Theses of Ph.D. dissertation

REDESIGN OF THE COLICIN E7 NUCLEASE DOMAIN INTO A CONTROLLED AND SPECIFIC ARTIFICIAL ENZYME

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I. INTRODUCTION

The ability to cleave nucleic acids at a selected specific site is a current demand of biotechnology and gene therapy. The cleavage of chromosomal DNA at a sequence related to a disease can initiate the natural correction of DNA, and this phenomenon could be exploited in the cure of certain genetic diseases. The cleavage of nucleic acids is an important part of the cell machinery, also in normal conditions. Nature has evolved the nuclease enzymes to perform this task. However, only a few DNA sequences can be targeted by these molecules, limiting the applications for gene therapy where the sequence to target is determined by the disease. In order to cleave DNA at a desired sequence new engineered enzymes are necessary.

Zinc-finger nucleases are the best studied modular enzymes designed for the above purpose. They consist of a nonspecific catalytic center (the nuclease domain of the FokI bacterial enzyme) attached to a tunable recognition domain (three or four zinc-finger units). By the mutation of the residues that interact with the DNA bases the recognition sequence can be altered and custom-designed enzymes can be developed. Such enzymes were widely applied, but a minor cytotoxicity was found hindering the human therapeutic applications.

In this thesis the design of a novel type of zinc-finger nucleases is introduced. Instead of the common catalytic center (FokI) we proposed to use the nuclease domain of colicin E7. The choice of this protein relied on previous investigations showing that the N-terminally truncated NColE7 is inactive. We hypothesized that the missing N-terminus could be applied to develop a novel allosteric activation mechanism. These attempts proved to be successful and the results presented here may contribute to the development of a new strategy that can be generally applied for the artificial nuclease design.

II. AIMS AND OBJECTIVES

The aim of this research is to design new ZFN molecules with a novel type of control mechanism as a proof of concept. The building blocks for the models contained a three zinc-finger array and NColE7, a nonspecific bacterial nuclease. This is the first case to use NColE7 as a part of a chimeric artificial nuclease. Therefore, in my work first I aimed at understanding and influencing the function of the NColE7 protein, to be followed by the redesign of the protein for construction of the new ZFN molecule. The main idea is shown in Fig. 1. According to this scheme NColE7 would be divided into two parts, each fused to opposite termini of the ZF array. This arrangement can provide a control mechanism: none of the NColE7-parts has a nuclease activity on its own, but they function if the ZF-s orient them to get sterically close.

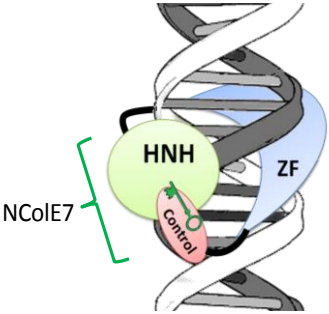

Fig. 1. *Scheme of the chimeric enzyme that we intend to design. The zinc-fingers (in blue) bind specifically to DNA, and the HNH-motif (catalytic site) containing part of NColE7 (green) is responsible for the cleavage. However, it can only hydrolize DNA, if the N-terminal controlling part (red) that is fused to the opposite end of the ZF-s precisely fits to induce the catalytic activity.*

1. Understanding the properties of the NColE7 protein through point mutations with emphasis on the N-terminus and metal-binding properties

NColE7 was extensively studied in the literature, but the potential control function of the N-terminus has not been investigated so far. Since NColE7 is a metalloprotein, it forms ternary (three component) complex with the substrate DNA. The metal-binding properties may change upon mutations altering the nuclease activity leading to a complex bioinorganic chemical problem. Since the N-terminal four residues (446-KRNK-449) proved to be essential for the nuclease activity, we can get a deeper insight into the role of these residues through their mutations. The questions addressed in this point include:

- How many of the three positively charged residues in the N-terminal section is needed for the nuclease activity?
- Is the nuclease activity abolished when all the positive residues are missing but the backbone is present?
- Can K446 or K449 promote the reaction in the absence of R447?

