

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 20th till the 25th of June 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. Although the protein hydrolysis was successful the 1MEY#-Ni(II) complex's DNA cleavage activity was not detectable. 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 and the results showed that GGH-Cu(II) is much more effective so we must try to form the copper complex of the zinc finger protein as well.
2. During my stay I will be studying the Cu(II) induced hydrolysis of the 1MEY# protein. To follow the interactions I combined electrophoretic, HPLC, and ESI-MS methods. As another part of our project I will demonstrate some further DNA cleavage experiments to optimise its conditions.
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.