Experiences with zinc(II) containing artificial metalloproteins and metalloenzymes

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A biologist can do:

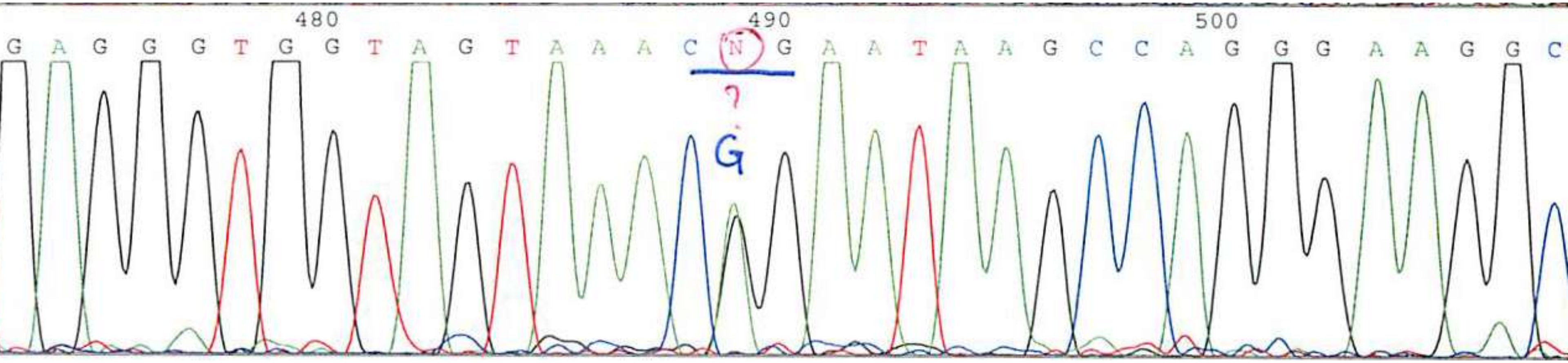
- ***Beautiful synthetic reactions: e.g. protein synthesis***

- ***Efficient catalysis: even of the thermodynamically unfavoured reactions – coupling of the reactions by the catalyst***

- ***Extraordinary selectivity: better than the best chemical separation methods.***

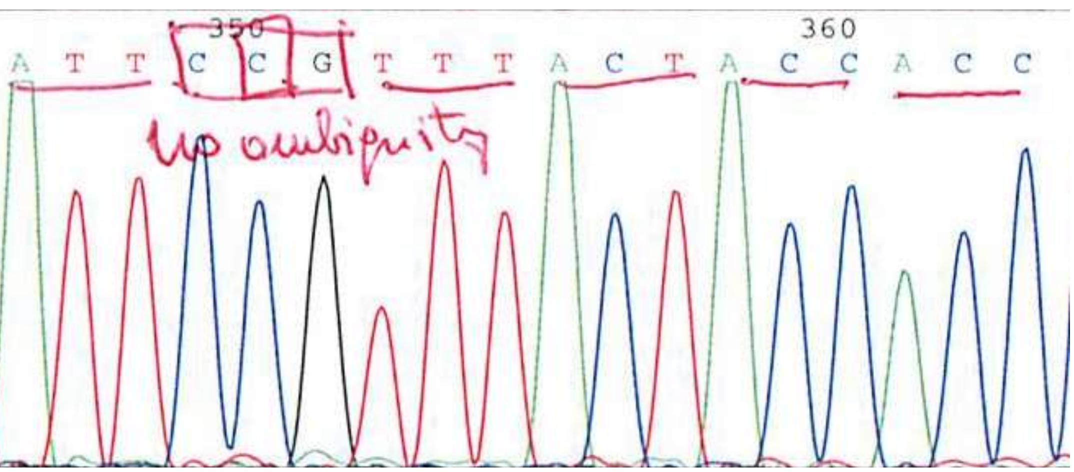
A chemist can do:

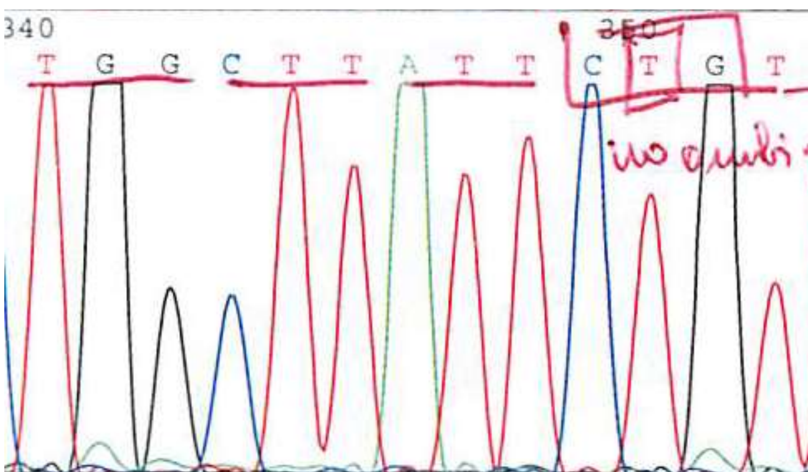
Capillary electrophoresis for DNA sequencing



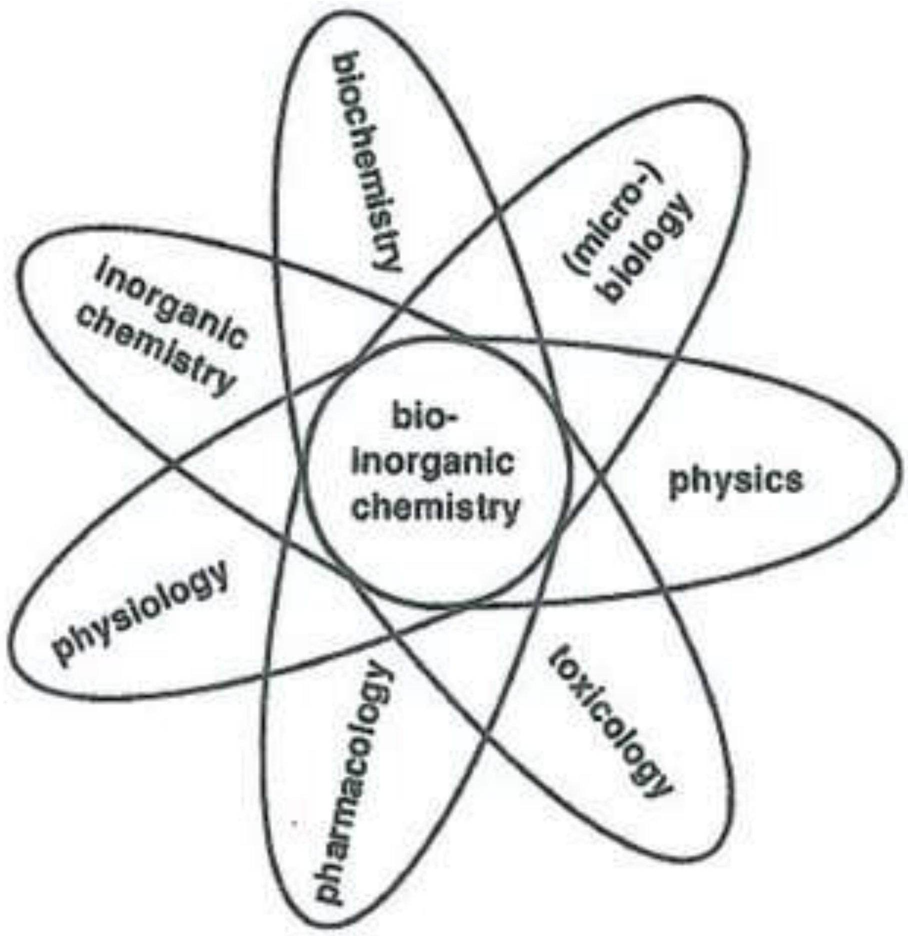
A biologist can do:

Separation of the two large DNA molecules (Same size: ~5000 bp)

