THESES OF DOCTORAL (PH.D.) DISSERTATION

Investigation of metal ion and DNA binding of zinc finger proteins, modification by Ni(II)-induced peptide hydrolysis

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I. Introduction and objectives

Metal ions play a crucial role in living organisms, for example in skeleton formation, signalling, enzymatic processes, and electron transport. Zinc is one of the most abundant essential trace elements. Due to the saturated *d* subshell of Zn^{2+} ion, it forms complexes with various coordination numbers and geometries. It does not participate in redox reactions, while it is a strong Lewis acid. Therefore, Zn(II) can be found in the active centre of numerous hydrolytic enzymes. There are also a large number of proteins where Zn(II) participates in stabilizing the functional structure by coordinating to the side chains of cysteine and histidine amino acids, usually in a tetrahedral geometry.

Zinc finger proteins contain such structural zinc binding sites, among them the classical Cys_2His_2 type fingers can specifically recognize DNA target sequences. Their biotechnological significance is provided by the fact that a zinc finger unit recognizes three nucleobases, but several units can be fused together, thus the specificity can be increased (**Figure 1 a,**). Due to their modular structure, zinc finger proteins can be redesigned, so the recognized DNA sequence can be modified relatively easily. As a result, they were applied as DNA recognition domains in the first artificial metallonucleases. Beside nuclease development, researchers focuses on the investigation of natural zinc finger proteins with various endogenous and exogenous metal ions as well. Such metal ions can reduce, cease or modify the original function of the proteins, which can significantly affect the viability of cells. The toxic soft metal ions such as Ag(I), Cd(II), Hg(II) pose the highest risk, but the literature on the interaction of zinc finger proteins with these metal ions is rather sparse and there are many contradictions. Therefore, it is not surprising that although the first member of the protein family was discovered in 1985, natural and artificial zinc finger proteins are still an important research field.

The investigation of interactions of further metal ions with peptides revealed that Ni(II) and Cu(II) promote selective peptide bond hydrolysis before an (S/T)XH sequence motif (**Figure 1 b,**).

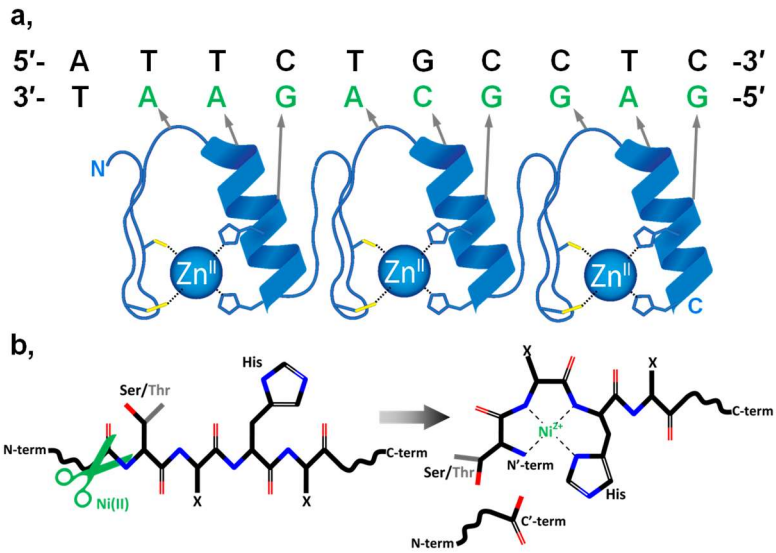

Figure 1. Schematic representation of **a,** a three-subunit zinc finger protein and its DNA recognition. **b,** the Ni(II)-induced peptide bond cleavage at the X(S/T)XH amino acid sequence.

