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

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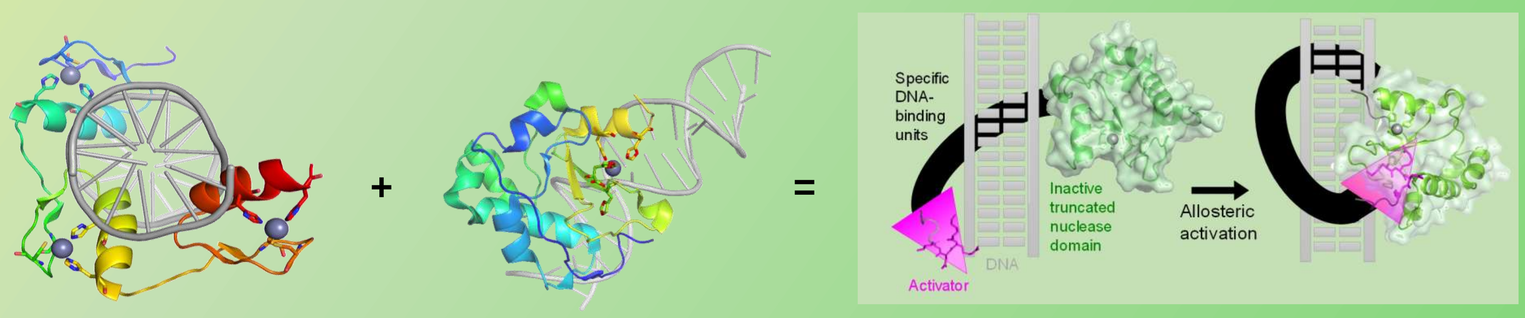
INTRODUCTION

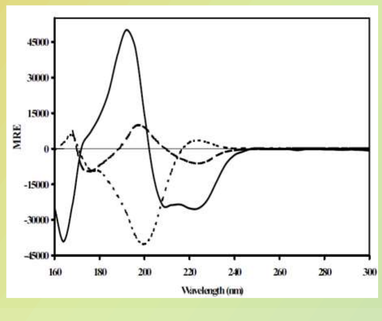
The aim of our work is to construct a new type of artificial nuclease, based on zinc finger scaffold and the nuclease domain of the Colicin E7 enzyme (NColE7). The latter needs the cooperation of the C-terminal zinc-binding catalytic motif and the N-terminal basic amino acid sequence for its function, providing opportunity for the enzyme regulation. We have studied the crystal and/or solution structures of the components of the new enzyme, to better understand their properties for the design optimization.

The concept of the artificial nuclease design

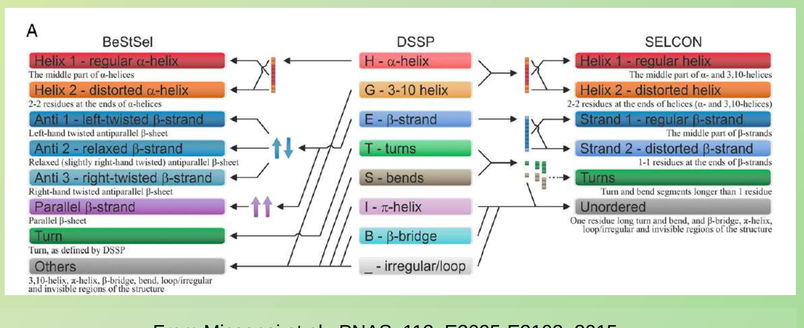


X-ray diffractometry dominates protein studies, as it can provide 3D structures of these diverse macromolecules or their molecular complexes with interacting partners. Disordered proteins are difficult to crystallize on their own. Therefore, the standard procedure involves the cocrystallization with substrates, inhibitors and/or cofactors. In this work we show that under cocrystallization conditions, the results could reflect induced protein folds instead of the (partially) disordered original structures (E. Németh, R.K. Balogh, K. Borsos, A. Czene, P.W. Thulstrup, B. Gyurcsik, Protein Sci., 25, 1977-1988, 2016).