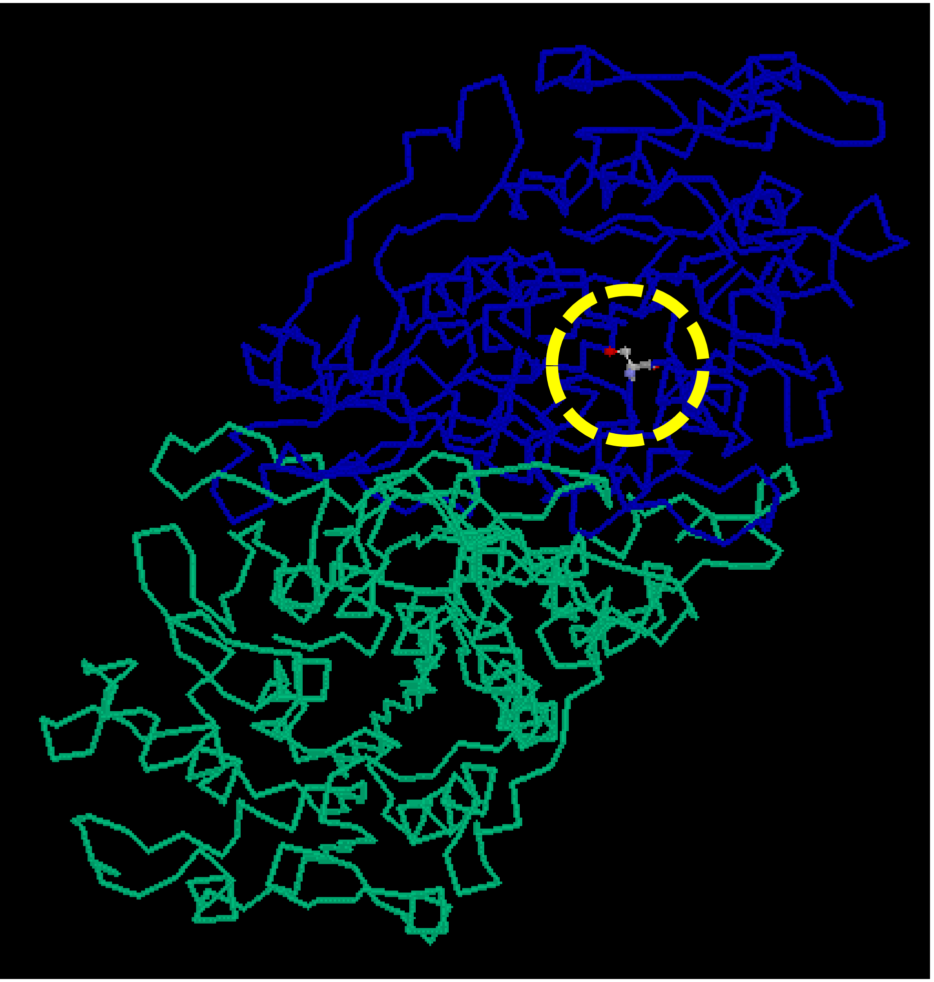




Bioinorganic Chemistry



The role of the metal ions



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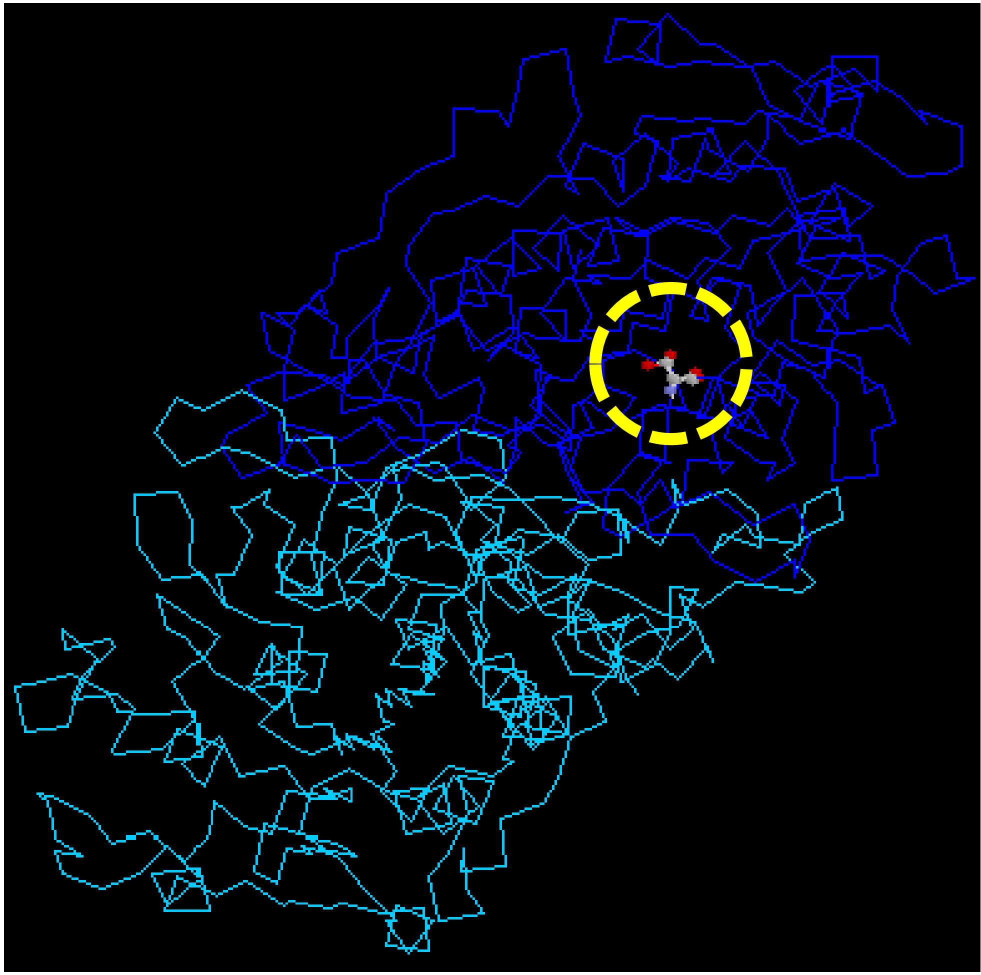
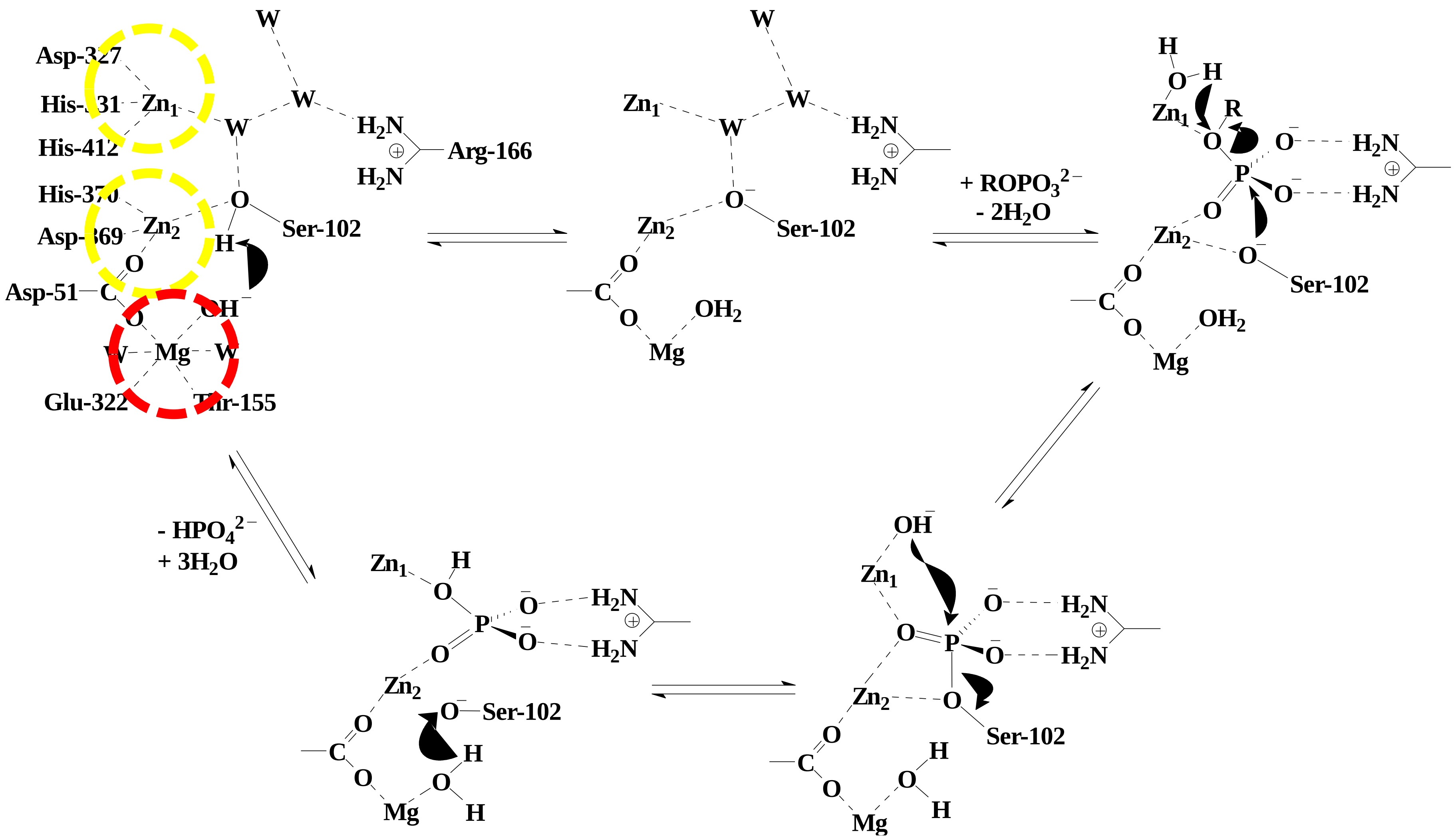