Many human proteins contain the sequence required for the hydrolytic cleavage, including zinc finger proteins. This observation may be one explanation for the toxicity of Ni(II). In addition, this type of selective hydrolysis is also interesting from chemical point of view, since Ni(II) can replace enzymes that are otherwise required for the sequence-specific cleavage of proteins. Based on this background knowledge, I tried to answer the following questions during my doctoral work:

1. Is Ni(II)- or Cu(II)-induced selective peptide hydrolysis feasible in a zinc finger protein?

My initial goal was to study how the hydrolysis with the above mentioned two metal ions takes place in zinc finger proteins, and what is the consequence of the process on the structure of the zinc finger subunits and on the properties of the zinc finger protein. I compared the reaction with the hydrolysis carried out using a protein-based protease enzyme. I also examined whether the complex formation properties of

the ATCUN motif and the zinc finger subunits can be distinguished in the reaction product.

2. What is the relationship between Zn(II) and DNA binding of an artificial zinc finger protein, model peptides and natural zinc finger proteins?

In the literature, the Zn(II)-affinity of the single zinc finger subunits were investigated widely, but only few data are available on zinc finger proteins consisting of more than one fingers. Therefore, it is difficult to compare the data. Quantitative interpretation of the DNA binding of natural zinc finger proteins is also challenging. I aimed to compare the thermodynamic properties of the zinc finger motifs published in the literature and the artificial 1MEY# zinc finger protein that I purified. Furthermore, I planned to investigate the affinity and specificity of this three-subunit zinc finger protein toward DNA sequences, and to compare these results with the DNA-binding properties of natural zinc finger proteins. I aimed to find relationship between the protein's DNA and Zn(II) binding.

3. How do zinc finger proteins interact with toxic metal ions such as Ag(I), Cd(II) and Hg(II)?

Several contradictions can be discovered in the literature regarding the competition between zinc finger proteins and toxic metal ions. My aim was to study these interactions thoroughly from a qualitative and quantitative perspective, especially focusing on the effect of the DNA target sequence.

4. Can Ni(II)-induced selective peptide hydrolysis be applied as a protein purification method to generate native amino acid sequences?

Affinity tags are frequently applied in protein purification processes, but the subsequent removal of this tag is often essential in order to obtain the native protein with the exact sequence for further studies. My goal was to investigate whether Ni(II) can be used for this purpose instead of much more expensive proteases, and to optimize the application of such a process in solution or directly on the affinity resin.

5. Can Ni(II)-induced peptide hydrolysis be applied in the allosteric regulation of enzymes?

My aim was to studied whether Ni(II)-induced peptide bond hydrolysis can be used to regulate enzymes in case where only the protein with the native sequence is functional, i.e., the affinity tag influences the enzyme activity. If so, is this regulatory mechanism purely allosteric, or coordinative properties may also play a role in the process.

6. Can the Ni(II) or Cu(II) complex of zinc finger proteins be used as a specific artificial DNA-cleaving enzyme?

One of my goals was to investigate whether the DNA-cleavage induced by an ATCUN-zinc finger protein presented in the literature can be reproduced using proteins hydrolysed by Ni(II) or Cu(II). Furthermore, I planned to analyse the activity and specificity of such enzymes, and the influence of the linker amino acid sequence between the ATCUN motif and the zinc finger subunits on their properties.