2. Finding the most suitable way to control the NColE7 nuclease

To explore the use of the N-terminus to control NColE7 we need to be able to influence its function. This objective involves computational study of the contribution of each residue to the structural stabilization of the proteins and selection of the mutations to be performed experimentally. The questions to answer were:

- What is the role of the 25 residues long loop at the N-terminus? Is it responsible for keeping the KRNK motif in place?
- How does the mutation of selected residues in this seemingly not important part of the sequence influence the nuclease activity?
- How can we induce the reaction if the nuclease activity is decreased upon the mutations weakening the interactions of the loop?

3. Design of NColE7-based ZFN-s by computational methods

The next goal is to design a ZF-NColE7 artificial nuclease, based on the results of the previous part of the research. Starting out from the published crystal structures of the NColE7/DNA and ZF/DNA complexes the computational design includes the following points:

- Construction of an initial model by approaching the NColE7 and the selected ZF protein simultaneously binding to a DNA substrate.
- Selection of the controlling part from the N-terminus and the catalytic part from the C-terminus.
- Design of appropriate short linker sequences to fuse the proteins.
- Study of the constructed models by MD simulations.

4. Experimental study of the designed ZFN-s

The main objective of this thesis work is to express, purify the computationally designed ZFN-s as recombinant proteins and study their folding, metal-binding and reactivity. These properties may be significantly affected within the redesigned nuclease domain, therefore the the following questions concerning the new enzymes need to be answered:

- Do the ZF-s maintain the sequence-specificity after the fusion of the NColE7 segments?
- Is there any observable nuclease activity? If yes, is it specific, and is it a single strand or double strand cleavage?
- Is the model good enough to serve as a starting point for optimization to obtain a nuclease that is applicable in practice?

III. PRECEDING RESEARCH

Prior to this work we studied the N-terminally truncated NColE7 proteins ΔN4-NColE7 and ΔN25-NColE7 [6]. None of these proteins have nuclease activity pointing out the important role of the N-terminus. Experimental data and computational modeling showed that ΔN4-NColE7 has an intact structure and strong Zn^{2+}-binding, in contrast to ΔN25-NColE7. These results inspired the further investigation of the role of the N-terminal loop.

IV. METHODS

The methods applied in this work involved various experimental techniques related to bioinorganic chemistry augmented by computational modeling. The scheme that was followed is shown in Fig.2.

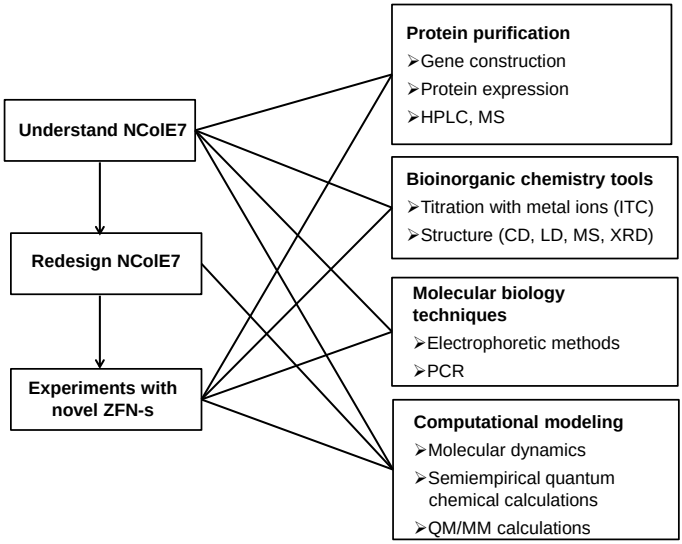

Fig.2. *Workflow of the Ph.D. research project.*

Experimental methods

The mutants and designed proteins were obtained by bacterial expression. For this the genes were produced using recombinant DNA technology. The proteins were purified from the bacterial culture by FPLC (Fast Protein Liquid Chromatography) methods. The purified proteins were validated by SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) and by the determination of their molecular weight with ESI-MS (Electrospray Ionization Mass Spectrometry).

The metal-binding of the proteins was quantified by ITC (Isothermal Titration Calorimetry). The structural changes related to metal-ion binding were followed by SRCD (Synchrotron Radiation Circular Dichroism) spectroscopy and ESI-MS.

The nuclease activity and DNA-binding were studied by gel electrophoretic methods and FLD (Flow Linear Dichroism) spectroscopy as well as AFM (Atomic Force Microscopy). For the detection of single strand cleavages also PCR (Polymerase Chain Reaction) was applied.