Pdb id: 1ED8

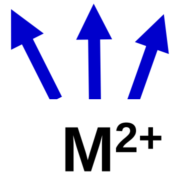
The role of the metal ions

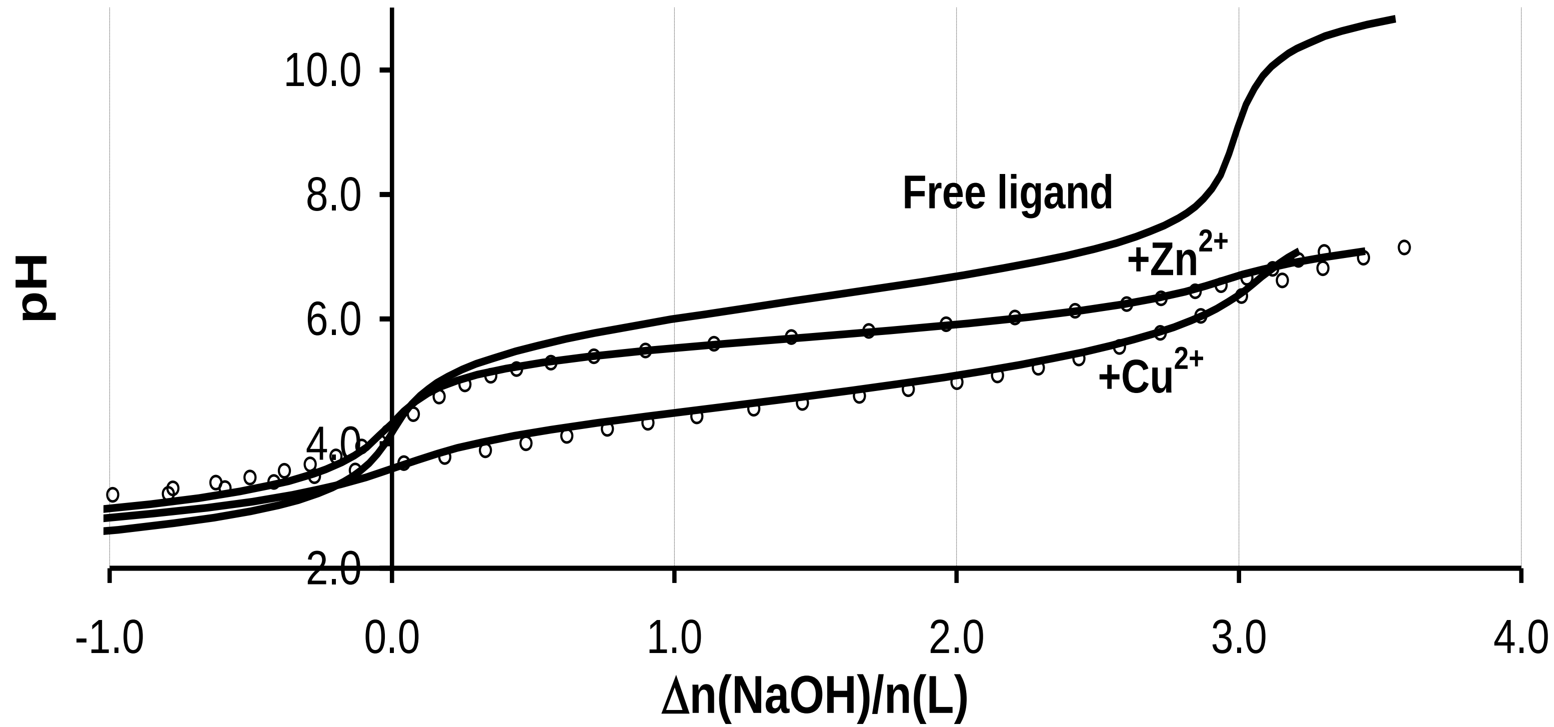


Models vs. Native biomolecules

Histidine triad enzymes: HXHXH motif

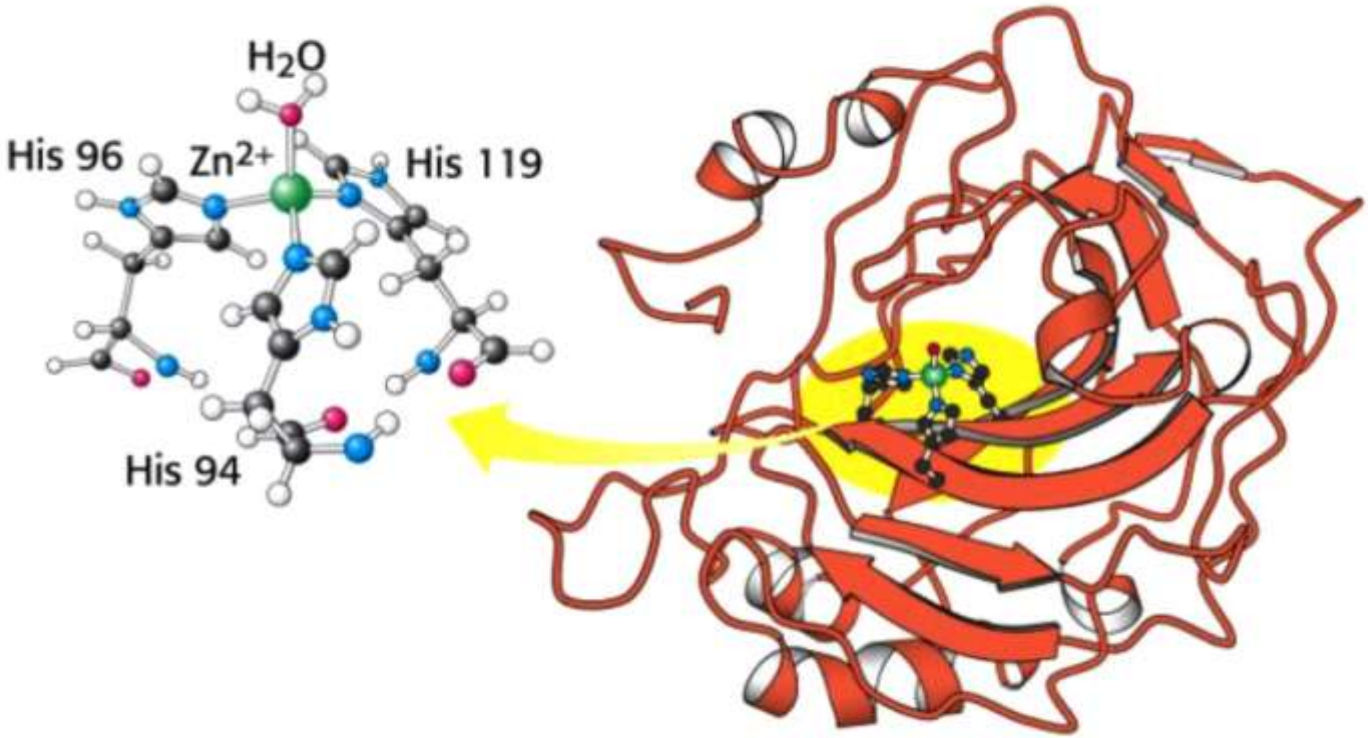
Copper(II) and zinc(II) complexes of the AcHPHPH-$CONH_2$ peptide





Jakab et al., Dalton Trans., 6987-6995 (2008).

Models vs. Native biomolecules



Bioinorganic zinc chemistry

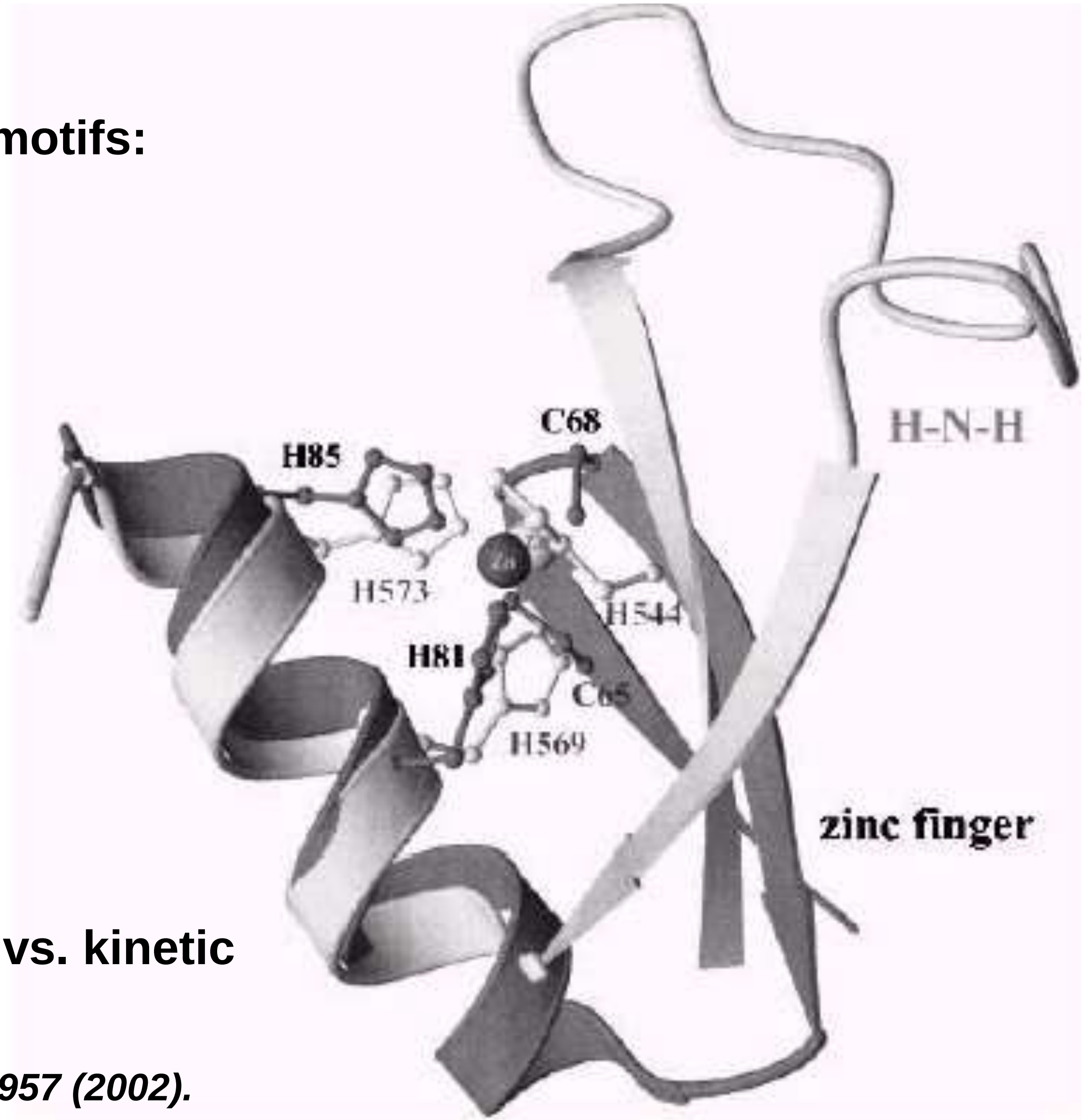
ββα-metal ion binding motifs:

Cys_2His_2 zinc finger

vs.

HNH:

$HHX_{14}NX_8HX_3H$



Redox properties
Role of metal ion
Geometry
Stability – thermodynamic vs. kinetic

Sui et al., Protein Sci., 11, 2947-2957 (2002).

Chris Oostenbrink | Sine Larsen | Lars Hemmingsen | Peter Thulstrup | Hans Christensen | Masamitsu Asaka | Kyosuke Nagata | Wojciech Bal