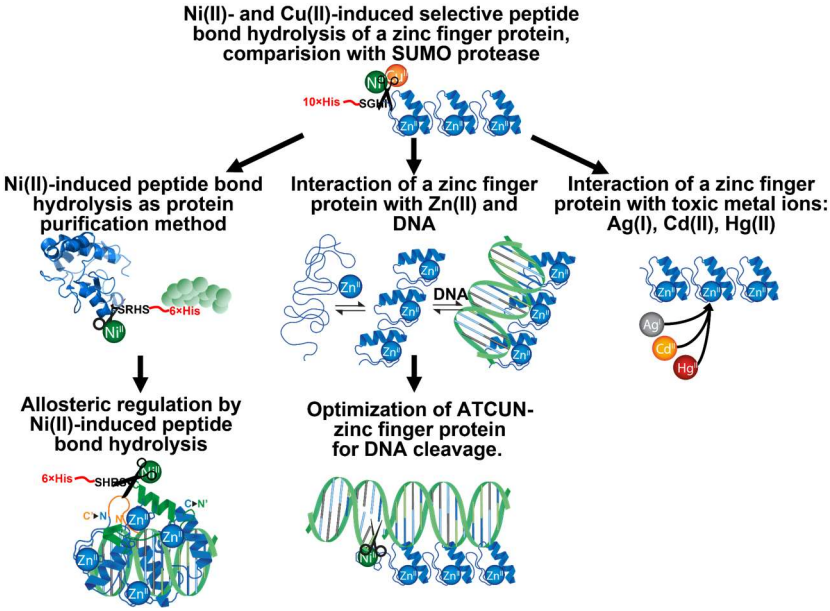

Figure 2. Schematic representation of the main aims and objectives of my doctoral thesis.

II. Experimental methods

The bacterial DNA carriers and genes of the proteins used in our experiments were established using recombinant DNA technology. The proteins were expressed in *E. coli* bacterial cells and batch or HPLC Ni(II)-affinity purification methods were applied for their purification. The purification steps and the hydrolysis of the proteins were monitored using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The integrity of the reaction products was verified by circular dichroism (CD) spectroscopy and electrospray ionization mass spectrometry (ESI-MS). Metal ion binding of the proteins was investigated using UV-Vis absorbance, CD, and fluorescence spectroscopy as well as isothermal titration calorimetry (ITC). ESI-MS measurements were also performed for this purpose, as well. DNA recognition of proteins was studied with short DNA molecules, using electrophoretic mobility shift assay (EMSA), CD and fluorescence anisotropy spectroscopy. DNA cleavage of the proteins was monitored using agarose gel electrophoresis.

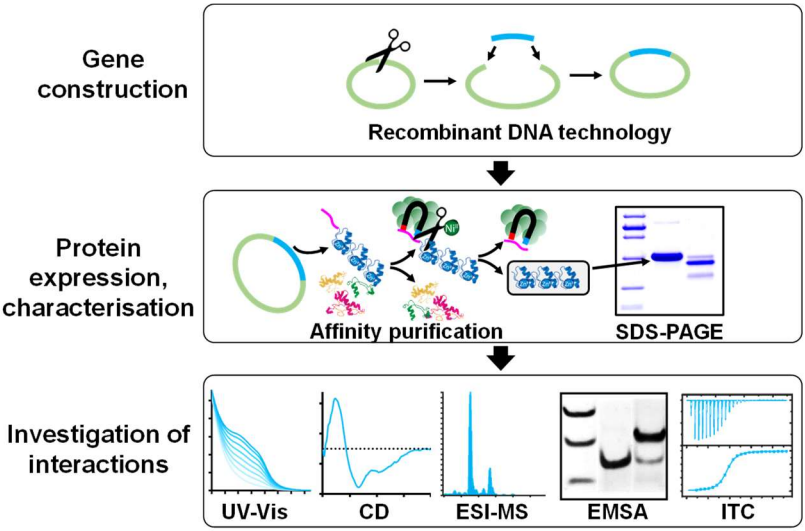

Figure 3. Schematic representation of the experimental methods used in my doctoral thesis.

III. New scientific results

T1. Ni(II)-induced selective peptide bond hydrolysis can be applied for zinc finger proteins without changing the structure and function of the zinc finger subunits, while the reaction with Cu(II) results in a collapsed non-functional zinc finger protein.

T1.1 We proved by SDS-PAGE analysis that zinc finger proteins consisting of several subunits and containing the (S/T)XH sequence can be selectively and quantitatively cleaved along the peptide bond preceding the (S/T) amino acid in 3 days at 50 °C (pH 8.2) through Ni(II)-induced peptide bond hydrolysis.

T1.2 If the (S/T)XH amino acid sequence is located outside the zinc finger units, the zinc finger units remain intact, retain Zn(II) and the secondary structure and function of the protein remain unchanged after the hydrolytic reaction.

T1.3 Ni(II)-induced hydrolysis results in identical final product with that obtained using the ULP1 protease in protein purification.

