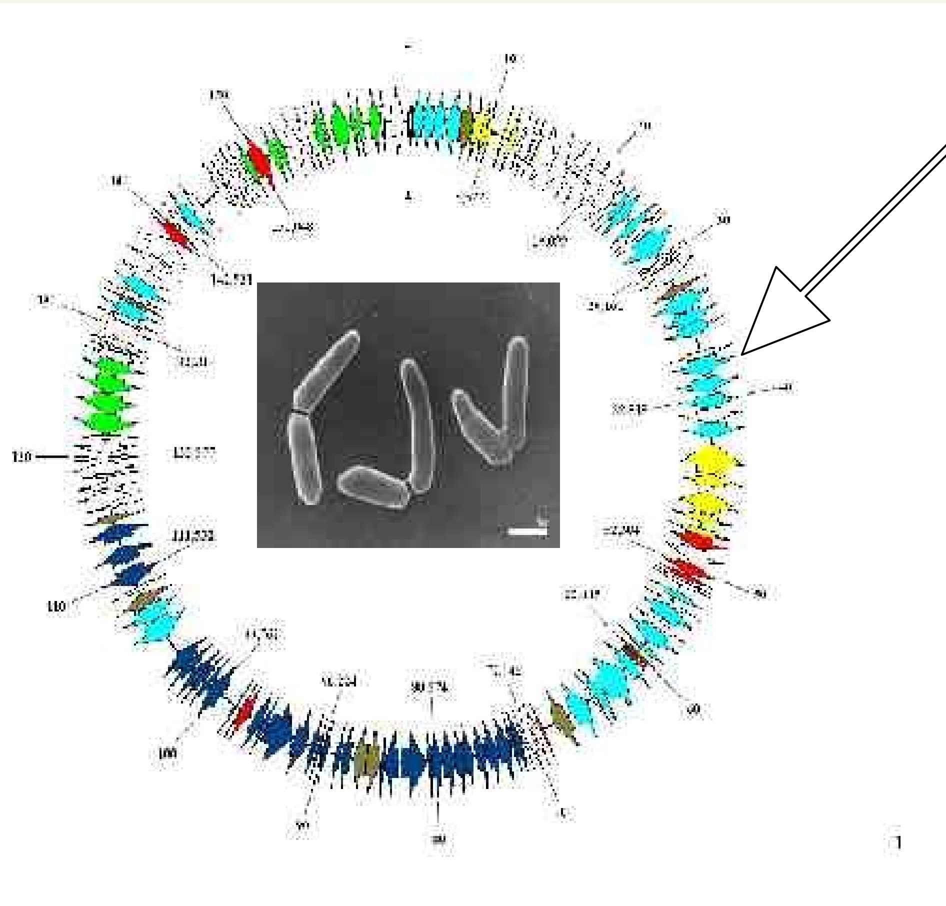
IN-SILICO IDENTIFICATION OF KEY RESIDUES FOR SHIFTING THE COENZYME SPECIFICITY OF A ALDEHYDE-DEHIDROGENASE

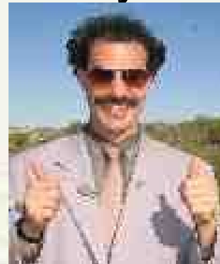
AlDH of *Arthrobacter nicotinovorans* pAO1



ORF39 (AlDH)

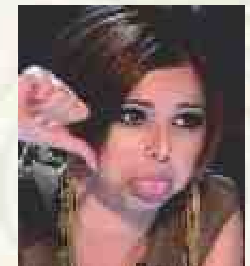
- Monomeric protein
- Cys in the catalitic site
- Aldehyde dehydrogenase activity
- Acts on glutaraldehyde

  verry good !!!



- $NADP^+$ dependent

  not good!!!