BOKU

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筑波大学
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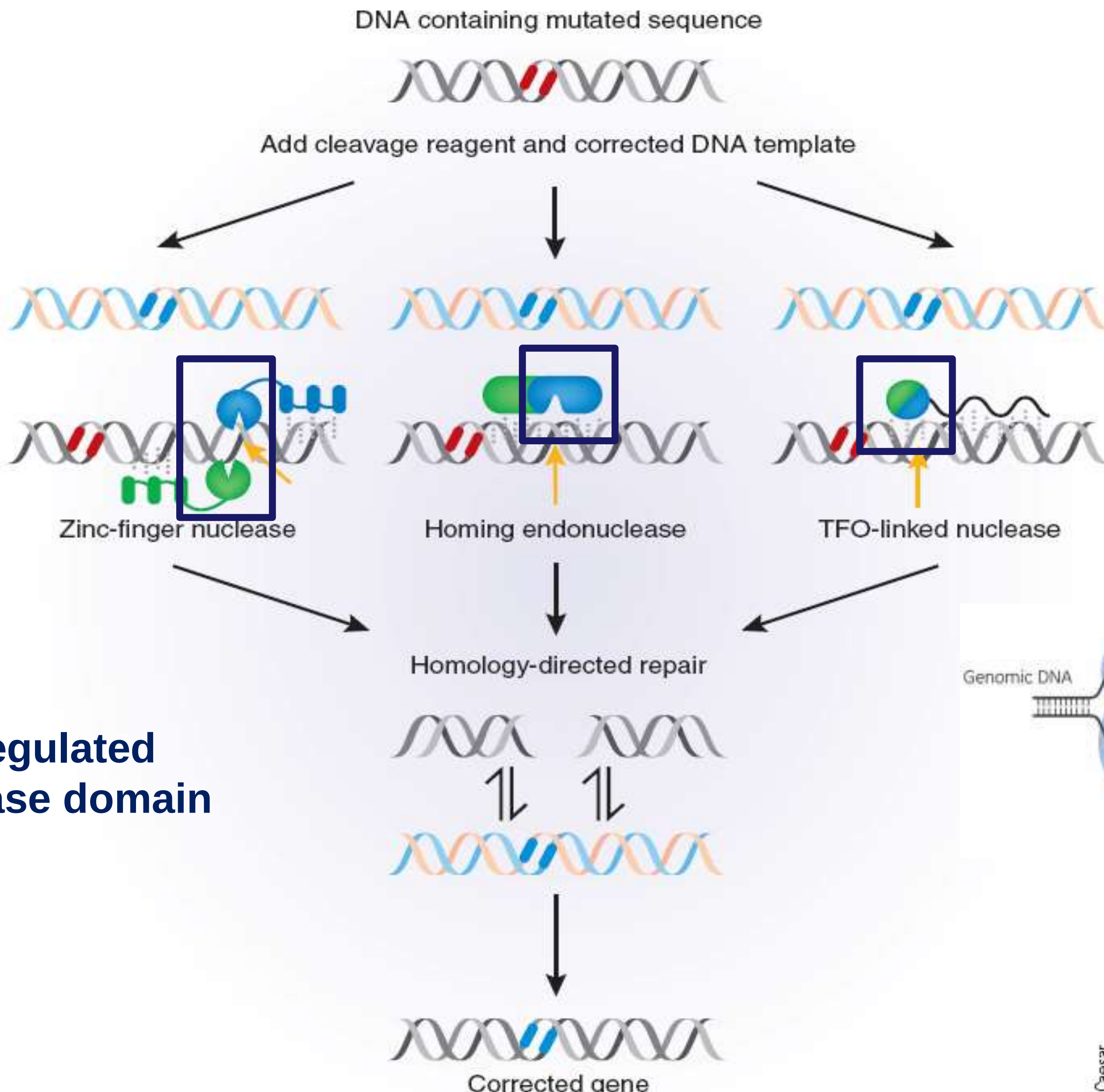
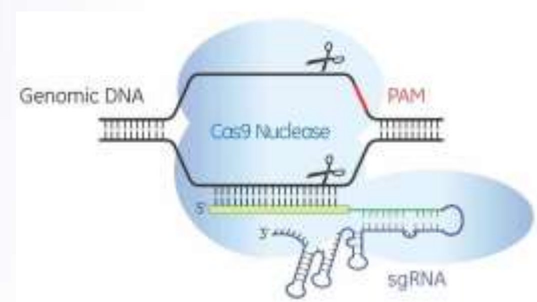
Artificial nucleases



Non-regulated nuclease domain



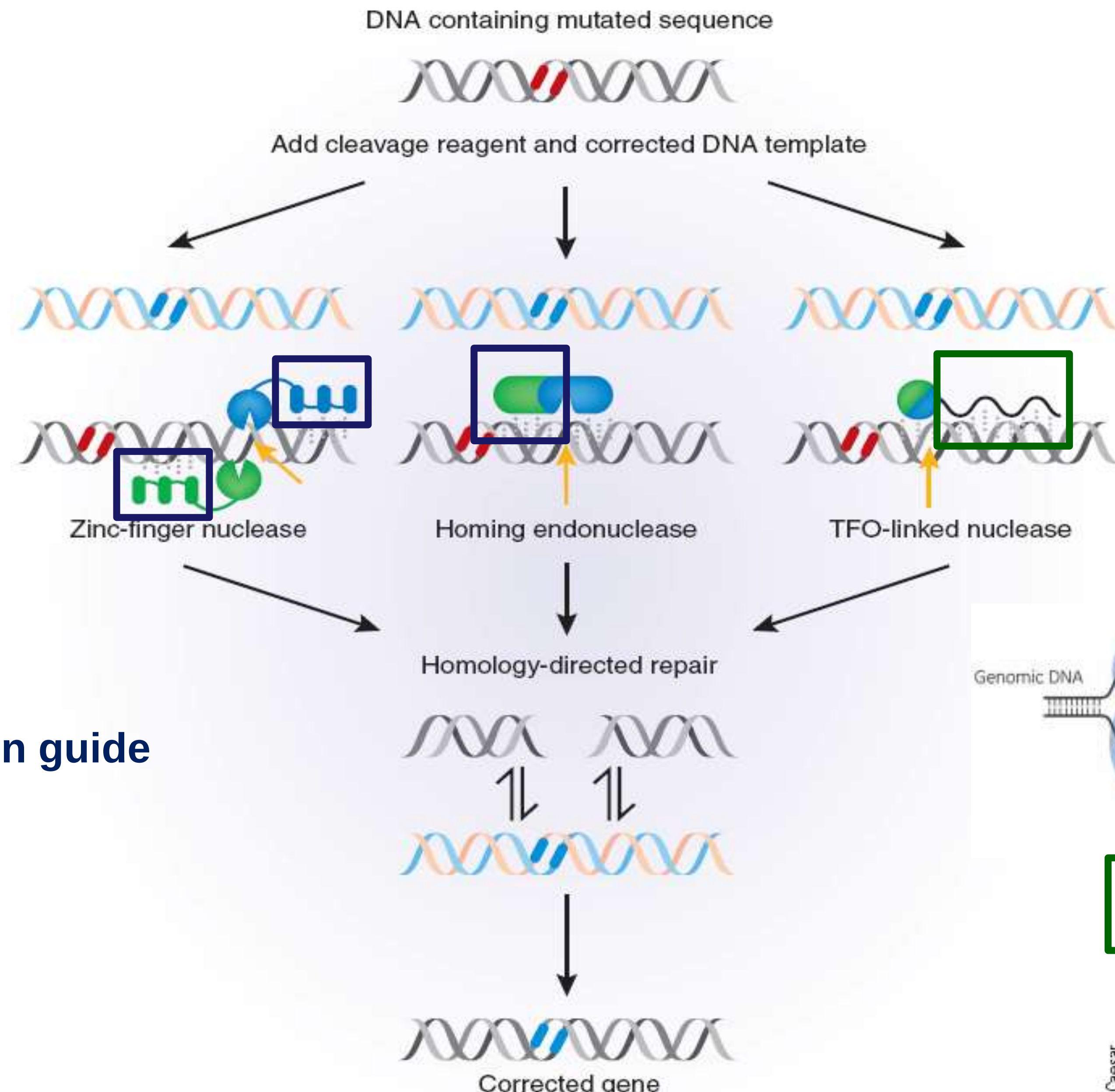
CRISPR/Cas9

A. Pingoud, G.H. Silva: Precision genome surgery; Nature Biotechnol., 25 (2007) 743.

Artificial nucleases

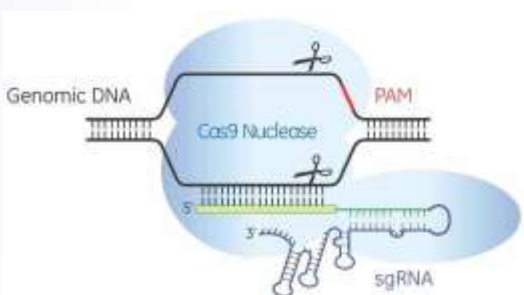
Artificial nucleases

Artificial nucleases



RNA guide

Protein guide



CRISPR/Cas9

A. Pingoud, G.H. Silva: Precision genome surgery; Nature Biotechnol., 25 (2007) 743.

Artificial nucleases

A guide to genome engineering with programmable nucleases

Table 2 | **Comparison of three classes of programmable nucleases***

	ZFNs	TALENs	RGENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	*FokI*	*FokI*	Cas9
Success rate‡	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate§	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb × 2	~3 kb × 2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)

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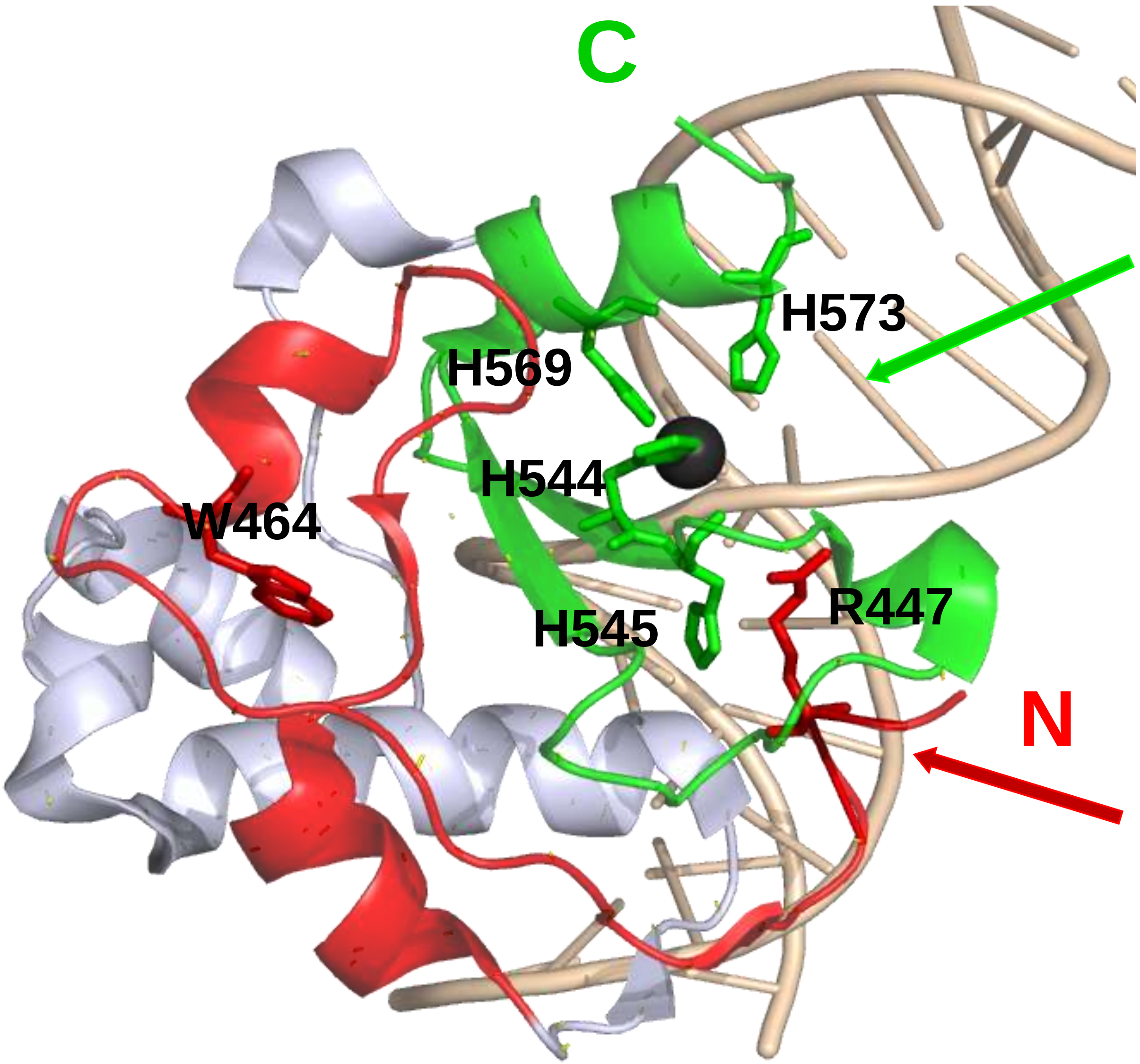
Aim of the work

To develop an artificial nuclease with a safe control mechanism:

The nuclease has to stop cleaving DNA in case of any damage of the enzyme within the cells.