T1.4 The protein binds an additional Ni(II) in the newly formed ATCUN motif at the N-terminus of the protein after the hydrolysis. Due to its kinetic inertness, the Ni(II) complex of the N-terminal ATCUN motif does not affect the investigation of zinc finger subunits in most solution equilibrium studies. Even at 10× excess Ni(II) cannot efficiently compete with Zn(II) for zinc the finger subunits.

T1.5 Based on CD measurements we demonstrated that the hydrolytic reaction cannot be carried out with Cu(II). The secondary structure of the zinc finger subunits collapses and then the protein precipitates in the presence of Cu(II).

T1.6 We proved that if the ATCUN motif was metal ion-free, while the zinc finger subunits were saturated with Zn(II), Cu(II) coordinated to the ATCUN motif first, and a gradual collapse of the protein structure started only after addition of excess Cu(II) over the zinc finger protein.

T2. We demonstrated that the thermodynamic properties of the individual subunits in the 1MEY# artificial zinc finger protein show high similarity with the CP1 model peptide, however, the Zn(II) binding is further stabilized in the presence of the DNA target sequence of the protein.

T2.1 Based on competitive ITC and CD titrations, we found that the apparent stability constant ($\log\beta' = 12.2$) and the characteristic enthalpy ($\Delta H_{\text{binding site}} = -23.5$) for Zn(II) binding of the zinc finger subunits in 1MEY# zinc finger protein are similar to the values determined for CP1 model peptide (from which the amino acid sequence of 1MEY# subunits was derived). This suggests that linking the zinc finger subunits into a zinc finger protein does not significantly affect their behaviour, so that they can be considered to be independent.

T2.2 Based on EMSA and fluorescence anisotropy studies, we have shown that the 1MEY# zinc finger protein recognizes its DNA target sequence and the association constant characterising this interaction is in the range of 10^9. There was a two orders of magnitude difference in the protein–DNA affinity for the recognition of specific and non-specific but guanine-rich sequences.

T2.3 We proved that the DNA binding of the protein thermodynamically stabilizes the Zn(II) binding. In the presence of the DNA target sequence, the apparent stability constant of the binding site Zn(II) complex increased by 3.4 orders of magnitude.

T3. We proved that Ag(I) can inhibit the DNA recognition of a zinc finger protein, and in the case of Cys_2His_2 zinc finger proteins, 2 Ag(I) displaces a Zn(II) from a zinc finger binding site. In addition, we showed that additional Ag(I) can coordinate to the protein at Ag(I) excess, but the exact structure of these clusters is difficult to determine.

T4. We clearly proved that in zinc finger proteins with high Zn(II) affinity, Cd(II) coordinates to the Cys_2His_2 binding sites with 1-2 orders of magnitude lower affinity than Zn(II). The structure of the formed complex is very similar

to the Zn(II) complex, and it also recognizes the target DNA sequence of the protein, but with a reduced affinity by ~0.6 orders of magnitude.

T5. In a chloride ion-free medium, Hg(II) coordinates to the Cys_2His_2 zinc finger subunits by at least 4 orders of magnitude stronger than Zn(II), forming a disordered protein structure. Thus, the protein is no longer able to bind to its target DNA sequence. The 1MEY# protein can bind more than 12 Hg(II) in the presence of excess Hg(II). The exact coordination of the metal ions is not known, but based on competition measurements followed by fluorimetric measurements, the coordination of each metal ion can be characterized by a stability constant of at least 10^9 magnitude.

T6. We demonstrated that the Ni(II)-induced selective peptide hydrolysis can be used as a protein purification method. After purification of the target protein with a C-terminal his-tag, the affinity-tag could be removed by Ni(II)-induced selective peptide hydrolysis both in solution and on Ni-NTA resin without leaving any residual amino acids. Based on CD spectroscopy and ESI-MS measurements, the N-terminal fragment of the hydrolytic reaction is the native protein itself, so we proved that it is possible to produce and purify a protein with a native sequence by using Ni(II) ions instead of expensive and complicated proteases.

