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Arbre-Mobieu Warsaw 2018 plenary meeting

INTRINSIC PROTEIN DISORDER COULD BE OVERLOOKED IN COCRYSTALLIZATION CONDITIONS: AN SRCD CASE STUDY

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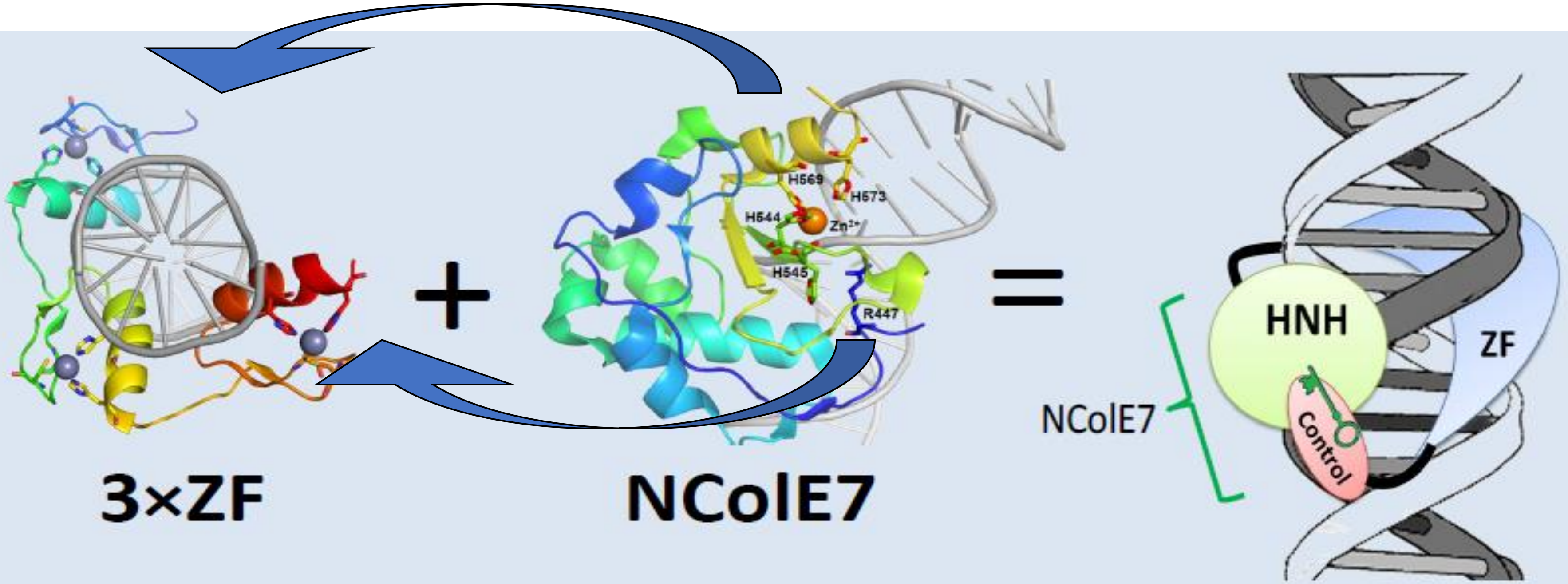
The concept of the new artificial nuclease design