Crystal structure of an NColE7 mutant in its DNA complex



The C-terminal HNH motif with Zn(II) is situated within the minor-groove.

The N-terminal KRNK sequence.

Y.-T. Wang, J.D. Wright, L.G. Doudeva, H.-C. Jhang, C. Lim, H.S. Yuan, J. Am. Chem. Soc. 131 (2009) 17345-17353; Protein Data Bank (PDB) ID: 3FBD

NColE7

1. ***Interaction between the N- and C-termini***

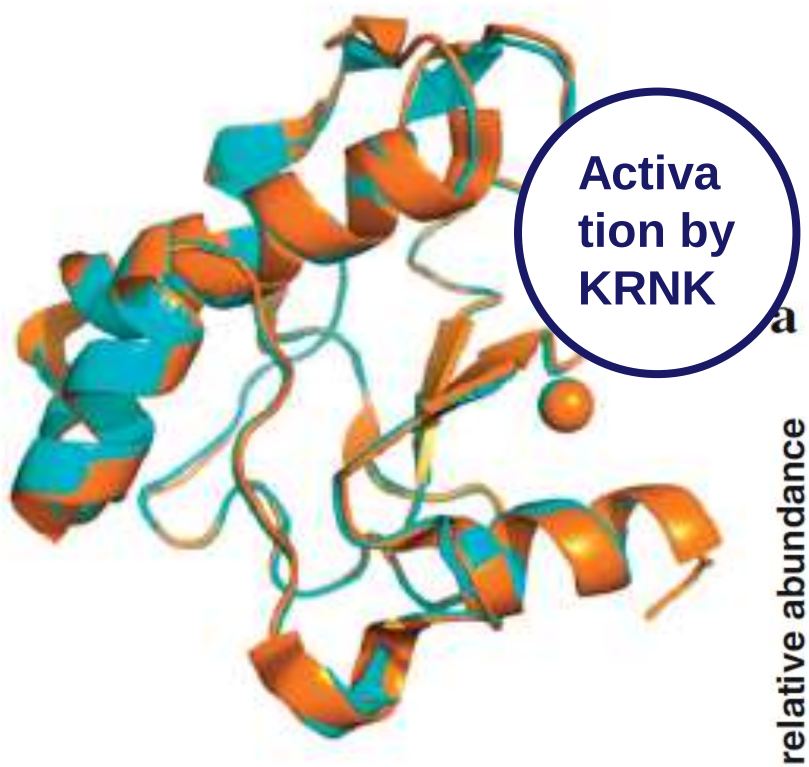
2. ***Fine tuning of the nuclease activity***

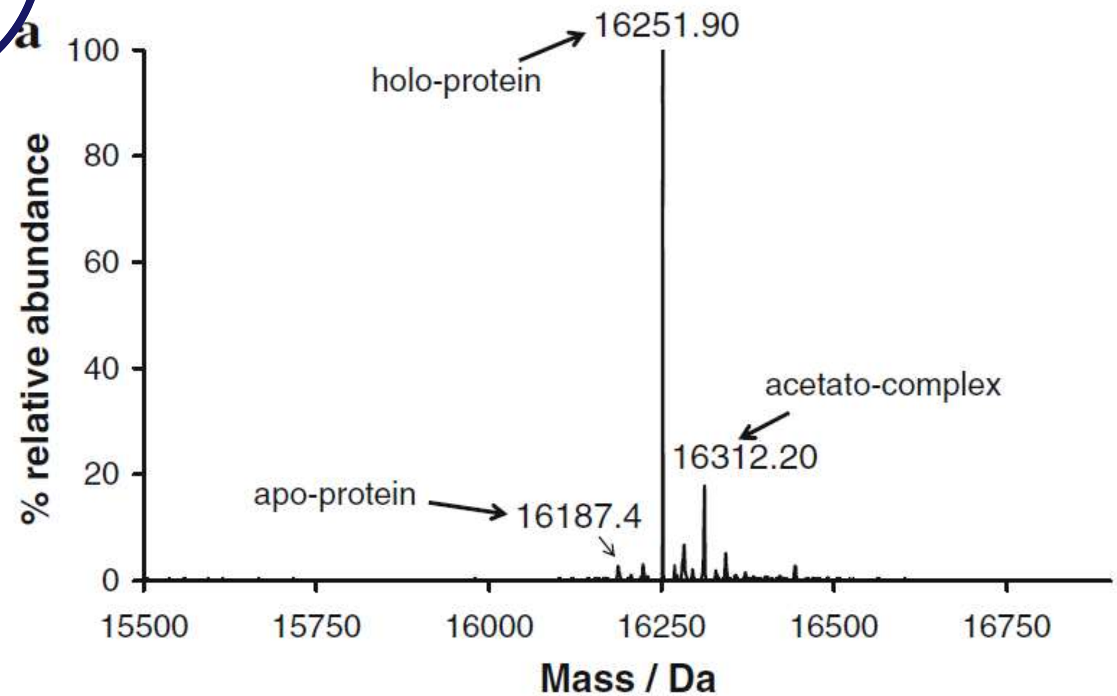
3. ***W464: stabilization of the structure of NColE7***

