AFFINITY PROTEIN PURIFICATION RESULTING IN PROTEIN SEQUENCE WITHOUT REMAINING AMINO ACID RESIDUES

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INTRODUCTION

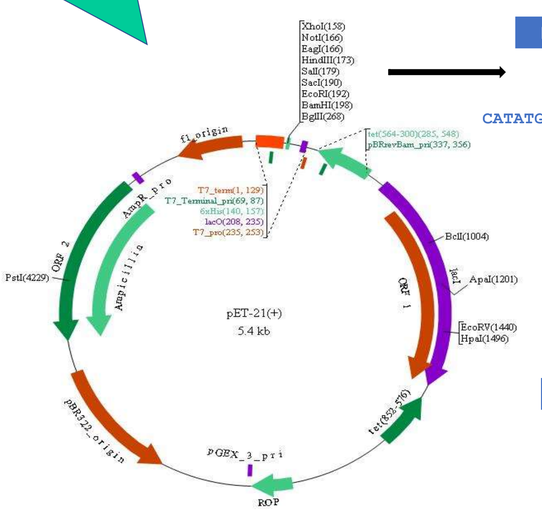
Purification of proteins is efficiently carried out by affinity chromatographic methods. The specific interaction with the appropriate resins requires fusion affinity tags, such as e.g. the oligo-His, the maltose binding protein or glutathione-S-transferase tag. These sequences are encoded by the plasmids used for protein expression. The affinity tags have to be cleaved off after the target protein is selectively bound to the solid support. This is performed by specific proteases. These enzymes are expensive and mostly they leave few extra amino acids at the terminus, which may interfere with the structure and function of the purified protein.

Our recent studies focused on the protein constituents of new artificial nucleases. Zinc finger nucleases (ZFNs), proteins designed to cut DNA at specific sequences are becoming powerful tools in gene targeting. The computer design of such enzymes requires the precise protein sequence to be obtained. Using the Ni(II)-affinity chromatography for e.g. zinc finger protein purification requires the complete removal of the oligo-His affinity tag. However, this was not possible to achieve by the traditional methods. Therefore, we redesigned the cloning region of pET21a plasmid for expression of fusion proteins that can be cleaved by nickel(II) ions to obtain the desired target sequence.

RESULTS - 1. DESIGN OF THE PLASM ID

The new approach is to cleave the His-tag by Ni(II) instead of proteases. Therefore, the new plasmid encodes the affinity tag with a Ni(II)-sensitive cleavage site at its N-terminus. The precise gene of the protein is inserted into the cloning region by the help of BsmBI restriction endonuclease, so that the recognition site is deleted in the cleavage/ligation procedure.

pET21a plasmid



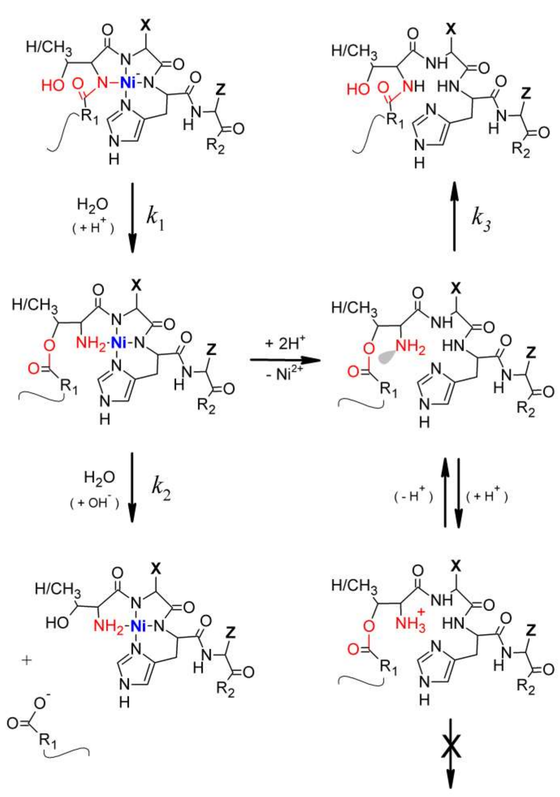
NdeI	Restriction enzymes	6X His-tag	Stop