DNA and metal ion binding was further studied by cocrystallization of a mutant nuclease with DNA and solution of the crystal structures.

Computational methods

Protein structure was studied by semiempirical quantum chemical calculations, MD (Molecular Dynamics) simulations. The DNA-binding was investigated by hybrid QM/MM calculations.

The initial models of NColE7-based zinc finger nucleases were constructed by structural alignments. The linkers joining the NColE7 parts to zinc finger proteins were designed based on protein loop databases and the stability of the scanned loops was estimated by empirical based methods. The four selected models were studied by MD simulations.

V. NEW SCIENTIFIC RESULTS

1. NColE7 as a possible building block for a controlled zinc-finger nuclease: N-terminal point mutants

1.1. The genes of NColE7 and its mutants within the pGEX-6P-1 plasmid showed their cytotoxicity in the cloning process. The genes were successfully cloned in this system in the presence of the Im7 immunity protein. [4]

1.2. NColE7 and its four point mutants with the KGNK, KGNG, GGNK or GGNG sequence at the N-terminus, replacing KRNK were successfully expressed and purified. According to ESI-MS the proteins possess the expected molecular weights. The measurements also indicated that the proteins can bind Zn^{2+}. [1]

1.3. We investigated the nuclease activity of the new mutants and the factors that may influence it.

1.3.1. Agarose gel electrophoresis and flow linear dichroism monitoring of the DNA cleavage pointed out that the mutants have low activity in the order of KRNK >> KGNK > KGNG ~ GGNK > GGNG. [1]

1.3.2. According to CD-measurements the solution structure of the proteins is not significantly changed. [1]

1.3.3. The Zn^{2+}-affinity was determined by ITC. The K_d of NColE7 (16.6 ± 3.5 μM) did not significantly change in the mutants (~13 ± 3 μM). [1]

1.3.4. The dynamics of the structure slightly changed according to MD simulations. The results suggested that K446 may partially take over the role of R447. [1]

1.3.5. The structure of proteins was optimized on the PM6 semiempirical quantum-chemical level. Slight changes in DNA binding and catalytic distances were predicted. [1]

1.3.6. The electrophoretic mobility shift assays (EMSA) with a short 13 bp dsDNA showed that the DNA-binding affinity of the mutant proteins was not significantly affected by the decrease of the number of positive charges at the N-terminus. [1]

1.3.7. DNA binding was computationally modeled by a QM/MM method. The absence of R447 caused minor changes in DNA-binding that may lead to the decrease in nuclease activity. The possibility that a bound water

molecule in the coordination sphere of the Zn^{2+}-ion can take part in the reaction was suggested. [2]

1.3.8. The KGNK protein was cocrystallized with a 18 bp dsDNA. Two data sets have been evaluated: one for the KGNK/DNA complex and one for the KGNK/DNA/Zn^{2+} complex, as a result of soaking the crystal in a zinc solution prior to freezing.

1.3.9. DNA-binding was further studied with long DNA substrates (188, 306, 777 bp) in EMSA and AFM experiments. We showed that the DNA-binding of NColE7 is cooperative, and this feature is changed in the mutants.

1.4. We suggested that the N-terminus could be used to control the nuclease activity of NColE7. [1]

2. The function of the N-terminal chain can be altered: further mutants in the N-terminal loop

2.1. A computational alanine scan was performed to identify the residues in the 25 residues long N-terminal loop that are most important for the stability of the protein. The T454A, K458A and W464A mutations were selected for experimental study. [3]

2.2. After the construction of the new genes four NColE7 mutants combining the mutation of the above residues to alanine (TKW [3], TK, TW, W) were expressed and purified. According to ESI-MS the proteins have the accurate expected molecular weights.

2.3. We showed that the mutations in the N-terminal chain lead to decreased nuclease activity in the mutants containing the W464A mutation (TKW [3], TW, W). We investigated the reasons behind this phenomenon.

