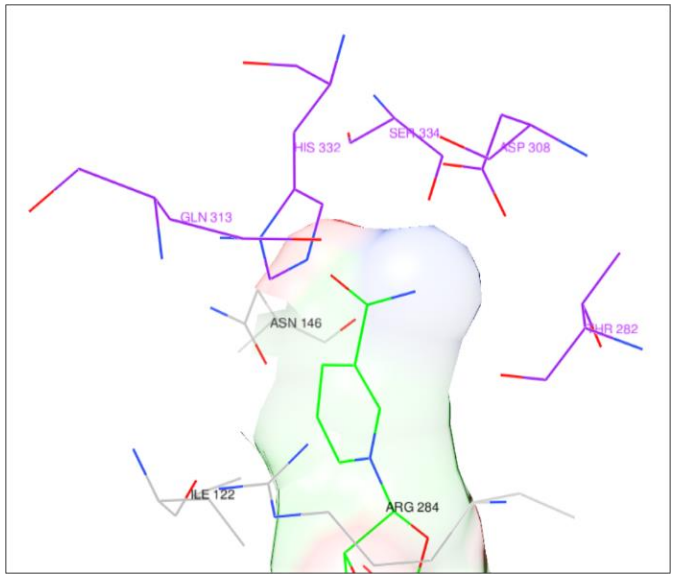
Master’s Thesis Defense Presentation

Engineering of formate dehydrogenase for the acceptance of a biomimetic nicotinamide-based cofactor in the reduction of CO_2

by Vanesa Teijeiro Seijas

January 2020



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University of Barcelona

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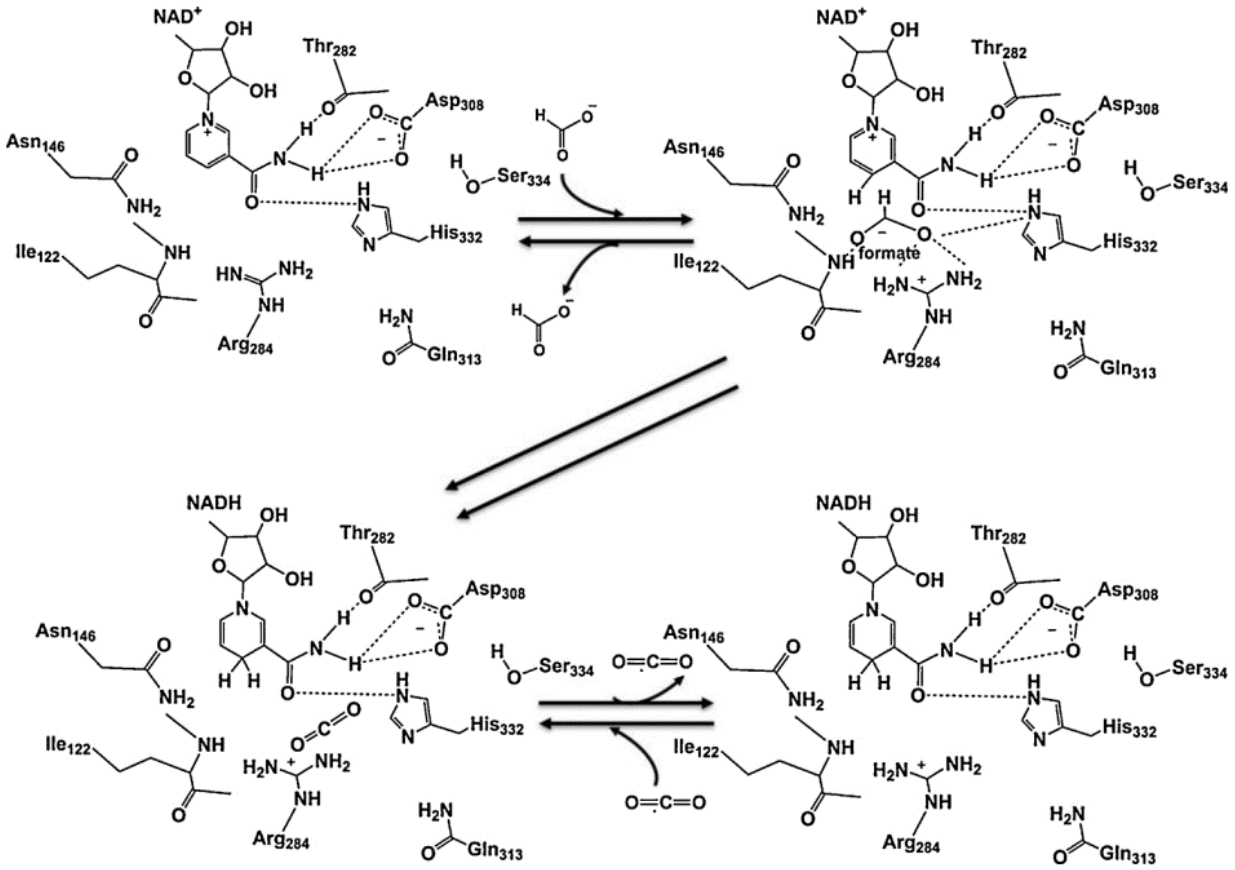
1. Introduction

