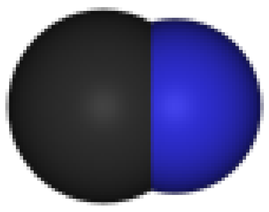
MOLECULAR DYNAMICS AND IN SILICO ANALYSIS OF OLIGOMERIZATION SURFACES OF CYND ENZYMES

Aldhair Médico[1], Cristian Bustamante[1], Roberto Pineda[1], Santiago Justo[1]
1: Laboratorio de Computo Avanzado, Grupo de Bioinformática y Sistemas Complejos, Facultad de Ciencias Biológicas, Universidad Ricardo Palma.
E-mail: aldhairmedico@gmail.com, sanjusare_712@hotmail.com, robertopineda75@gmail.com

INTRODUCTION

Cyanide is a toxic compound widely used in mining to extract precious metals like gold, silver or bronze.



CN-

Naturally some bacterias are capable to degrade these molecules. CynD is a type of prokaryote enzyme able to degrade cyanide. Its active form seems to be an oligomer of variable length according to the species

Several attempts to obtain structural models of CynD by crystallography has not success mainly due the insolubility of the oligomeric state of this protein. In this work we aimed to use *in silico* tools to obtain models of CynD from *Bacillus pumilus* to perform molecular dynamics that will allow us to identify the aminoacids implied in the oligomerization surfaces. This knowledge could lead us to rationally design CynD variants that are not able to form tights oligomers stabilizing other forms such as dimers or monomers that could be better in purification and crystallization assays.

METHODS

Sequence obtaining
GenBank-NCBI (AAN77004.1)

National Center for Biotechnology Information NCBI

Structural modelling
Sending sequences to online servers

SWISS-MODEL
Phyre²
I-TASSER Protein Structure & Function Predictions
ROBETTA BETA Full-chain Protein Structure Prediction Server

Model validating
Based on chemical characteristics using online tools

RAMPAGE
PDBsum
The ModFOLD (Version 6.0)

Choice of models
Based on the best validation results

Energy Minimization and Molecular dynamics

GROMACS FAST. FLEXIBLE. FREE.

Docking and analysis of surfaces

PRELIMINARY RESULTS

	SWISS 1	I TASSER 1	I TASSER 2	I TASSER 3	I TASSER 4	I TASSER 5
% Favoured	93.6	75.3	77.4	76.5	77.4	73.5
% Allowed	3.9	17.2	14.2	16.3	16.0	18.4
% Outliers	2.5	7.5	8.4	7.2	6.6	8.1

A

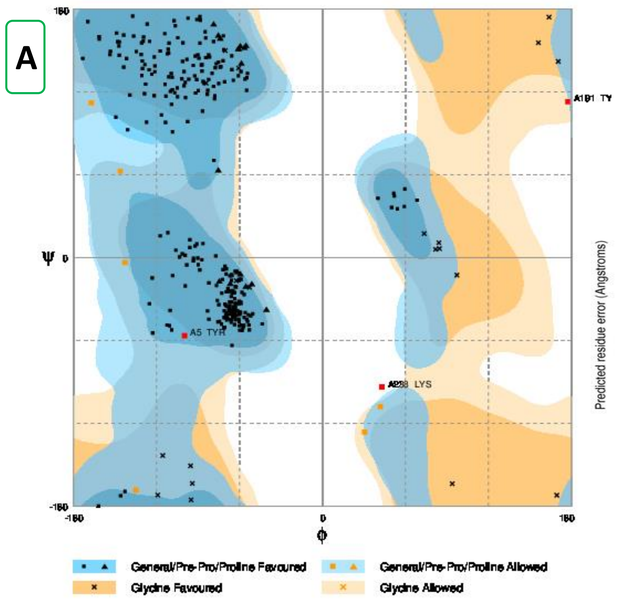
	PHYRE 1	ROBETTA 1	ROBETTA 2	ROBETTA 3	ROBETTA 4	ROBETTA 5
% Favoured	92.0	97.3	95.4	97.0	94.5	98.2
% Allowed	5.3	1.8	3.7	1.8	4.9	1.2
% Outliers	2.7	0.9	0.9	1.2	0.6	0.6