T7. During the investigation of the NColE7 nuclease and its modified versions, we showed that the production of these extremely toxic enzymes became possible with the newly developed protein expression and purification method, even though introducing the gene of the protein into a bacterial cell kills it immediately in the absence of the affinity-tag.

T7.1 The inhibited NColE7-type enzyme can be reactivated by Ni(II)-induced selective hydrolysis, which allows the regulation of nucleases.

T7.2 Nuclease activity and CD spectroscopic measurements proved that the inhibition can not be explained solely by allosteric effect. The his-tag binds to the free

coordination site of Zn(II) in the active centre of the enzyme, which prevents binding of the substrate. This could be verified with imidazole-containing low molecular weight model compounds.

T8. We have shown that DNA can be cleaved using the Ni(II) or Cu(II) complex of the 1MEY# zinc finger protein. The reaction was influenced by the length of the linker section between the ATCUN motif and the zinc finger subunits.

T8.1 We purified the 1MEY# zinc finger-ATCUN fusion protein whose Ni(II) and Cu(II) complexes were able to induce DNA cleavage in circular DNA carriers. However, the activity and specificity of the protein complexes were moderate.

T8.2 We designed new target DNA carriers in which there are several protein recognition sites, thereby facilitating the specific cleavages during the nuclease activity experiments.

T8.3 We successfully purified 9 modified 1MEY# proteins, in which the length and amino acid composition of the linker section connecting the zinc finger subunits and the ATCUN motif were varied. The structure, size and metal content of the proteins were as expected based on SDS-PAGE, ESI-MS and CD measurements, confirming that the length of the linker has no effect on the zinc finger subunits.

T8.4 With the help of the redesigned 1MEY# protein variants, we demonstrated that DNA cleavage can be enhanced if the linker section is shorter and/or contains more positively charged amino acids.

IV. Scientific publications

The Hungarian Scientific Bibliography (MTMT) identifier: 10054660

Full papers related to the dissertation:

1. A. Belczyk-Ciesielska, B. Csipak, **B. Hajdu**, A. Sparavier, M.N. Asaka, K. Nagata, B. Gyurcsik, W. Bal: Nickel (II)-promoted specific hydrolysis of zinc finger proteins, *Metallomics,* DOI: 10.1039/C8MT00098K IF = 3.571
2. H.A.H. Abd Elhameed, **B. Hajdu**, R. K Balogh, E. Hermann, É. Hunyadi-Gulyás, B. Gyurcsik: Purification of proteins with native terminal sequences using a Ni (II)-cleavable C-terminal hexahistidine affinity tag, *Protein Expr. Purif.*, DOI: 10.1016/j.pep.2019.03.009 IF = 1.513
3. H.A.H. Abd Elhameed, **B. Hajdu**, A. Jancsó, A. Kéri, G. Galbács, É. Hunyadi-Gulyás, B. Gyurcsik: Modulation of the catalytic activity of a metallonuclease by tagging with oligohistidine, *J. Inorg. Biochem.,* DOI: 10.1016/j.jinorgbio.2020.111013 IF = 4.155
4. K. Kluska, G. Veronesi, A. Deniaud, **B. Hajdu**, B. Gyurcsik, W. Bal, A. Krezel: Structures of silver fingers and a pathway to their genotoxicity, *Angew. Chem. Int. Ed.,* DOI: 10.1002/anie.202116621 IF = 16.823 (2021)
5. **B. Hajdu**, É. Hunyadi-Gulyás, B. Gyurcsik: Interactions of an artificial zinc finger protein with Cd(II) and Hg(II): Competition and metal and DNA binding, *Inorganics,* DOI: 10.3390/inorganics11020064 IF = 3.149 (2021)
6. **B. Hajdu**, É. Hunyadi-Gulyás, K. Kato, A. Kawaguchi, K. Nagata, B. Gyurcsik: Zinc binding of a Cys2His2-type zinc finger protein is enhanced by the interaction with DNA, *J. Biol. Inorg. Chem.,* DOI:10.1007/s00775-023-01988-1 IF = 3.862 (2021)

