them have valuable biological and medicinal activities. These cyclic compounds are synthesized or cleaved with the help of various natural catalysts, i.e. enzymes, many of which belong to a class of iron-dependent enzymes. Since still many of such enzymes have not been studied thoroughly, chemical "tricks" used by them to produce their valuable products are not understood, yet such knowledge would be very helpful, e.g. for designing new catalysts for production of novel compounds or cleaning the environment from recalcitrant pollutants. This project focuses on selected iron-dependent enzymes that participate in ring formation and ring cleavage reactions leading to valuable chemicals. One example is the DODA enzyme that catalyzes transformation of L-DOPA to betalamic acid, which is the bioactive core of betalain pigments (see Figure). Betalains are produced by some plants and fungi and they fall into two major categories: yellow betaxanthins and violet betacyanins. Betalains play protective roles in plants but also display various health-promoting properties in animals, including antitumor and life-prolonging activities. It is currently supposed that these properties are due to the high antioxidant activity of betalamic acid and its derivatives.

Our research aims include determining 3D atomic structures of these metalloproteins with the use of X-ray diffraction methods and thorough analysis of reaction mechanisms by a combined experimental and computational approach. More specifically, we will use stopped-flow techniques to identify reaction intermediates and to determine reaction rates for elementary steps. Reaction intermediates will be characterized with the use of Mossbauer spectroscopy, which is a technique selective for iron capable of providing valuable information on oxidation state, electronic structure and geometry around the metal ion. Interpretation of experimental data will be enhanced by computer simulations, whereby structures, energies and spectral properties are computed by methods rooted in quantum mechanics. Moreover, we will try to obtain stop-motion pictures of the reaction by means of time-resolved synchrotron crystallography methods. We will use the knowledge gained in this way to rationally redesign enzymes so as to change their selectivity.

It is expected that the results obtained within this project will provide a detailed atomic picture of the studied cyclization or ring cleavage reactions, which will form a groundwork for further exploitation of cyclic compounds and enzymes producing or cleaving them.