B

	ROBETTA 1	ROBETTA 3	ROBETTA 5
Confidence and p-value	$1.874\ x\ 10^{-8}$	$2.128\ x\ 10^{-8}$	$4.214\ x\ 10^{-8}$
Global model quality score	0.6951	0.6922	0.6764

Fig. 1: Results of the analysis of the Ramachandran's plots of the 12 models generated using the RAMPAGE tool **(A)** Results of the analysis of the 3 best models generated using the ModFold6 tool **(B)**

A



B

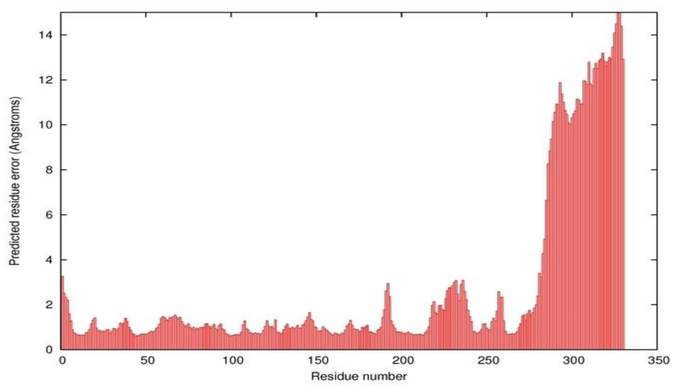
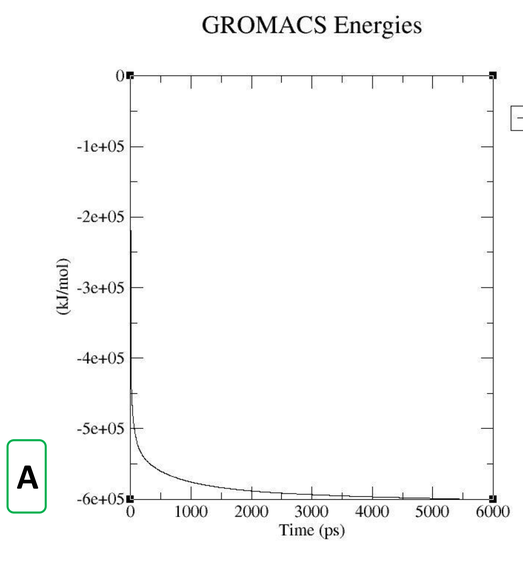


Fig. 2: Ramachandran's Plot of the Robetta 1 model obtained from the Rampage tool (A). Residue error plot of the protein obtained from ModFold6 tool (B)