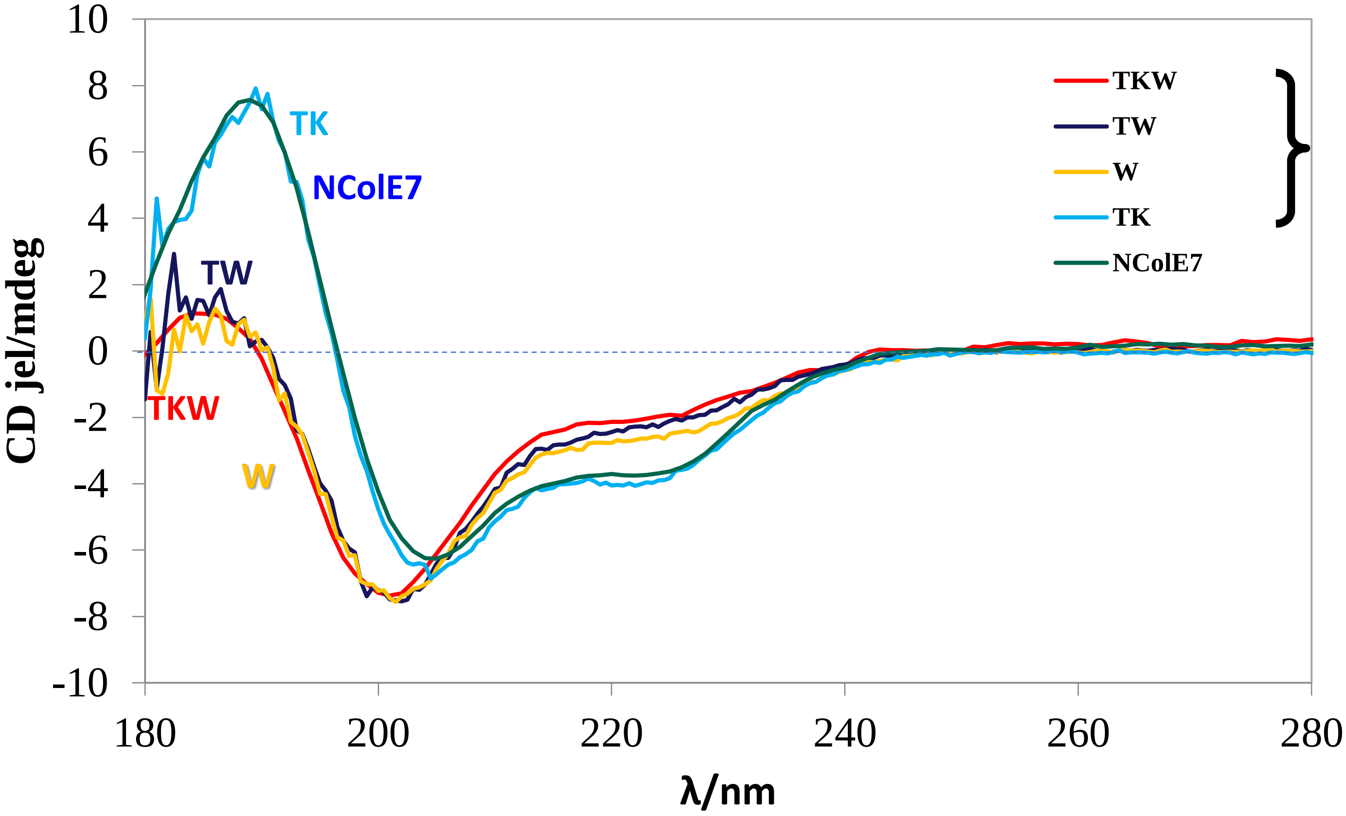


Protein design by means of computational techniques using the crystal structures of NColE7/DNA and ZF/DNA as starting structures

Gyurcsik et al. J Comput Aided Mol Des., 2014, 28, 841-850 .