Protein Circular Dichroism

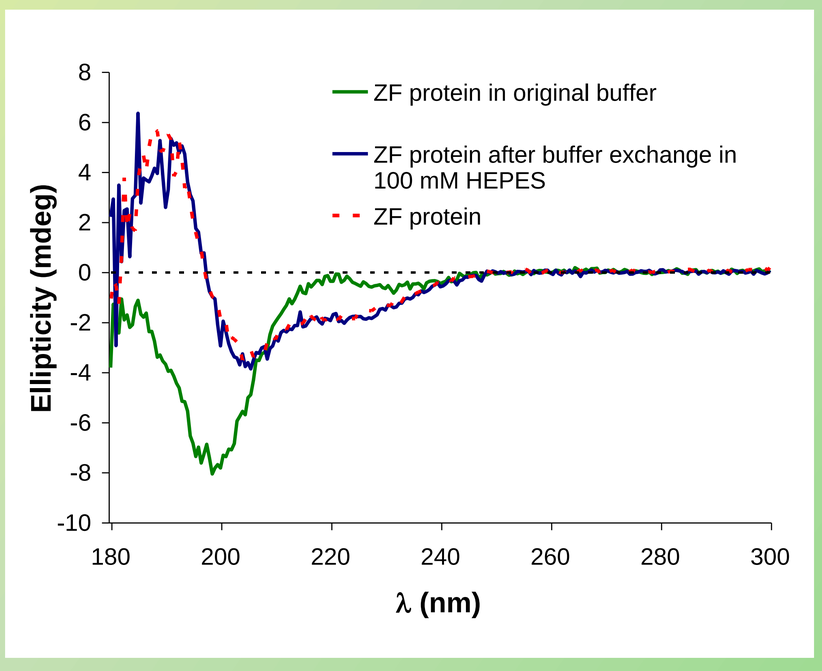


From B.A. Wallace and R.W. Janes, 2009.



From Micsonai et al., PNAS, 112, E3095-E3103, 2015.

Zinc finger proteins



The apo zinc finger protein displays disordered structure. Buffer exchange with solution made from commertial HEPES results in structure stabilization – the CD spectrum is identical to that of the holo-protein.

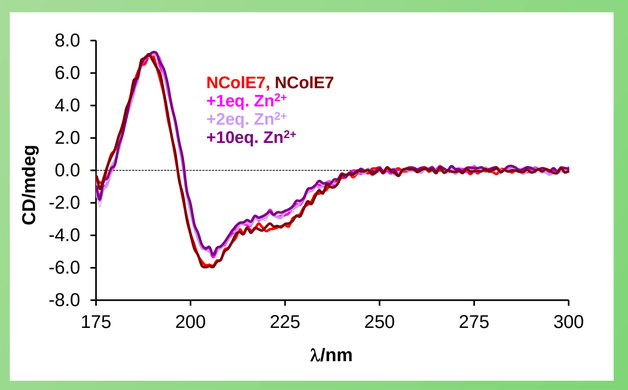
The metal ions can be removed from the zinc finger protein resulting in a disordered structure. The interaction with DNA, however, stabilizes the original protein structure. Is the metal ion removed by EDTA?

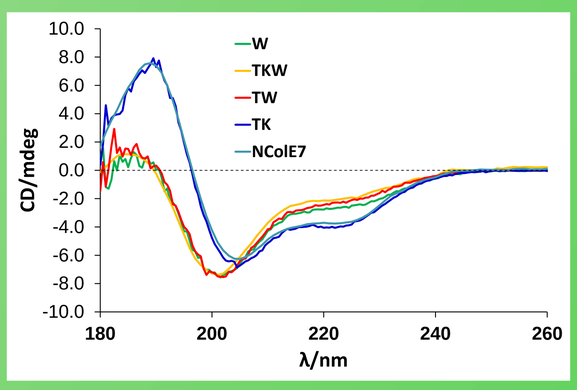
CONCLUSIONS

Our results draw attention to the possible structural consequence of protein modifications, which is often hidden by compensational effects of intermolecular interactions. Induced folding is an essential way of protein regulation. To better understand these processes, we emphasize the importance of understanding the structure and interactions of intrinsically disordered proteins. This research demands more extensive complementary experiments in solution phase with the unligated form of the protein of interest.

NColE7 and its mutants

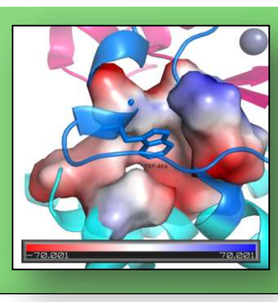
The analysis of synchrotron radiation circular dichroism spectra revealed that the Im7 immunity protein stabilizes the native-like solution structure of unfolded NColE7 nuclease mutants via complex formation. This is consistent with the fact that among the several available crystal structures with its inhibitor or substrate, all NColE7 structures are virtually the same.