∑IF = 33.073

Full papers not related to the dissertation:

1. **B. Hajdu**, G. Czakó: Benchmark ab initio characterization of the complex potential energy surfaces of the $X^- + NH_2Y$ [X,Y = F, Cl, Br, I] reactions, *J. Phys. Chem. A*, DOI: 10.1021/acs.jpca.7b11927 IF = 2.641
2. N. Ivošević DeNardis, J. Pečar Ilić, I. Ružić, N. Novosel, T. Mišić Radić, A. Weber, D. Kasum, Z. Pavlinska, R.K. Balogh, **B. Hajdu**, A. Marček Chorvátová, B. Gyurcsik: Algal cell response to laboratory-induced cadmium stress: a multimethod approach, *Eur. Biophys J.*, DOI: 10.1007/s00249-019-01347-6 IF = 2.094
3. V. Pósa, **B. Hajdu**, G. Tóth, O. Dömötör, C. R. Kowol, B. K. Keppler, G. Spengler, B. Gyurcsik, Éva A. Enyedy: The coordination modes of (thio)semicarbazone copper(II) complexes strongly modulate the solution chemical properties and mechanism of anticancer activity, *J. Inorg. Biochem.,* DOI: 10.1016/j.jinorgbio.2022.111786 IF = 4.336 (2021)
4. T.V. Petrasheuskaya, F. Kovács, N. Igaz, A. Rónavári, **B. Hajdu,** L. Bereczki, N.V. May, G. Spengler, B. Gyurcsik, M. Kiricsi, É. Frank, É.A. Enyedy: Estradiol-based salicylaldehyde (thio)semicarbazones and their copper complexes with anticancer, antibacterial and antioxidant activities, *Molecules,* DOI 10.3390/molecules28010054 IF = 4.927 (2021)

$\sum$IF = 13.998

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Oral presentations and posters related to the dissertation:

1. **Hajdu B.,** Kiricsi M., Moncol J. Gyurcsik B. (Hungarian oral presentation)
   Kismolekulák és fehérjék kölcsönhatása DNS-sel fémionok jelenlétében
   52. Komplex Kémiai Kollokvium, 2018.05.22-24 Balatonvilágos, Hungary
2. **Hajdu B.,** Kato K., Kyosuke N., Gyurcsik B. (Hungarian oral presentation)
   Fémtartalmú fehérje alapú nukleázok DNS specifitása
   53. Komplex Kémiai Kollokvium, 2019.05.21-23 Velence, Hungary
3. **B. Hajdu,** H. Abd Elhameed, E. Hermann, É. Hunyadi-Gulyás, K. Kato, N. Kyosuke, W. Bal, B. Gyurcsik (English poster)
   Applications of Ni(II)-Induced Peptide Bond Cleavage
   19. ICBIC, 2019.08.11-16 Interlaken, Switzerland
4. **B. Hajdu,** R. Csáki, K. Kato, N. Kyosuke, B. Gyurcsik (English oral presentation)
   NOVEL ZINC FINGER-BASED ARTIFICAL NUCLEASES
   ARBRE-MOBIEU, 2020.02.24-26 Prague, Czech Republic
5. **Hajdu B.,** Gyurcsik B. (Hungarian oral presentation)
   Hogyan szerkeszthetünk DNS-t?
   XV. PSAK, 2020.10.08-10 online
6. **Hajdu B.,** Gyurcsik B. (Hungarian oral presentation)
   Cinkujj fehérjék kölcsönhatása fémionokkal
   54. Komplex Kémiai Kollokvium, 2021.05.26-27 online
7. **B. Hajdu,** B. Gyurcsik (English poster)
   Metal binding of a potential zinc finger nuclease
   35th Anniversary Protein Science Symposium, 2021.07.07-14 online
   Protein Sci. 2021, 30, 69.
8. **B. Hajdu,** B. Gyurcsik (English poster)
   Interaction of an artificial zinc finger protein with toxic metal ions.
   Workshop on Structural Biophysics, 2021.12.06-10 Bordeaux, France
9. **B. Hajdu,** B. Gyurcsik (English poster)
   Interaction of an artificial zinc finger protein with toxic metal ions
   1st MOSBRI scientific conference, 2022.06.20-22 Paris, France
10. **B. Hajdu,** É. Hunyadi-Gulyás, B. Gyurcsik (English poster)
    Interaction of a Cys2His2 zinc finger protein with toxic metal ions
    16th EuroBIC, 2022.07.17-21 Grenoble, France
11. **Hajdu B.,** Gyurcsik B. (Hungarian oral presentation)
    Mesterséges cinkujj fehérje kölcsönhatása toxikus fémionokkal
    XVI. PSAK, 2022.10.13-15 Szeged, Hungary