1.1. Formate Dehydrogenase

- EC 1.17.1.9
- Types
  - Metal-independent
  - Metal-dependent
- Applications
  - Cofactor regeneration
  - CO_2 conversion (under research)



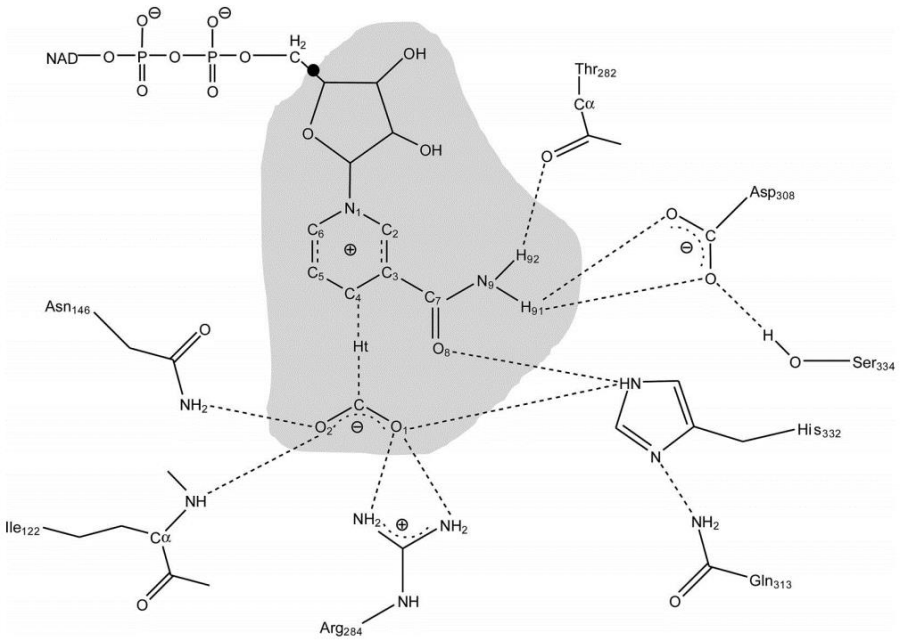
(Amao, 2018)