The effect of W464 on the solution structure of NColE7

Circular dichroism spectroscopy



Amino acids mutated to A

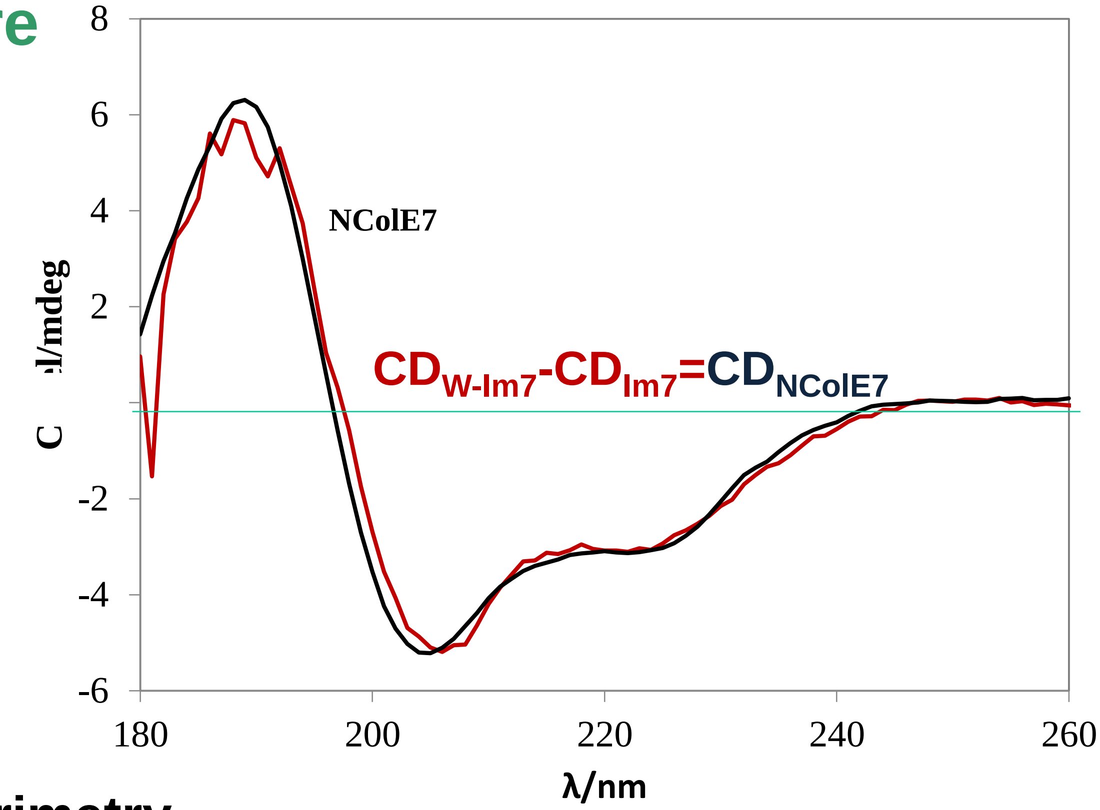
CDPro: 16% α-helix 22% β-sheet, 21% turn, 41% of unordered sequence

BeStSel: 11% α-helix 20% β-sheet, 18% turn, 51% of unordered sequence

Gyurcsik et al., J. Inorg. Biohem., 151, 143-149 (2015)