The plan for shifting the NAD/NADP preference of AlDH

1. Obtain the putative structure by means of homology modeling using SWISS-model (Arnold *et al.*,2006)

2. Perform *in-silico* mutagenesis with TRITON (Prokop et al., 2000) and MODELLER 9v8 (Eswar *et al.*,2007) for various aminoacids

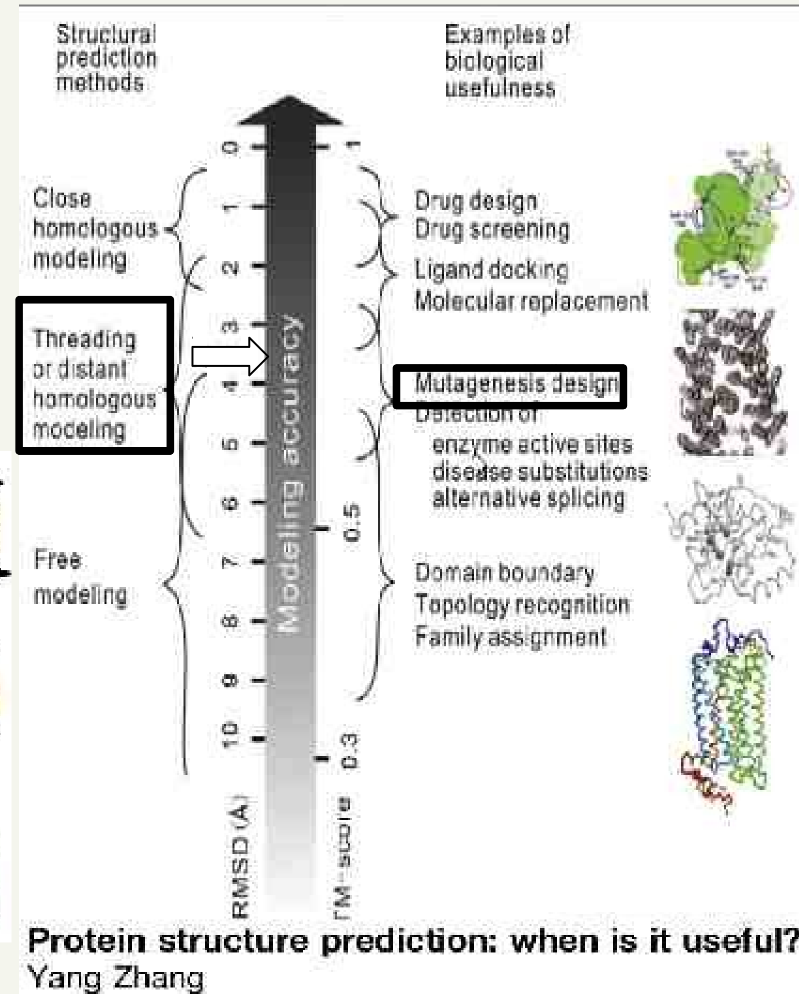
3. Screen for the most favorable mutation by *in-silico* docking with AutoDock 4 (Morris *et al.*,1998)

4. *Perform PCR mutagenesis for the selected residues*

5. *Calculate Km and Kcat for the mutants*

Putative structure of AlDH

PDB ID	Function	Sequence identity with AlDH
3efvA	Putative protein	44 %
3jz4A (Langendorf *et al.*,2010)	**NADP dependent succinic semialdehyde dehydrogenase**	**35 %**
3eklA	Putative protein, unpublished	34 %
3ifgE	Putative protein, unpublished	33 %
1bxs (Moore *et al.*, 1998)	Sheep liver cytosolic aldehyde dehydrogenase	32 %



Selected residues for mutagenesis

The catalytic mechanism for aldehyde dehydrogenases is the same disregarding the preference for NAP$^+$ or NADP$^+$. The key difference between NAP$^+$ and NADP$^+$ preferring enzymes resides in the shape and size of the cofactor binding pocket.

Residue	Role and reference	Mutation
K158	Interacts with 2 ' phosphate of NADP + (Langendorf *et al.* ,2010)	Changed to a bulky residue - H, W as well as a neutral one A
Ser161	NAD+ enzymes are characterized by a dicarboxilic residue in this position, while NADP+ enzymes have a small residue (Perozich et al. , 2000)	Mutated to a dicarboxilic residues - E or D
G215	Part of the GxGxxG motif of NAD(P) utilizing enzymes. In NADP enzymes the last G is replaced by bulky residues (Bellamacina R.,1996)	Mutated into a more bulky aminoacid - S or P

The X-Ray Crystal Structure of *Escherichia coli* Succinic Semialdehyde Dehydrogenase; Structural Insights into NADP$^+$/Enzyme Interactions