2.3.1. As shown by CD-spectroscopy the decrease in activity is related to the change in the protein structure. [3]

2.3.2. The solution structure in the W464A mutants is characterized by a decreased Zn^{2+}-binding affinity by 3 orders of magnitude as measured by ITC. This indicated that the structure of HNH-motif is also changed. [3]

2.3.3. We showed that the folding of the mutant protein can be induced by the binding of DNA or the Im7 protein. [3]

2.3.4. The protein with induced structure (in presence of Im7) has a rescued Zn^{2+}-binding affinity. This proves that the metal-binding site of NColE7 is preorganized in solution.

2.4. Based on the results we understood how we can influence the activity and metal-binding of the NColE7-protein. [3]

3. Computational design of novel ZFN-s

3.1. The initial models of the ZFN-s were set up based on structure alignment by PyMol. They contained the NColE7 protein, a ZF protein (three fingers), each bound to the DNA molecule containing the recognition sequence of the ZF domains. [4]

3.2. Using loop databases and protein stability prediction methods linkers were designed to connect the separated C- and N-terminal parts of NColE7, to the ZF protein sequence. The four selected final models contained a part of the N-terminus (N*X*) and that of the C-terminus (C*Y*) fused to the opposite ends of the ZF array, where *X* and *Y* are the number of NColE7 residues involved. The "straight" N*X*-ZF-C*Y* models included N4-ZF-C105, N4-ZF-C45 and N46-ZF-C45. The fourth, C*Y*-ZF-N*X* "reverse" model was denoted as C123-ZF-N7. [4]

3.3. The 4 selected models were studied by MD simulations. The computations suggested that N4-ZF-C105 and C123-ZF-N7 are the most promising models. [4]

4. Experimental study of the designed ZFN-s

4.1. After the construction of genes the proteins corresponding to the three "straight" models were expressed and purified. ESI-MS confirmed that the molecular weights of the proteins are accurate. [5]

4.2. EMSA experiments showed that all three proteins bind DNA molecules containing the ZF recognition sequence with high affinity and the specificity is also maintained. [5]

4.3. Plasmid cleavage experiments (with plasmids containing the ZF recognition sequence) showed that N4-ZF-C105 has well detectable single strand cleaving activity. According to primer extension experiments the cleavage occurs at the site expected based on computational modeling. [5]

4.4. We established a novel platform for NColE7-based artificial nucleases that can be used for the specific cleavage of DNA at the desired site. This strategy opens a new perspective for the design of safe artificial nucleases intended for use in gene therapy.

VI. PUBLICATION LIST

Identification number in the Hungarian Collection of Scientific Publications (MTMT): 10040185

Publications related to the dissertation

[1] **E. Németh**, T. Körtvélyesi, P.W. Thulstrup, H.E.M. Christensen, M. Kožíšek, K. Nagata, A. Czene, B. Gyurcsik: Fine tuning of the catalytic activity of colicin E7 nuclease domain by systematic N-terminal mutations. *Protein Sci.*, 23, 1113–1122 (2014)
IF = 2,861

[2] A. Czene, E. Tóth, **E. Németh**, H. Otten, J.-C.N. Poulsen, H.E.M. Christensen, L. Rulíšek, K. Nagata, S. Larsen, B. Gyurcsik: A new insight into the zinc-dependent DNA-cleavage by the colicin E7 nuclease: a crystallographic and computational study.
Metallomics, (2014); DOI: 10.1039/c4mt00195h
IF = 3,978

[3] **E. Németh**, T. Körtvélyesi, M. Kožíšek, P.W. Thulstrup, H.E.M. Christensen, M. Asaka, K. Nagata, B. Gyurcsik: Substrate binding activates the designed triple mutant of the colicin E7 metallonuclease.
J. Biol. Inorg. Chem., (2014); DOI 10.1007/s00775-014-1186-6
IF = 3,164

[4] **E. Németh**, G.K. Schilli, G. Nagy, C. Hasenhindl, B. Gyurcsik, C. Oostenbrink: Design of a colicin E7 based chimeric zinc-finger nuclease.
J. Comp-Aid. Mol. Des., 28, 841–850 (2014)
IF = 2,782

[5] **E. Németh**, M. Asaka, K. Nagata, C. Oostenbrink, B. Gyurcsik: Specific nicking of DNA by a colicin E7 based zinc finger nuclease - a new design strategy. Angew. Chem. Int. Ed. (2014) – submitted