The effect of the Im7 protein on the solution structure and Zn(II) binding

Circular dichroism spectroscopy



$CD_{W\text{-}Im7}\text{-}CD_{Im7}=CD_{NColE7}$

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	

Inducible preorganized metal ion binding site

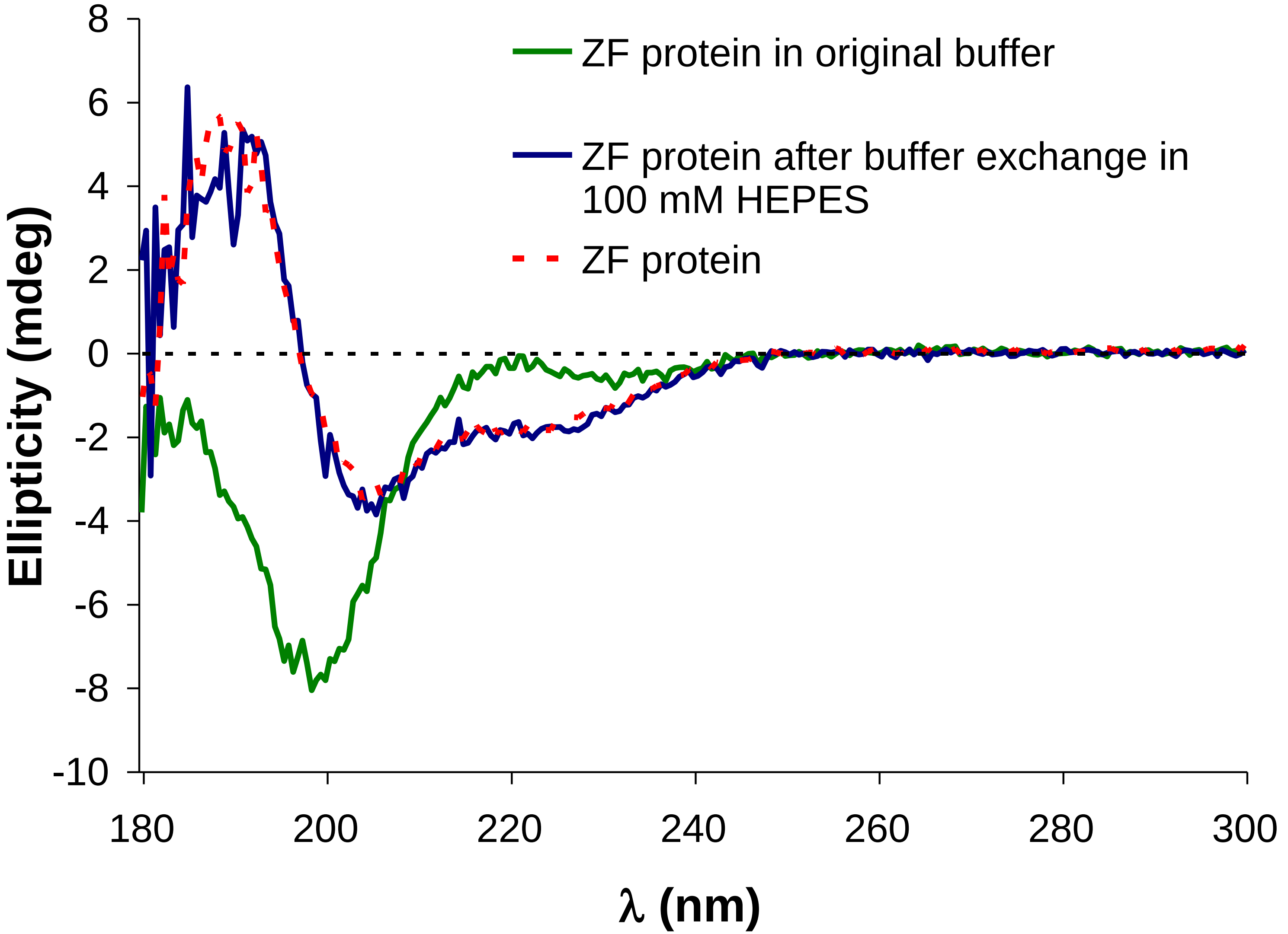
Effect of the interacting partners and metal ion on the structure of the NColE7 protein

- Im7 or DNA induced the protein folding of the NColE7 nuclease mutants.

