

Research Plan – Bálint Hajdu

The goal of our experiments is to design new types of artificial nucleases that can specifically recognize, bind and cleave DNA for various purposes. Zinc finger proteins are modular specific DNA binders that can be designed to recognize any desired DNA base sequence. As such, they can control the function of genes, but they also can be turned into specific DNA manipulating agents. A hydrolytic method by means of which we can specifically cleave proteins using nickel(II) and copper(II) ions results in square planar metal complex at the N-terminus of the C-terminal cleavage product. The latter complex is able to cleave DNA in the presence of oxidants. Based on these facts, the combination of the two experiments will, according to our expectations, lead to the development of in situ prepared specific nucleases. For this, we need to establish optimal conditions of the specific cleavage of the zinc finger proteins both in the absence and presence of DNA. During my research stay in Warsaw, in the laboratory of Professor Wojciech Bal from the 25th of July till the 5th of August I will perform experiments on this topic.

1. Previously we demonstrated that the hydrolysis of the 1MEY# zinc finger protein by nickel(II) ions is specific, and the formed complex binds one nickel(II) and three zinc(II) ions. Furthermore the incubation doesn't harm the protein's structure, it is still able to recognise its DNA target sequence. We elaborated a test system using GGH-Cu(II) and -Ni(II) model peptides to study the DNA cleavage of the square planar metal complex. The results showed that GGH-Cu(II) is much more effective although in the presence of Cu(II) the zinc finger's structure collapses so this complex can't be achieved using zinc finger proteins.
2. During my stay I will be studying the hydrolysis of the 1MEY# protein with Ni(II) in the presence of small specific and nonspecific oligonucleotides. To follow the interactions I combined electrophoretic, and ESI-MS methods. As another part of our project I will demonstrate some DNA cleavage experiments with model peptides to optimise the reaction conditions. Furthermore I am going to examine the interactions of the 1MEY#-Ni(II) with DNA to find out if the former complex is able to cause specific DNA cleavage.
3. It has to be emphasized, that in a case of success of our research the results will lead to a platform that will allow us to construct a library of ANs based on the already available knowledge of the zinc finger design. The resulting ANs will be able to introduce a specific cleavage at any chosen DNA target sequence. An enzyme with such property may find extensive applications i.e. in molecular biology research, or in various

biotechnological processes. Moreover, such ANs may be used for the targeted disruption of genes e.g. in cancer cells. This raises the prospect of applications in diverse fields.