4. ***Inducible preorganized zinc(II)-binding site***

1. Interactions between the N- and C-termini

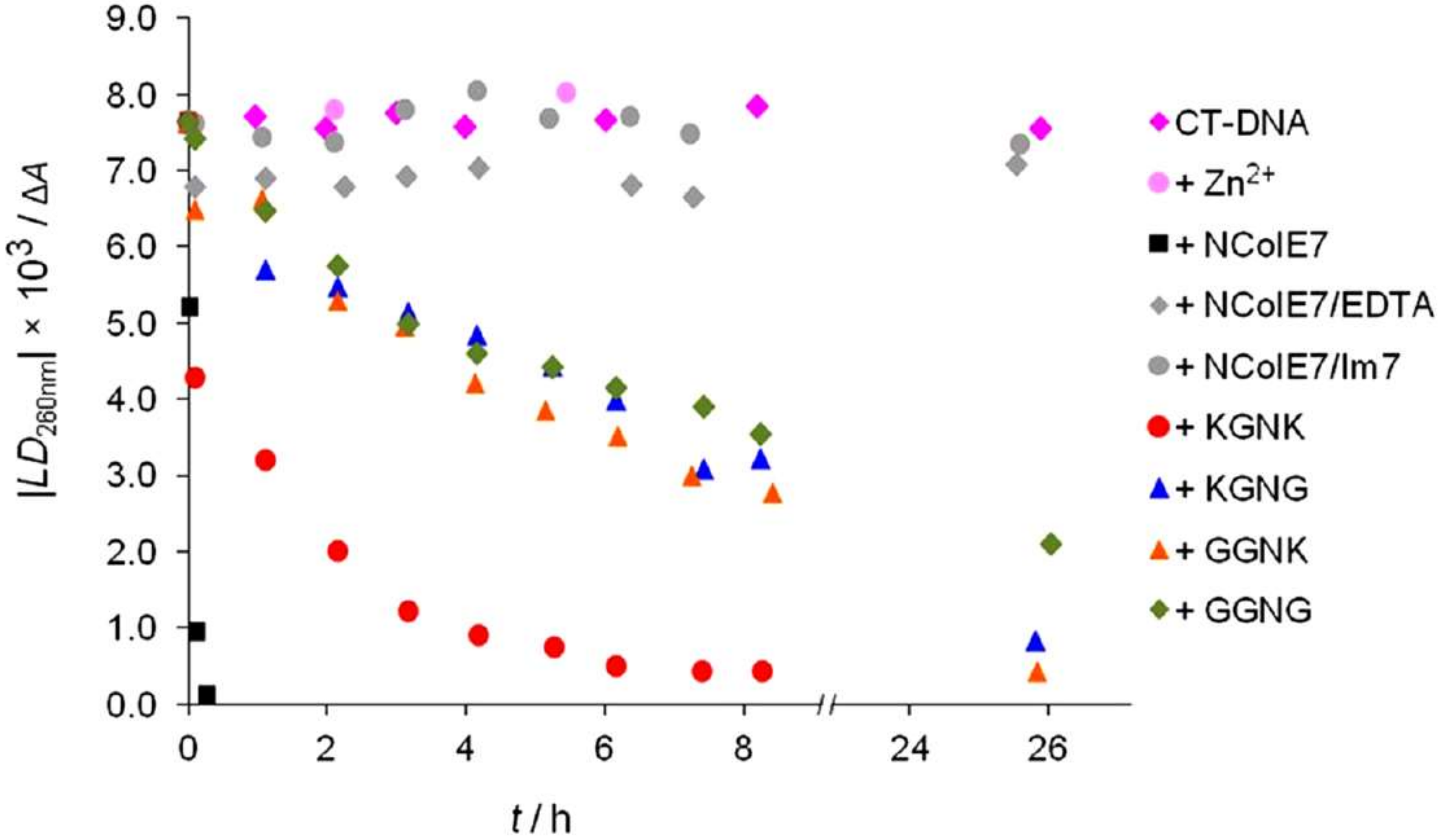
ΔN4-NColE7(-C*): ~~KRNK~~





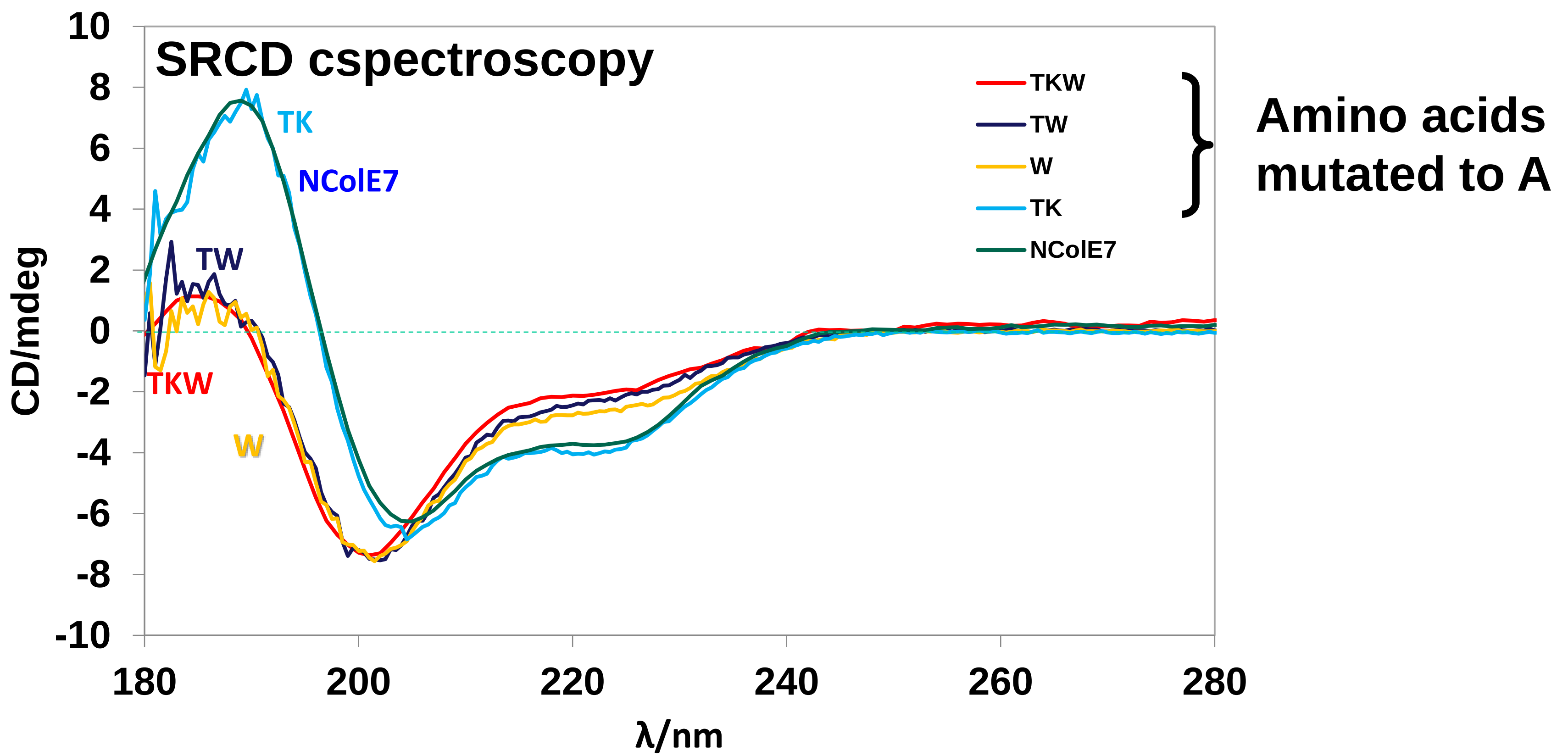
Gyurcsik, et al., J. Biol. Inorg. Chem., 18, 309-321 (2013); Acta Cryst. Sect F., 69 (2013) 551-554; Metallomics, 6 (2014) 2090-2099.

2. Fine tuning of the nuclease activity



Gyurcsik, et al.: Protein Sci., 23, 1113–1122 (2014)

3. W464: stabilization of the structure of NColE7

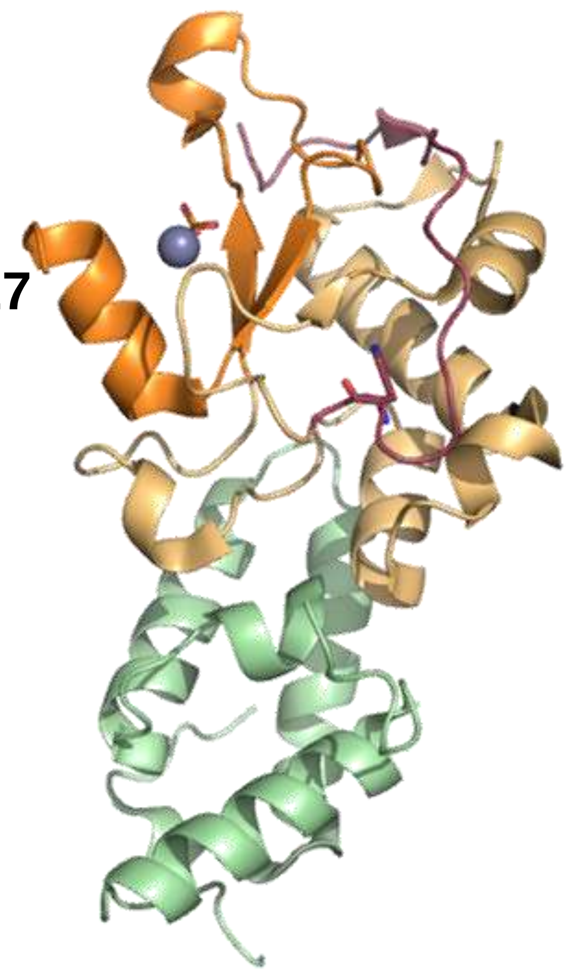


<u>K R N K</u> P G K A T G K G K P V N N K W L N N A G K D L G S P V P D R I A N K L R D K E F K S F D D F R K K F W E E V S K D P E L S K Q F S R N N N D R M K V G K A P K T R T Q D V S G K R T S F E L <u>H</u> <u>H</u> E K P I S Q N G G V Y D M D N I S V V T P K R <u>H</u> I D I <u>H</u> R G K

Gyurcsik, et al., J. Biol. Inorg. Chem., 19 (2014) 1295-1303; Gyurcsik, Curr. Prot. Pept. Sci., 17 (2016) 191-197; Gyurcsik, et al., J. Inorg. Biochem. 151 (2015) 143-149; Protein Sci., 25 (2016) 1977-1988.