ΣIF = 12,785

Further publications

[6] A. Czene, **E. Németh**, I.G. Zóka, N.I. Jakab-Simon, T. Körtvélyesi, K. Nagata, H.E.M. Christensen and B. Gyurcsik: On the role of the N-terminal loop in the function of colicin E7 nuclease domain.
J. Biol. Inorg. Chem., 18, 309-321 (2013)
IF = 3,164

[7] T. Körtvélyesi, K. E. Kövér, **E. Németh**, B. Penke, L. Szilágyi, M. Kasperkiewicz, A. Orosz: Study of the Binding Peptide-Derivatives on HSP90 Protein which have Anti-Cancer and Autoimmune Inhibitor Effects, Int. J. Drug. Des. Discov., 5, 1308-1317 (2014)

ΣΣIF = 15,949

Conference publication with impact factor

1. E**. Németh**, C. Oostenbrink, B. Gyurcsik: Design of a novel type of enzymatic control in NColE7-based zinc finger nucleases.
J. Biol. Inorg. Chem., 19, S833–S852 (2014 - S838–S839)
IF = 3,164

Publications in national journals

1. Németh Eszter: Áramlásos lineáris dikroizmus spektroszkópia alkalmazása nukleázok tanulmányozására. Magyar Kémikusok Lapja, 2014. február

Presentations at international conferences

1. **E. Németh**, B. Gyurcsik, A. Czene, I.N. Simon, I.Gy. Zóka, T. Körtvélyesi, H.E.M. Christensen, K. Nagata: Allosteric control in the nuclease domain of colicin E7. *QBIC-3 3rd Quantum Bioinorganic Chemistry Conference*, Cesky Krumlov, Czech Republic, 25-28 June, (2011) – poster
2. B. Gyurcsik, A. Czene, I.N. Simon, **E. Németh**, I.Gy. Zóka, T. Körtvélyesi, H.E.M. Christensen, K. Nagata: Allosteric control in the nuclease domain of colicin E7, International Workshop on Metal Containing Drugs, Szeged, Hungary, 30-31 August (2011) – lecture

3. **E. Németh**, B. Gyurcsik, T. Körtvélyesi, P. W. Thulstrup, H.E.M. Christensen: Computational design, expression and spectroscopic study of a mutant NColE7 metallonuclease. *XII International Symposium on Inorganic Biochemistry, Collaboration and Beyond*, Wrocław, Poland, August 28 – September 1 (2013) - lecture

4. **E. Németh**, T. Körtvélyesi, P. W. Thulstrup, H.E.M. Christensen, B. Gyurcsik: Fine tuning of the catalytic activity by the positively charged residues at the N-terminus of the ColE7 nuclease. *6th Central Europe Conference - Chemistry towards Biology*, Trieste, Italy, September 10-13 (2013) – lecture

5. **E. Németh**, C. Oostenbrink, B. Gyurcsik: Design of a novel type of enzymatic control in NColE7-based zinc finger nucleases. *EUROBIC12, 12th European Biological Inorganic Chemistry Conference*, Zürich, Switzerland, August 24-28 (2014) – poster

6. **E. Németh**, J. Kopniczky, E. Balázs, B. Gyurcsik: Cooperative DNA-binding of the colicin E7 nuclease domain. *Significance of Knotted Structures for Function of Proteins and Nucleic Acids - Biophysical Society meeting*, Warsaw, Poland, September 17-21 (2014) - poster

7. **E. Németh**, M. Asaka, K. Nagata, C. Oostenbrink, B. Gyurcsik: Redesign of the colicin E7 nuclease domain into a controlled and specific artificial enzyme.*Tsukuba Global Science Week 2014*, Tsukuba, Japan, September 28-30 (2014) – invited lecture

Coauthor at international conferences

1. B. Gyurcsik, A. Czene, N.I. Simon, **E. Németh**, I.G. Zóka, G. Nagy, T. Körtvélyesi, H.E.M. Christensen, K. Nagata: Study on the HNH motif as a possible component of a controlled zinc-finger nuclease. *International Conference on Gene Targeting*, Vienna, Austria, 9-12th February (2011), - lecture