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Docking Results

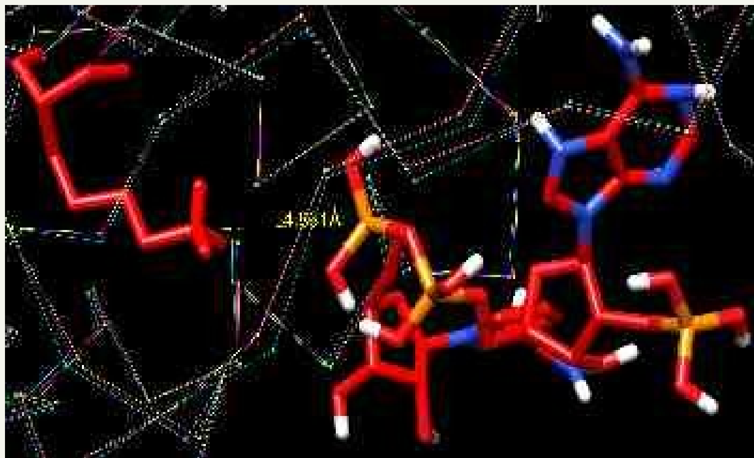
	Mutant	Free energy (kcal/mol)	
		NAD^+	$NADP^+$
*	K158W	-10,06	-9,74
***	K158H	-9,79	-6,76
**	G215P	-8,61	-5,71
	S161D	-8,4	-8,05
	K158A	-7,56	-6,83
	WT	**-7,38**	**-8,26**
	G215S	-6,98	-3,98
	S161E	-5,58	-4,42

NAD^+/$NADP^+$ preference shifted
Affinity for NAD^+ is higher

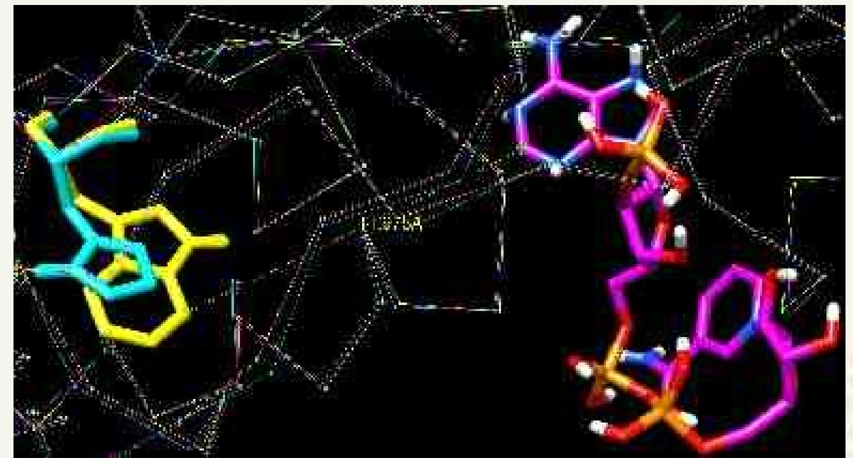
NAD^+/$NADP^+$ preference shifted
Affinity for NAD^+ is lower

Docking Results

NADP$^+$ binding and position in the wt protein.



NADP$^+$ binding is disrupted in the mutant proteins.