4. Inducible preorganized zinc(II)-binding site

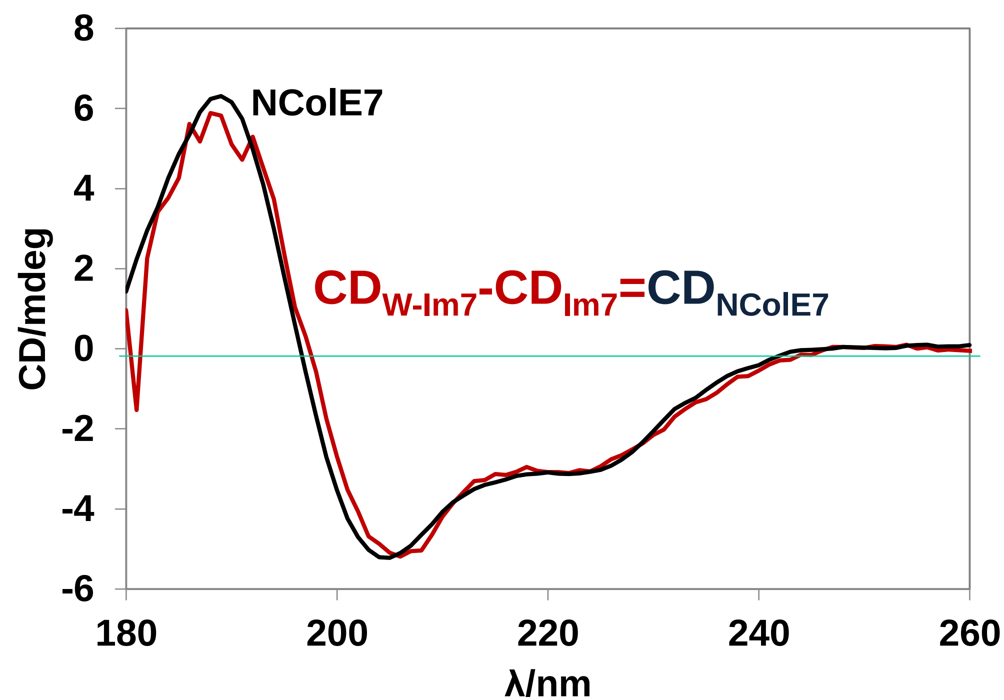
NColE7



Im7

Inducible preorganized metal ion binding site

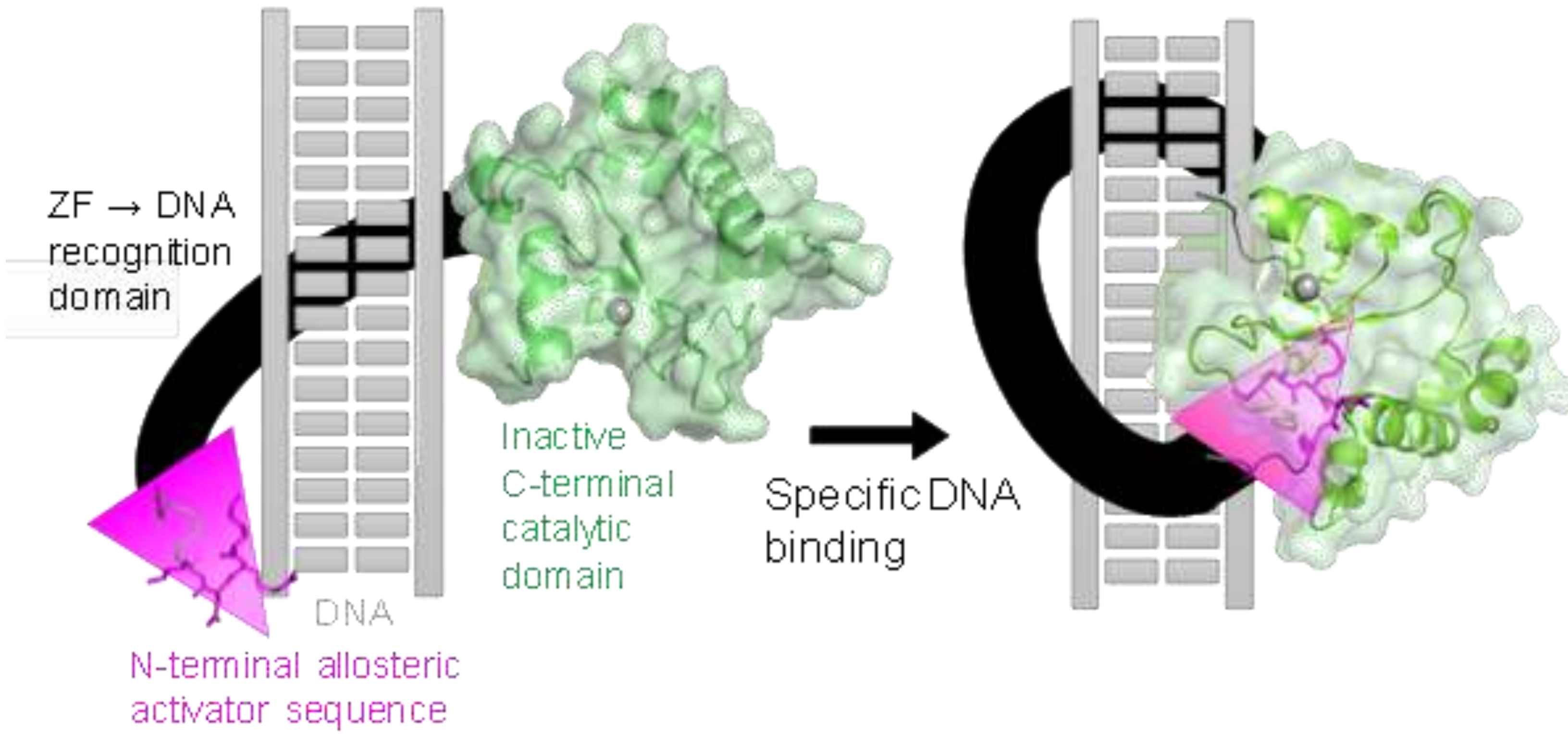
SRCD spectroscopy



Isothermal Titration Microcalorimetry Zn(II) binding

	$K_{d\ (Prot+Im7)}$	$K_{d\ (Prot)}$
NColE7	61 ± 18 nM	9.6 ± 3.2 nM
TKW	33 ± 23 nM	11 ± 1 μM
W	55 ± 25 nM	5.6 ± 0.3 μM
Im7	ND	

The concept of the NColE7-based artificial nuclease design



Split NColE7 into Nx regulatory and Cy catalytic segments and insert a zinc finger guide protein between them.

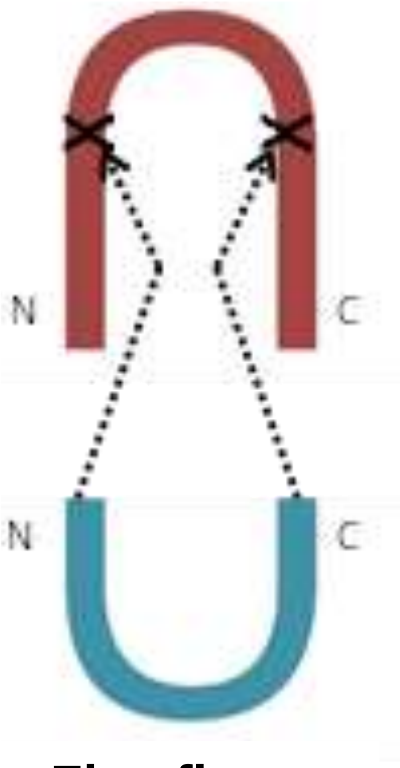
N-terminal control unit of NColE7	**Linker**	**Zinc Finger protein**	**Linker**	**C terminal catalytic unit of NColE7**

Protein design

Strategy:

Nx – ZF – Cy

NColE7

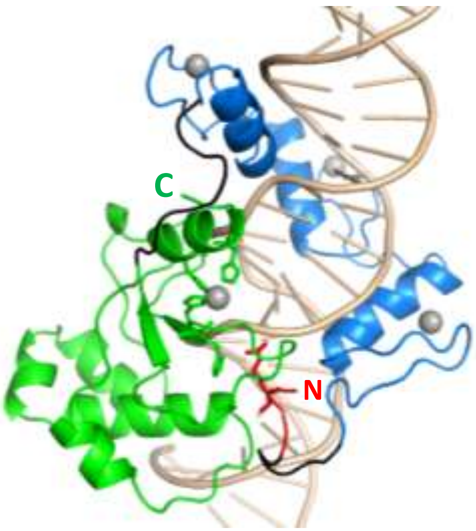


Zinc finger

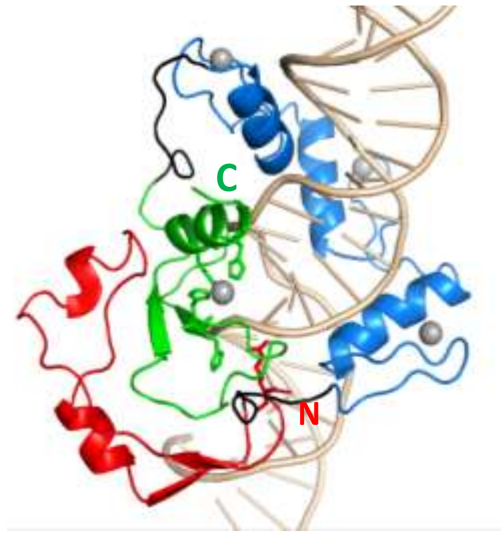
Gyurcsik, et al., J. Comp-Aid. Mol. Des., 28 (2014) 841-850.

Nx – ZF – Cy strategy

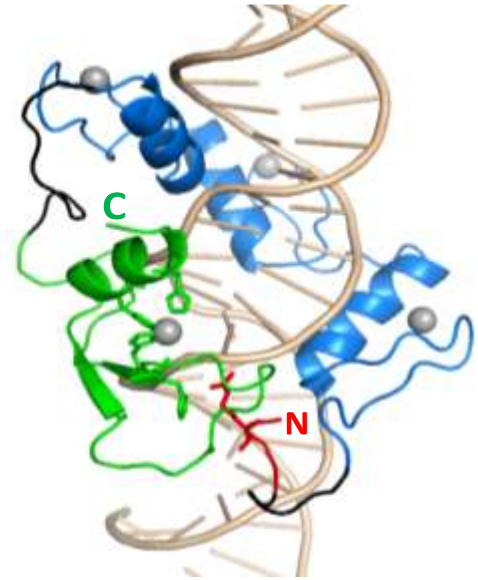
Protein expression and purification



N4-ZF-C105



N46-ZF-C45

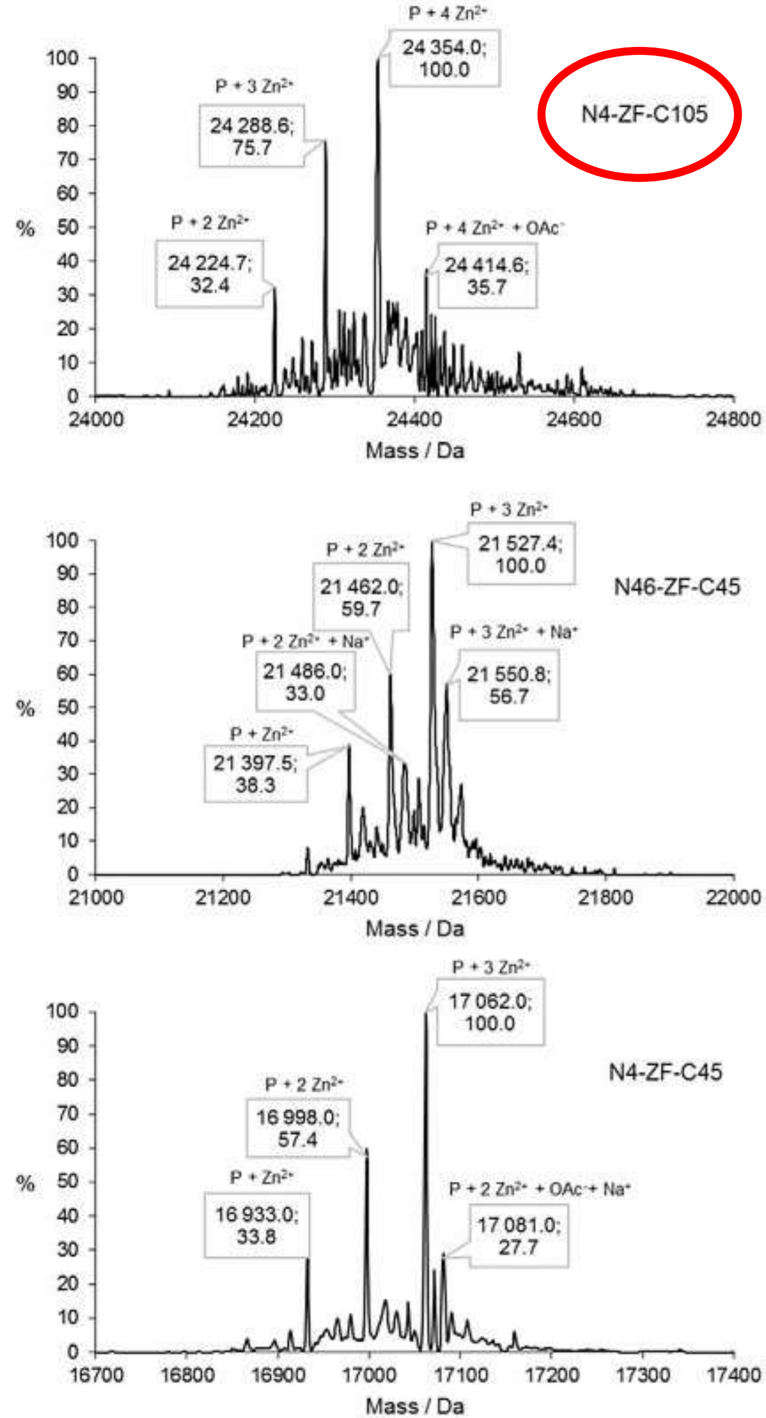


N4-ZF-C45

Gyurcsik, et al. ChemBioChem 19 (2018) 66-75.