2. B. Gyurcsik, A. Czene, N.I. Simon, **E. Németh**, I.G. Zóka, G. Nagy, T. Körtvélyesi, H.E.M. Christensen, K. Nagata: Study on the HNH motif as a possible component of a controlled zinc-finger nuclease. *New Trends in Coordination, Bioinorganic and Applied Inorganic Chemistry; XXIII. International Conference on Coordination and Bioinorganic Chemistry*, Smolenice, Slovak Republic, 5-10th June (2011), - lecture

3. B. Gyurcsik, A. Czene, N.I. Simon, **E. Németh**, I.G. Zóka, T. Körtvélyesi, H.E.M. Christensen, K. Nagata: Study on the HNH motif as a possible component of a controlled zinc-finger nuclease. *4th European Conference on Chemistry for Life Sciences (4ECCLS)*, Budapest, Hungary, August 31-September 3 (2011) - lecture

4. B. Gyurcsik, A. Czene, N.I. Simon, **E. Németh**, I.G. Zóka, T. Körtvélyesi, H.E.M. Christensen, K. Nagata: Design of a novel artificial nuclease based on the HNH. *ISABC11 - 11th International Symposium on Applied Bioinorganic Chemistry*, Barcelona, Spain, 2-5 December (2011)-poster

5. A. Czene, **E. Németh**, I.G. Zóka, N.I. Simon, B. Gyurcsik, H.E.M. Christensen, K. Nagata: Mutant Colicin E7 proteins reveal the conditions for allosteric control of the enzymatic action. *ISABC11 - 11th International Symposium on Applied Bioinorganic Chemistry*, Barcelona, Spain, 2-5 December (2011)-poster

6. B. Gyurcsik, A. Czene, **E. Németh**, I.G. Zóka, E. Endreffy, H.E.M. Christensen, K. Nagata: Targeting the breakpoint in duchenne muscular dystrophy. *Nobel75, 75th Anniversary of Albert Szent-Györgyi's Nobel Prize Award*, Szeged, Hungary, 22-25 March (2012)-lecture

7. B. Gyurcsik, A. Czene, **E. Németh**, I.G. Zóka, E. Endreffy, H.E.M. Christensen, K. Nagata: Targeting the breakpoint in Duchenne Muscular Dystrophy *ISMEC 2012, International Symposia on Metal Complexes*, Lisbon, Portugal, June 18th – 22nd, ISMEC Acta, Vol. 2, p.111 (2012)-poster

8. B. Gyurcsik, A. Czene, **E. Németh**, I.G. Zóka, E. Endreffy, H.E.M. Christensen, K. Nagata: Targeting the breakpoint in Duchenne Muscular Dystrophy by an artificial metallonuclease. *EUROBIC11, 11th European Biological Inorganic Chemistry Conference*, Granada, Spain September 12-16 (2012) - poster

9. B. Gyurcsik, A. Czene, **E. Németh**, I.Gy. Zóka, E. Endreffy, H.E.M. Christensen, K. Nagata: Design of a novel artificial metallonuclease for targeting the breakpoint in Duchenne Muscular Dystrophy. *The 3rd Leading Graduate Schools International Conference*, Tsukuba, Japan, November 1-2 (2012) - invited lecture

10. K. Borsos, **E. Németh**, B. Gyurcsik: The interaction between a truncated mutant of NColE7 nuclease and its immunity protein. *13th Edition of Academic Days Timisoara - New trends and strategies in the chemistry of advanced materials with relevance in biological systems, technique and environmental protection*, Timisoara, Romania, June 13-14 (2013) - poster

11. B. Gyurcsik, A. Czene, **E. Németh**, I.G. Zóka, E. Endreffy, H.E.M. Christensen, K. Nagata: The nuclease domain of colicin E7 as a potential template for artificial nuclease design. *13th Edition of Academic Days Timisoara - New trends and strategies in the chemistry of advanced materials with relevance in biological systems, technique and environmental protection*, Timisoara, Romania, June 13-14 (2013) - plenary lecture

12. B. Gyurcsik, **E. Németh**, P. W. Thulstrup, M. Kožíšek, H.E.M. Christensen, K. Nagata: Fine tuning of the allosteric activation in the nuclease domain of colicin E7. *Global Science Week 2013 - 'Integration of Chemistry and Life Science'*, Tsukuba, Japan, October 2-4 (2013) – invited lecture