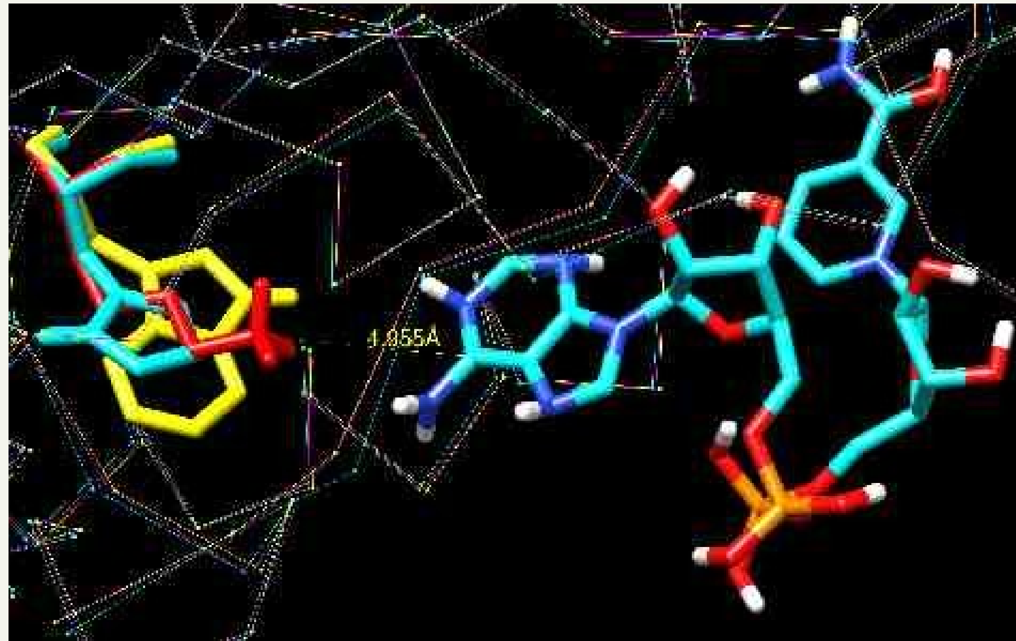


Superposition of the wt AlDH and the best scored mutants: K158W K158H.

The protein backbone is represented as wire in the background. The mutated residues are represented as sticks K158 is in red, W in yellow and H in cyan.

Docking Results

NAD^+ binds in the same manner in both the wt and the mutated proteins.



Superposition of the wt AlDH and the best scored mutants: K158W K158H.
The protein backbone is represented as wire in the background. The mutated residues are represented as sticks K158 is in red, W in yellow and H in cyan.

Conclusions

- Mutating the selected residues in the AlDH model lead to various responses.
- Although in all the mutants the preference for NAD/NADP was shifted as desired, in some cases the affinity for NAD was lower then the wt.
- The most favorable position for mutagenesis is K158, two of the mutants at this location K158W and K158H having an docking score for NAD higher than the score obtained by the wt protein.

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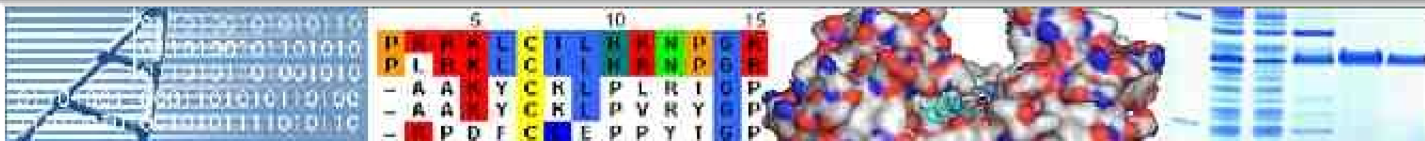

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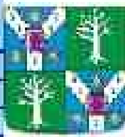

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Cloning and characterisation of ORF32 and ORF40 from *Arthrobacter nicotinovorans* pAO1 megaplasmid - putative tagatose-binding protein models

- Research project financed by the Ministry of Education, Research and Inovation - The National Authority for Scientific Research UEFISCSU - CNCSIS, contract nº 117/30.07.2010
- Funding amount - 339 000 roni (aprox. 81 000 euros)
- Funding period - 2 years (30.07.2010 - 30.07.2012)
- Project manager - dr. Marius Mihasan

Project summary

Tagatose is a proven anti-diabetic and obesity control agent (Moore, 2006, Lu et al., 2008). So far, the only feasable method of obtaining this monosacharyde is by chemical synthesis, using an isomerisation reaction. Identification of alternative enzymatic methods of obtaining this compound has proven to be an extremely difficult task, as the mechanism of tagatose-protein interaction has not been described. An experimental model of this interaction would provide an invaluable tool for *in-silico* screening of databases and identification of enzymes usefull for obtaining the foreword mentioned compound.
Using homology modeling and in silico docking, two putative tagatose-binding proteins were identified. The two genes are part of a gene cluster from the pAO1 megaplasmid of *Arthrobacter nicotinovorans*, coding an yet unknown putative catabolic pathway and consist of ORF32 – an transcriptional regulator and ORF40 – a sugar oxidoreductase.
The main goal of the project is to isolate and to characterize the two proteins as well as to evaluate their applications as models of tagatose-protein interactions.

- Objectives and activities (only in romanian, pdf file)

http://www.bio.uaic.ro/cercetare/contracte/PD337-Mihasan/pd337.html