DNA molecules as enzymes – how do they do it?

For a biochemist a DNA molecules is the synonym of stability and inertness. Nothing unexpected given the fact that it was developed by nature for one goal – long-term storage of genetic information. Without the risk of degradation or uncontrolled changed. Consequently, the idea of DNA acting as a catalysis – a facilitator of chemical reactions – sounds, at first glance, like a misconception. The role of enzymes (biological catalysts) was bestowed by nature upon proteins and, as it was proven in the 80s, in a limited scope also upon RNA molecules. It was the discovery of RNA catalysis that initiated the search for catalytically active molecules also among DNAs. Even though the existence of catalytic DNA in nature remains an open question even today, over the years many such molecules were created artificially in the laboratory, through a process known as *in vitro* selection. This methodology is a fascinating topic by itself. It relies on the combinatorial synthesis of a wide spectrum of random DNA sequences (in an ideal case of *all* the possible DNA molecules of a given length) followed by "fishing out" of those sequences that are able to catalyze the desired transformation. The design of the crucial selection step depends on the specific reaction for which the catalyst is sought and is beyond the scope of this text. The characteristic of in vitro selection most important for the current project is the fact that even though is able to pin point DNA sequences endowed with catalytic properties, it provides no information on which characteristics of a selected DNA sequence are the reason for its catalytic activity or on what is the mechanism of catalysis.

If DNAzymes (DNA enzymes) were to remain just a laboratory curiosity, one could perhaps overlook our current lacking in understanding of these molecules. Somewhat paradoxically though, the mentioned stability and inertness of DNA make enzymes constructed of this material promising candidates for applications in biotechnology, in industry and even in therapy. However, for this to happen the capability of rational design of catalytic DNA molecules is required. This sparks the need for their deep structural and functional understanding. Even though many studies in this direction were performed over the years, only very recently the first two crystallographic structures of catalycally active DNAs were solved. They have demonstrated that DNA enzymes exert their function as highly structured molecular machines, much more intricate than the previous partial data suggested. Two structures however constitute just a small drop compared to the overall number of DNAzymes identified so far.

The current project aims at considerably extending the amount of high-resolution structural information available for DNAzymes, through structural studies of two important DNA enzymes and feasibility test for similar studies for a range of other DNAzymes. The two main targets of the proposed research are the DNAzymes referred to as 8-17 and I-R2. The first of the two catalyzes site-specific cleavage of RNA strands, while the second catalyzes its own site-specific hydrolysis. For both molecules high-resolution structural studies will be performed using nuclear magnetic resonance (NMR) spectroscopy. NMR constitutes the only technique allowing for such studies to be performed in solution, that is the phase in which the DNAzyme activity actually takes place.