Coauthored oral presentations and posters related to the dissertation:

1. Z. Fábián, **B. Hajdu**, E. Hermann, H. Abd Elhameed, W. Bal, B. Gyurcsik (English poster)
   Affinity protein purification resulting in protein sequence without remaining amino acid residues.
   ISMEC2018, International Symposium on Metal Complexes, 2018.06.03-07 Florence, Italy
2. E. Németh, Z. Fábián, **B. Hajdu,** E. Hermann, R.K. Balogh, C. Oostenbrink, K. Nagata, B. Gyurcsik (English oral presentation)
   Design and investigation of novel zinc finger–NColE7-based artificial nucleases.
   ISMEC2018, International Symposium on Metal Complexes, 2018.06.03-07 Florence, Italy
3. B. Gyurcsik, Z. Fábián, E. Hermann, E. Németh, **B. Hajdu,** R.K. Balogh, H.A. Hosiny, C. Oostenbrink, K. Nagata (English oral presentation)
   Development of novel zinc finger-based artificial nucleases.
   43rd International Conference on Coordination Chemistry (ICCC2018), 2018.07.30-08.04 Sendai, Japan
4. B. Gyurcsik, **B. Hajdu,** Z. Fábián, E. Hermann, E. Németh, R.K. Balogh, H. Hosiny, C. Oostenbrink, K. Nagata (English poster)
   Multiple allosteric control in novel zinc finger-based artificial nucleases.
   14th European Biological Inorganic Chemistry Conference (EuroBIC 14), 2018.08.26-30 Birmingham, UK
5. Gyurcsik B., **Hajdu B.,** H.A.H. Abd Elhameed, Balogh R.K., Hermann E., Németh E. (Hungarian oral presentation)
   Szabályozott fehérjealapú mesterséges enzimek fejlesztése
   Reakciókinetikai és Fotokémiai Munkabizottság és a Koordinációs Kémiai Munkabizottság Munkabizottsági ülése, 2018.11.8-9 Veszprém, Hungary
6. B. Gyurcsik, **B. Hajdu,** E. Hermann, R.K. Balogh, H.A.H. Abd Elhameed (English oral presentation)
   Intramolecular allosteric control of NColE7 metallonuclease based on the specific protease action of nickel(II) ions.
   Molecular Biophysics: ABC of the puzzle of Life, ARBRE-MOBIEU Plenary Meeting, 2019.03.18-20 Zagreb, Croatia
7. Gyurcsik B., **Hajdu B.,** H.A.H. Abd Elhameed, W. Bal, K. Nagata (Hungarian oral presentation)
   Fémionok által szabályozott mesterséges nukelázok.
   53. Komplexkémiai Kollokvium és az MTA Koordinációs Kémiai Munkabizottság ülése, 2019.05.21-23 Velence, Hungary
8. Hermann E., H.A.H. Abd Elhameed, Németh E., Csáki R., **Hajdu B.,** C. Oostenbrink, Gyurcsik B (Hungarian oral presentation)

A C45-ZF-N85 összetett cinkujj-nukleáz és mutánsainak előállítása és vizsgálata.
MKE Vegyészkonferencia, 2019.06.24-26 Eger, Hungary

