

Research – 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 by nickel(II) ions – results in a square planar metal complex at the N-terminus of the C-terminal cleavage product. The latter complex is able to cleave DNA. 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 the 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 Prof. Woiciech Bal between 3-14 December, 2014 I was performing experiments on this topic.

1. In the first two days during my research stay in at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences I participated in a conference&workshop which was organised by Prof. Woiciech Bal and Nano Temper Technologies. This occasion was held to demonstrate the efficiency of a rather new promising method the Microscale Thermophoresis(MST). The event was special because of the participants could try this technique with their own samples in various instruments provided by the company. My experiments with the machines are quite good I could obtain clear reproducible data for protein – zinc(II) titrations with fluorescently labelled and non-labelled methods.
2. I also elaborated a test system and precise protocol to study the specific cleavage of the 1MEY# zinc finger protein - which is modified to contain only one cleavage site available - by nickel(II)ions in the presence of DNA, according to the project plan for the first year of the collaboration. Above at all I wanted to optimise the methods and finally achieved suitable system conditions. To study the interactions I combined electrophoretic, HPLC, CD and MS methods.
3. We also observed changes in the DNA substrate, but to clear up this system is the task of further experiments. It has to be emphasized, that in case of the success of our research the results will lead to a platform, that will allow 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.