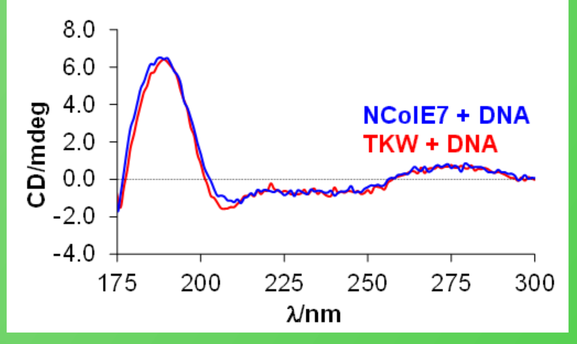




Protein	Data		α-Helix	β-Sheet	Turn	Unordered
NColE7	SRCD	CDPro	0.32	0.16	0.20	0.32
		CDNN	0.30	0.26	0.20	0.24
		BeStSel	0.20	0.21	0.16	0.43
	SRCD (with Zn^{2+})	CDPro	0.28	0.24	0.21	0.27
		CDNN	0.29	0.26	0.20	0.25
		BeStSel	0.16	0.24	0.16	0.44

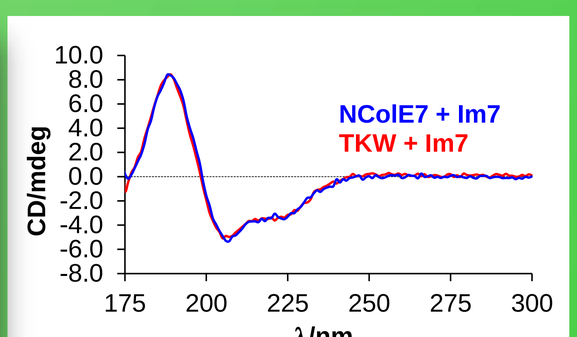
Protein	Data		α-Helix	β-Sheet	Turn	Unordered
TKW	SRCD	CDPro	0.16	0.22	0.21	0.41
		BeStSel	0.11	0.20	0.18	0.51

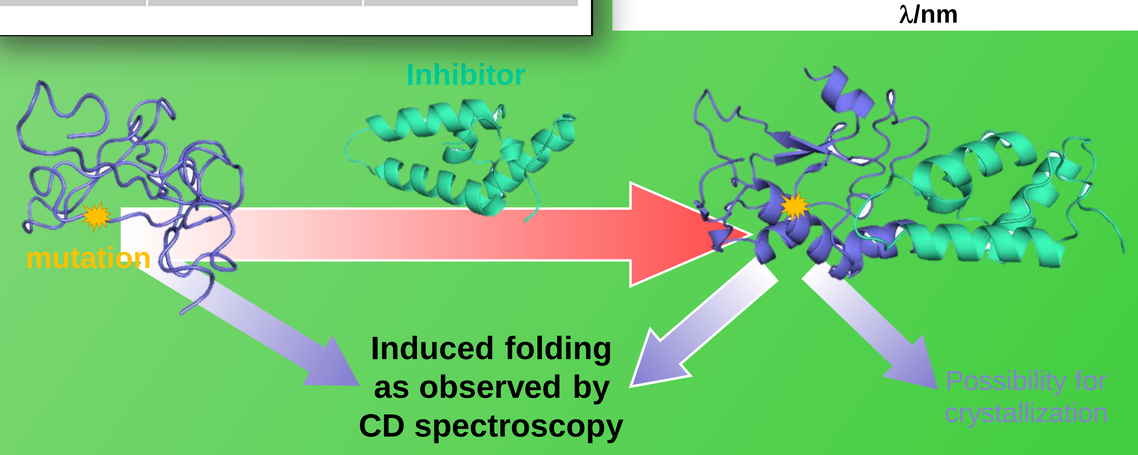




Zn^{2+}-AFFINITY

	K_d (Prot)	K_d (Prot+Im7)
NColE7	9.6 ± 3.2 nM	61 ± 18 nM
TKW	11 ± 1 μM	33 ± 23 nM
W	5.6 ± 0.3 μM	55 ± 25 nM





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