9. H.A. Abd Elhameed, **B. Hajdu,** E. Hermann, M.K. Goppisetty, M. Kiricsi, D.A. Ungor, E. Csapó, W. Bal, B. Gyurcsik (English oral presentation)
Metal ions as regulatory elements of artificial nucleases.
ISMEC2019, International Symposium on Metal Complexes, 2019.06.11-14 Debrecen, Hungary
10. H.A.H. Abd Elhameed, **B. Hajdu,** B. Gyurcsik (English poster)
Modulation of catalytic activity of the NColE7 metallonuclease.
ISMEC2019, International Symposium on Metal Complexes, 2019.06.11-14 Debrecen, Hungary
11. B. Gyurcsik, **B. Hajdu,** H.A. Abd Elhameed, W. Bal, K. Nagata (English oral presentation)
Metal ions as regulatory elements of artificial DNA cleaving enzymes.
15th International Symposium on Applied Bioinorganic Chemistry (ISABC15), 2019.06.02-05 Nara, Japan
12. E. Hermann, H.A. Abd Elhameed, E. Németh, R. Csáki, **B. Hajdu,** B. Gyurcsik (English oral presentation)
Purification and characterization of the C45-ZF-N85 artificial zinc-finger nuclease and its mutants.
XXVII. International Conference on Coordination and Bioinorganic Chemistry (XXVII. ICCBIC), 2019.06.02-07 Smolenice, Slovakia
13. B. Gyurcsik, **B. Hajdu,** H.A.H Abd Elhameed, W. Bal, K. Nagata (English poster)
Metal ions as regulatory elements of artificial DNA cleaving enzymes.
Serbian Biochemical Society, Ninth Conference with international participation, University of Belgrade, 2019.11.14-16 Belgrade, Serbia
14. H.A.H. Abd Elhameed, **B. Hajdu,** N. Igaz, M.K. Goppisetty, M. Kiricsi, D. Ungor, E. Csapó, B. Gyurcsik (English poster)
6×His tag modulates the catalytic activity of NColE7 nuclease.
Living Molecules: Towards Integrative Biophysics of the Cell, ARBRE-MOBIEU Plenary Meeting, 2020.02.24-26 Prague, Czech Republic
15. B. Gyurcsik, **B. Hajdu,** H.A. Abd Elhameed, K. Nagata (English oral presentation)
Metal ions as regulators of hydrolytic enzymes.
XXVIII. International Conference on Coordination and Bioinorganic Chemistry (XXVIII. ICCBIC), 2022.06.05-10 Smolenice, Slovakia
16. B. Gyurcsik, **B. Hajdu,** H.A. Abd Elhameed, A. Jancsó, É. Hunyadi-Gulyás (English oral presentation)
Interplay of multiple metal ion binding sites regulates the catalytic activity of metalloenzymes

EuroBIC-16, 16th European Biological Inorganic Chemistry Conference, 2022.07.17-21 Grenoble, France

Oral presentations and posters not related to the dissertation:

1. **Hajdu B.,** Kis M.L., Ivayla P., Gyurcsik B. (Hungarian oral presentation)
   Monensin A – egy ionofór antibiotikum fémkomplexei kétértékű fémionokkal
   54. Komplex Kémiai Kollokvium, 2021.05.26-27 online
2. **Hajdu B.,** Kis M.L., Ivayla P., Gyurcsik B. (Hungarian oral presentation)
   Monenzin A kölcsönhatása kétértékű fémionokkal
   XLIV. Kémiai Előadói Napok, 2021.10.26-27 Szeged, Hungary
3. V. Pósa, **B. Hajdu,** G. Tóth, O. Dömötör, C.R. Kowol, B.K. Keppler, G. Spengler, B. Gyurcsik, É.A. Enyedy (English poster)
   Effects of variations in coordination modes of copper(II) complexes of (thio)semicarbazones on solution chemical and biological properties
   COST NECTAR 4th Annual Conference, 2021.09.06-08 Ljubljana