A



B

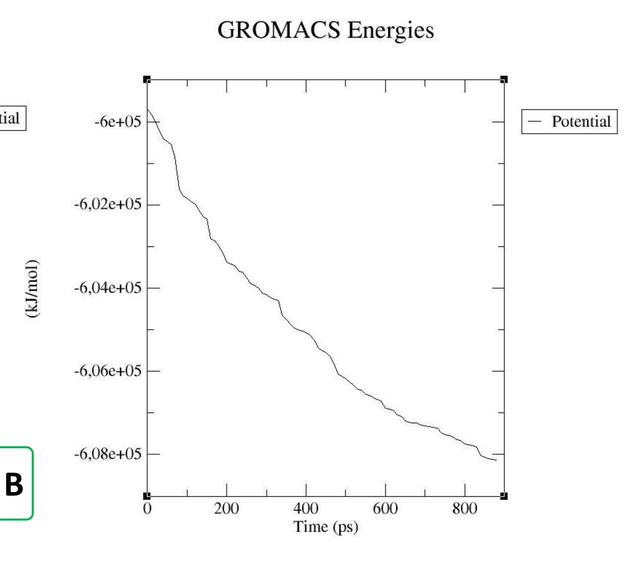
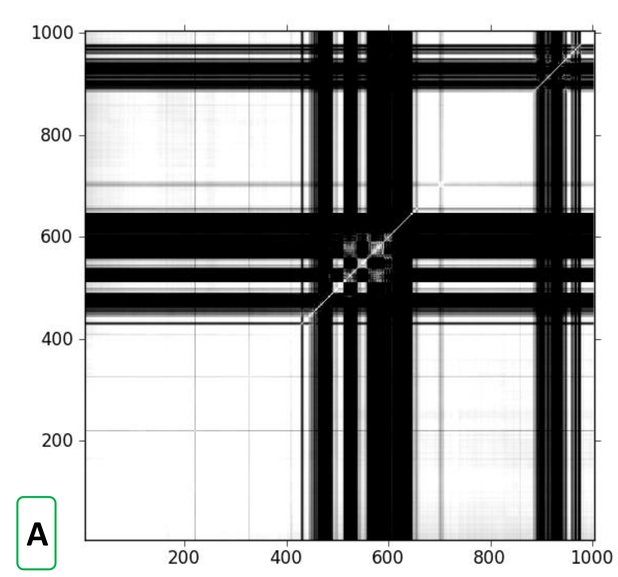
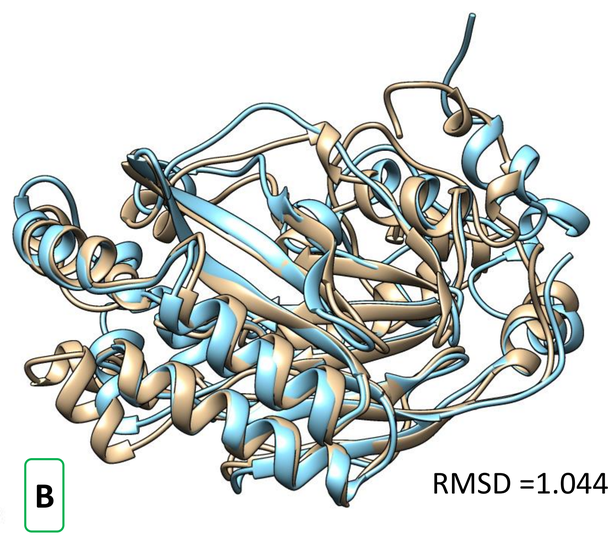


Fig. 3: GROMACS Energies Plot of the minimization of the Robetta 1 model using steepest descent **(A)**. GROMACS Energies Plot of the second minimization of the Robetta 1 model using conjugate gradients **(B)**

A



B



RMSD =1.044

Fig. 4: RMSD map that compares the different steps of molecular dynamics (each frame represents 50ps) **(A)**. Comparison between model 1 generated by Robetta (represented in ocher color) and the final conformation after a 50 ns molecular dynamics simulation (represented in light blue) **(B)**

CONCLUSIONS

CynD models were obtained from several online servers, Ramanchandran plots showed that the Robetta server generated models with higher percentage of residues in favored and allowed regions of the plot. The ModFold6 server showed that the C-terminal of CynD probably is a disordered region. We also performed a molecular dynamics simulations in order to analyze if this C-terminal region in fact is a intrinsically disordered region of CynD. We will analyze those results and generated longer simulations to try to better understand the dynamics of these enzymes We are also interested in understand how the oligomerization process occur.

REFERENCES

JANDHYLA, D.; Berman, M.;Meyeres, P.; Sewell, B.; Willson, R.; Benedik, M. cynD, the Cyanide Dihidratase from *Bacillus pumilus:* Gene Cloning and Structural Studies. Applied and Environmental Microbiology. Vol 69, No. 8. Aug. 2003. p 4794 - 4805