CATATG...GGATCCGAATTCGAGCTC...AAGCTT...CTCGAGCACCACCACCACCACCACTGA

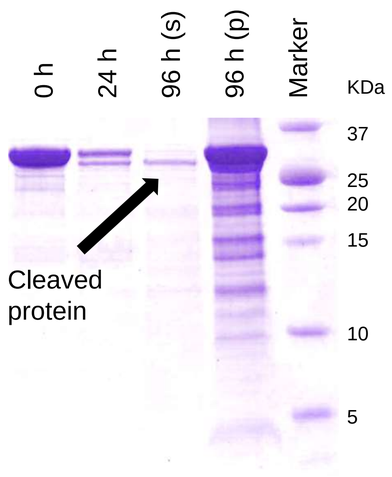
Cloning Region Redesign

NdeI	Restriction enzymes	SRHS code	Linker	6X His-tag	Stop

Ni(II)-dependent hydrolysis of the X-(Ser/Thr)-X-His-X sequence



Cleavage of the catx-ZF#-regy-SRHS-L-His-tag protein at the SRHS sequence was performed in a pilot experiment using a 100-fold excess of Ni(II) ions at 52 ºC in Tris buffer (pH = 7.9). A part of the protein precipitated.



Molecular mechanism from: E. Kopera, A. Krezel, A. M. Protas, A. Belczyk, A. Bonna, A. Wyslouch-Cieszynska, J. Poznanski, W. Bal, Inorg. Chem. 49 (2010) 6636-6645.

RESULTS - 2. DNA CLONING , PROTEIN EXPRESSION AND PURIFICATION

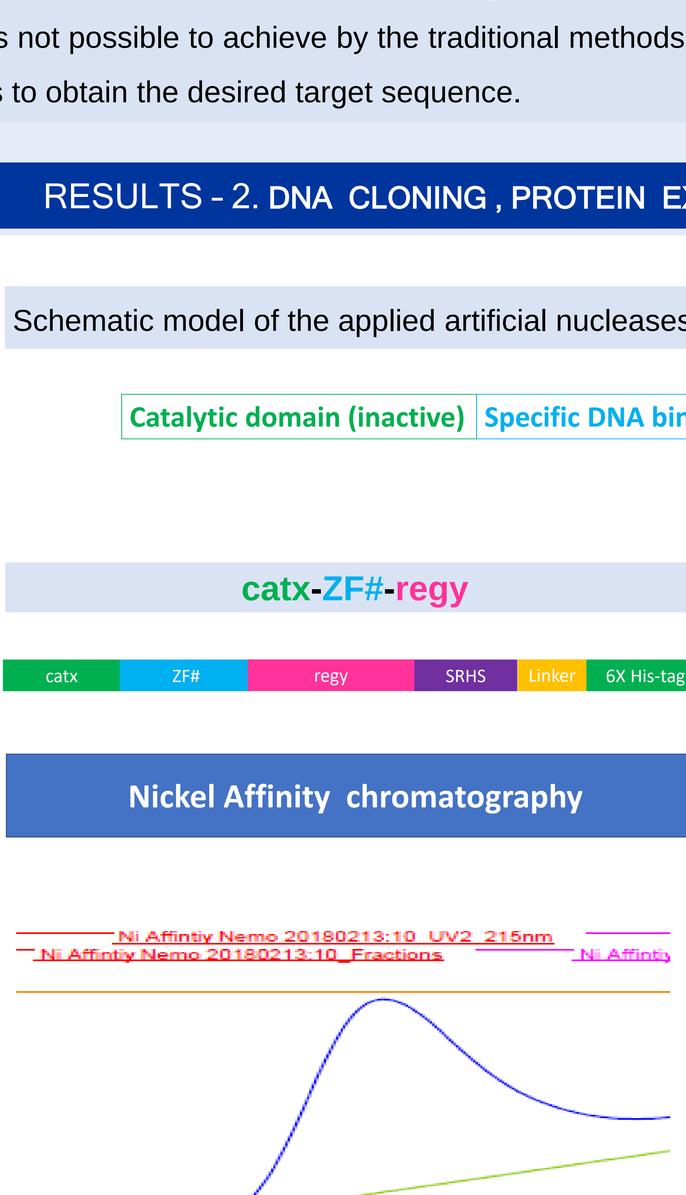
Schematic model of the applied artificial nucleases:

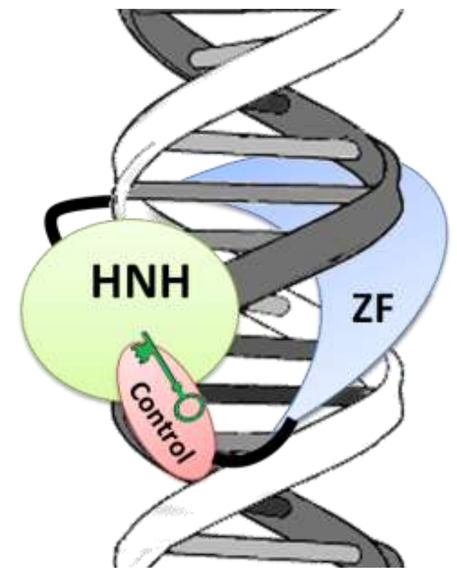
Catalytic domain (inactive)	Specific DNA binding domain	Control

catx-ZF#-regy

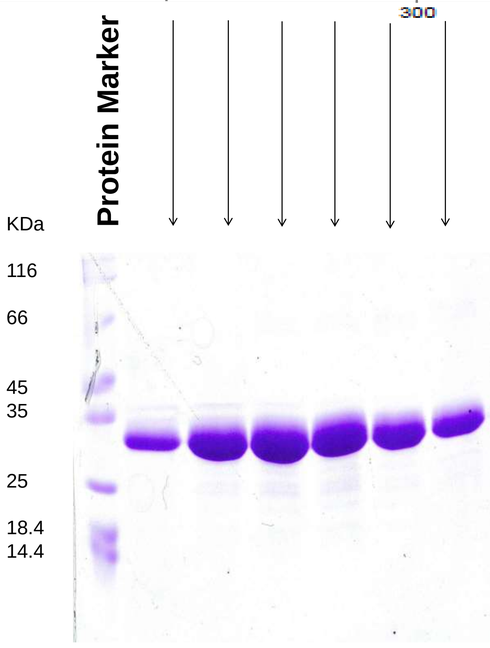
catx	ZF#	regy	SRHS	Linker	6X His-tag

Nickel Affinity chromatography

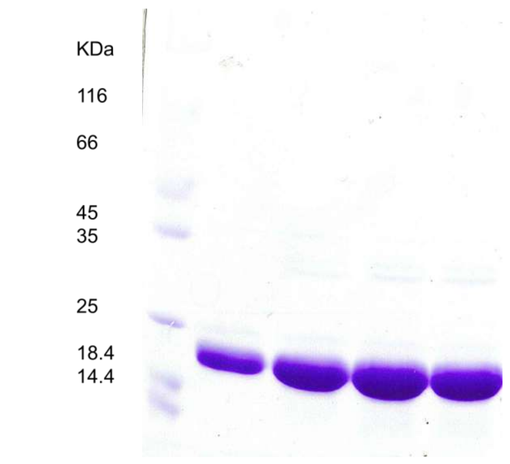




catx-ZF#



catx	ZF#	SRHS	Linker	6X His-tag



FUTURE PLANS

- Optimization of the Ni(II) cleavage conditions.
- Mass determination and characterization of proteins by Mass spectrometry.
- Redesign of zinc finger nucleases to cleave DNA at specific sequence for gene therapy.

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