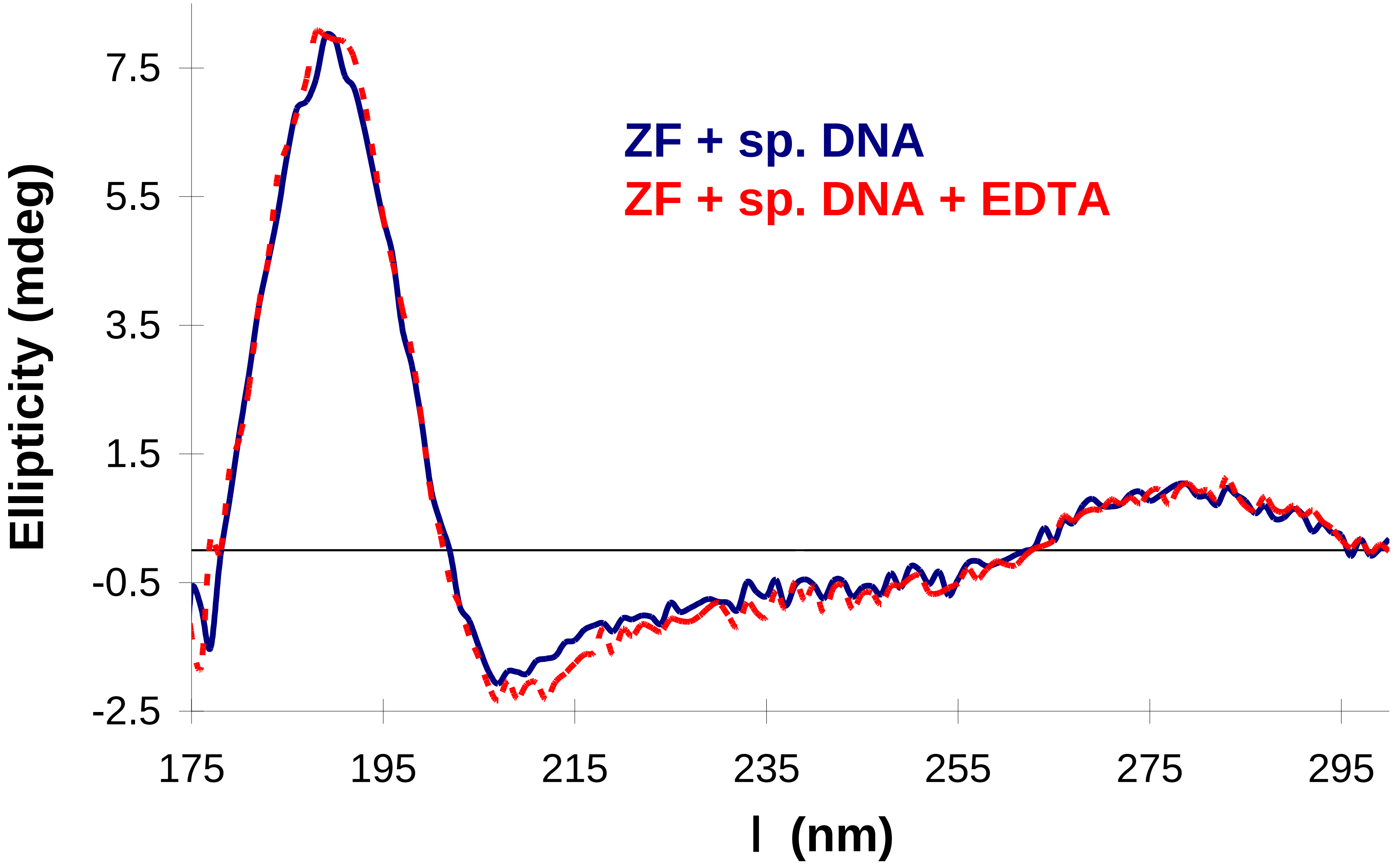
- 32 crystal structures of NColE7/9 or mutants, but only 4 without interacting partners

- Investigate the enzyme structures in solution in the absence of their inhibitors or substrate analogue compounds, which may cause structural changes of the enzyme.

CD spectra of the Zinc-Finger (ZF) protein with and without Zn(II)



Effect of the DNA on Zn(II) binding of the ZF protein



Buffers and intearcting partners may influence the metal ion binding and the structure of the proteins

- The minor metal ion contamination of buffers may cause significant changes in metal ion binding properties and thus, function of proteins.

- DNA may stabilize a protein fold and metal ion binding either kinetically or thermodynamically.

- Investigate the protein structures in solution in the absence of their interacting partners.

Acknowledgement

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