Nx – ZF – Cy strategy

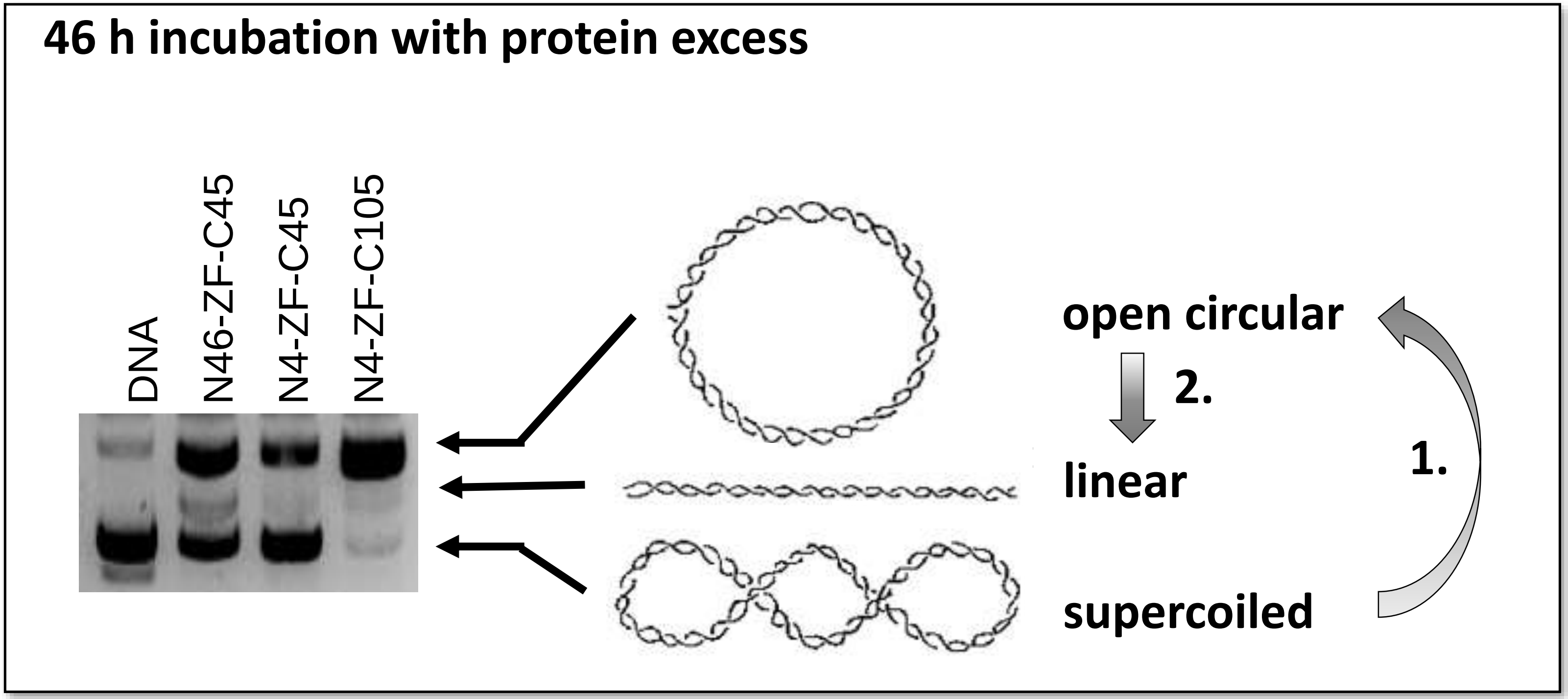
Protein expression and purification



Gyurcsik, et al. ChemBioChem 19 (2018) 66-75.

Nx – ZF – Cy strategy

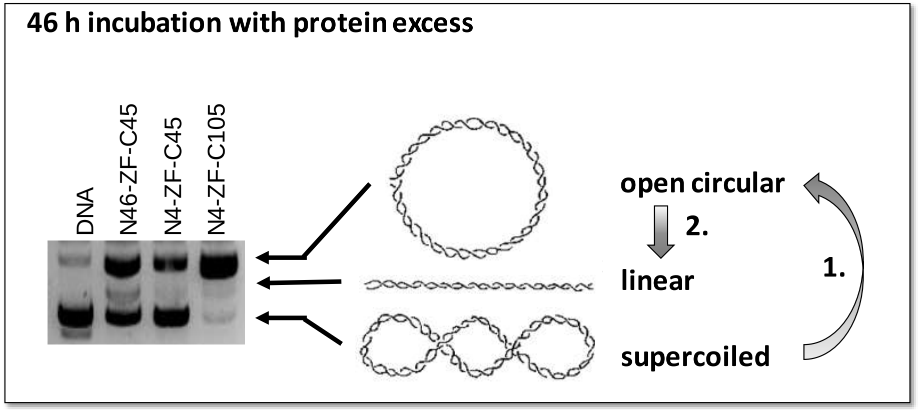
Catalytic activity - specificity

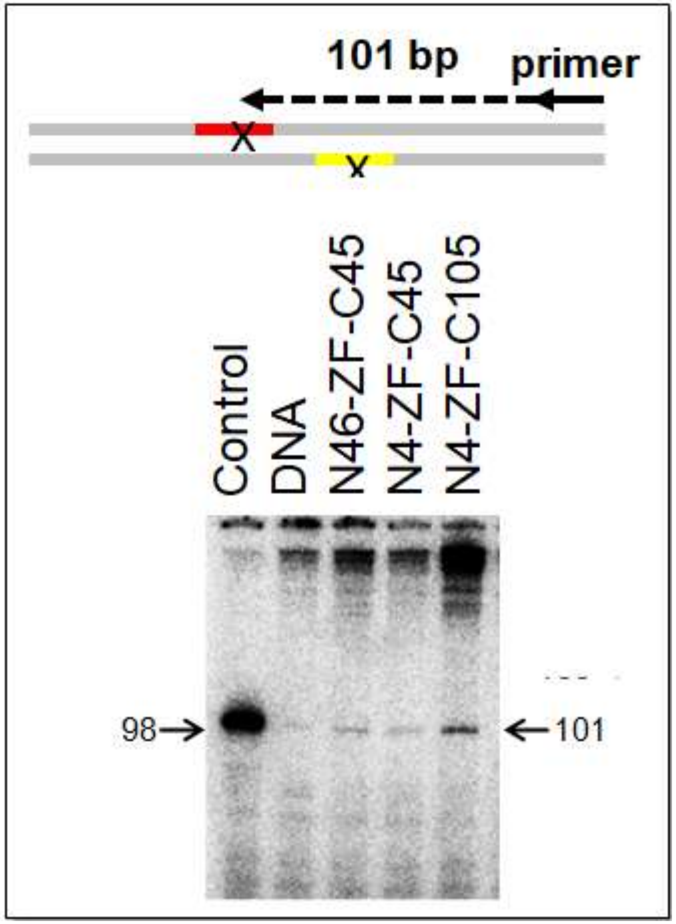


Gyurcsik, et al. ChemBioChem 19 (2018) 66-75.

Nx – ZF – Cy strategy

Catalytic activity - specificity

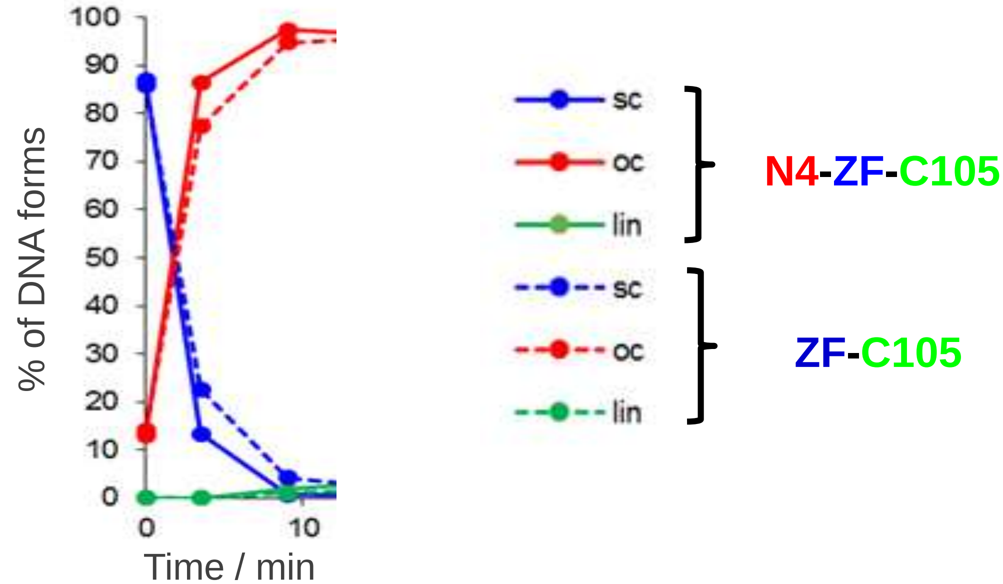




Gyurcsik, et al. ChemBioChem 19 (2018) 66-75.

Nx – ZF – Cy strategy

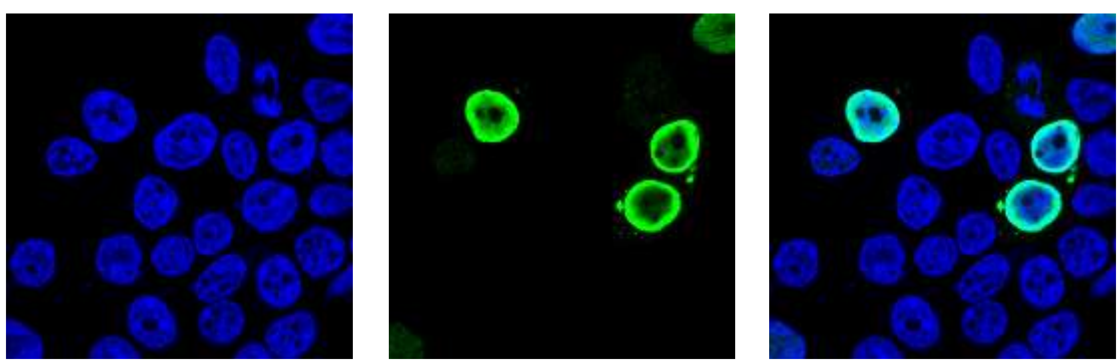
Allosteric control



Cellular localization on in HEK293T cells

DAPI | Anti-Flag | merged

N4-ZF-C105



Conclusions

- The formation of native-like structure of the artificial nucleases can be monitored through their zinc-binding ability.

- *The* Nx – ZF – Cy models showed low activity, they were specific, but not well regulated.

- The possibility of fine tuning and structure induction in combination with competition by the DNA binding proteins will lead us to a better artificial enzyme.

Acknowledgement

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BETWEEN ATOM AND CELL

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IN SCIENCE AND TECHNOLOGY

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