13. **E. Németh**, G. Schilli, C. Koncz, A. Czene, M. Kožíšek H.E.M. Christensen, K. Nagata, B. Gyurcsik: The effect of the metal ions on the active centre of colicin E7 nuclease. *ISMEC 2014, International Symposium on Metal Complexes*, Pavia, Italy, June 08-12 (2014) - lecture

14. **E. Németh**, C. Oostenbrink, M. Asaka, K. Nagata, B. Gyurcsik: Novel zinc finger nucleases based on colicin E7 nuclease domain. *CTB 2014 - 7th Central Europe Conference Chemistry Towards Biology*, Katowice, Poland, September 9-12 (2014) - lecture

Presentations at national conferences

1. Roósz B., **Németh E.**, Hoffmann A. E., Körtvélyesi T.: Kukurbit[7]uril "host-guest" komplexeinek elméleti vizsgálata. KeMoMo-QSAR szimpózium, Szeged (2010) - poszter

2. **Németh E.**, Keserű Gy. M.: A Caco-2 permeabilitás in silico predikciója. A Magyar Tudomány Ünnepe, Szeged (2010) - előadás

3. **Németh E.**, Keserű Gy. M.:A Caco-2 permeabilitás in silico predikciója, XXXIII. Kémiai Előadói Napok, Szeged (2010) - előadás

4. **Németh E.**, Zóka I.Gy., Czene A., Jakab-Simon I.N., Gyurcsik B., Körtvélyesi T., Nagata K., Christensen H.E.M.: A colicin E7 fehérje nukleáz doménjének vizsgálata. *MKE, 1. Nemzeti konferencia*, Sopron 2011. május 22-25 - előadás

5. **Németh E.**, Gyurcsik B., Körtvélyesi T: Allosztérikus kontroll a Colicin E7 fehérje nukleáz doménjében. *XXXIV. Kémiai Előadói Napok*, Szeged, 2011. November 2-4 - előadás

6. **Németh E.**, Schilli G., Gyurcsik B., Körtvélyesi T.: Pontmutációk tanulmányozása a colicin E7 fehérje nukleáz doménjében. *46. Komplexkémiai Kollokvium és az MTA Koordinációs Kémiai Munkabizottság ülése,* 2012. május 21-23, Mátrafüred - előadás

7. **Németh E.**, Gyurcsik B., Körtvélyesi T: A colicin E7 metallonukleáz domén finomhangolása. *XXXVI. Kémiai Előadói Napok*, 2013. október 28-30. Szeged - előadás

8. **Németh E.**, Czene A., Gyurcsik B.: Egy fehérjealapú mesterséges metallonukleáz szabályozásának lehetősége. *MTA Koordinációs Kémiai és Peptidkémiai Munkabizottság közös ülése*, 2013. október 28, Budapest - előadás.

Coauthor at national conferences

1. Zóka I. Gy., Gyurcsik B., Czene A., **Németh E.**: A colicin E7 nukleáz N-terminális végén rövidített mutáns fehérjéinek vizsgálata. *XXXIV. Kémiai Előadói Napok*, Szeged, 2011. November 2-4 - előadás

2. Gyurcsik B., Czene A., **Németh E.**, Tóth E., Zóka I.Gy.: Aktiváló allosztérikus szabályozás kialakításának lehetősége egy Colicin E7 alapú mesterséges metallonukleázban. *46. Komplexkémiai Kollokvium és az MTA Koordinációs Kémiai Munkabizottság ülése,* 2012. május 21-23, Mátrafüred - előadás

3. **Németh E.**, Czene A., Borsos K., P.W. Thulstrup, Gyurcsik B.: A colicin E7 metallonukleáz aktivitásának hangolása az N-terminális végen kialakított pontmutációk révén. *47. Komplexkémiai Kollokvium és az MTA Koordinációs Kémiai Munkabizottság ülése*, 2013. május 29-31, Mátraháza - előadás.

   Schilli G., **Németh E.**, Gyurcsik B.: Egy mutáns NColE7 metallonukleáz előállítása *XXXVI. Kémiai Előadói Napok*, 2013. október 28-30. Szeged - előadás