1. Introduction

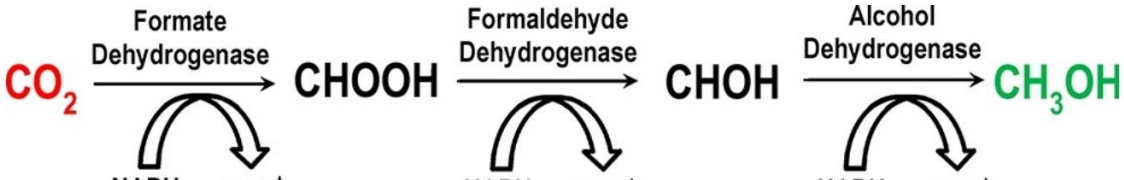
1.2. CO_2 Conversion by FDH

$$HCOO^- \rightleftharpoons CO_2 + H^+ + 2e^-$$

$$CO_2 + H^+ + 2e^- \rightleftharpoons HCOO^-$$



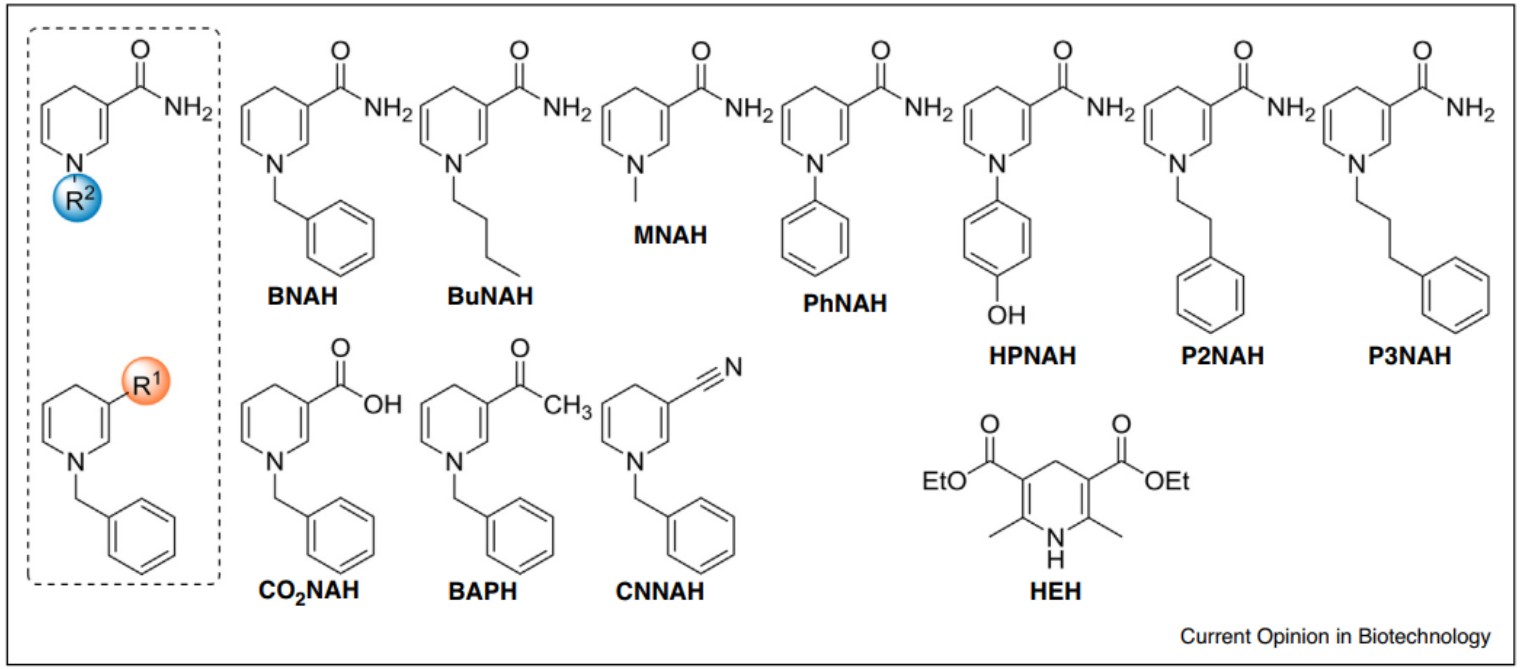
(Castillo et al., 2008)



(Marpani et al., 2017)

1. Introduction

1.3. Biomimetic Cofactors



(Guarneri et al., 2019)

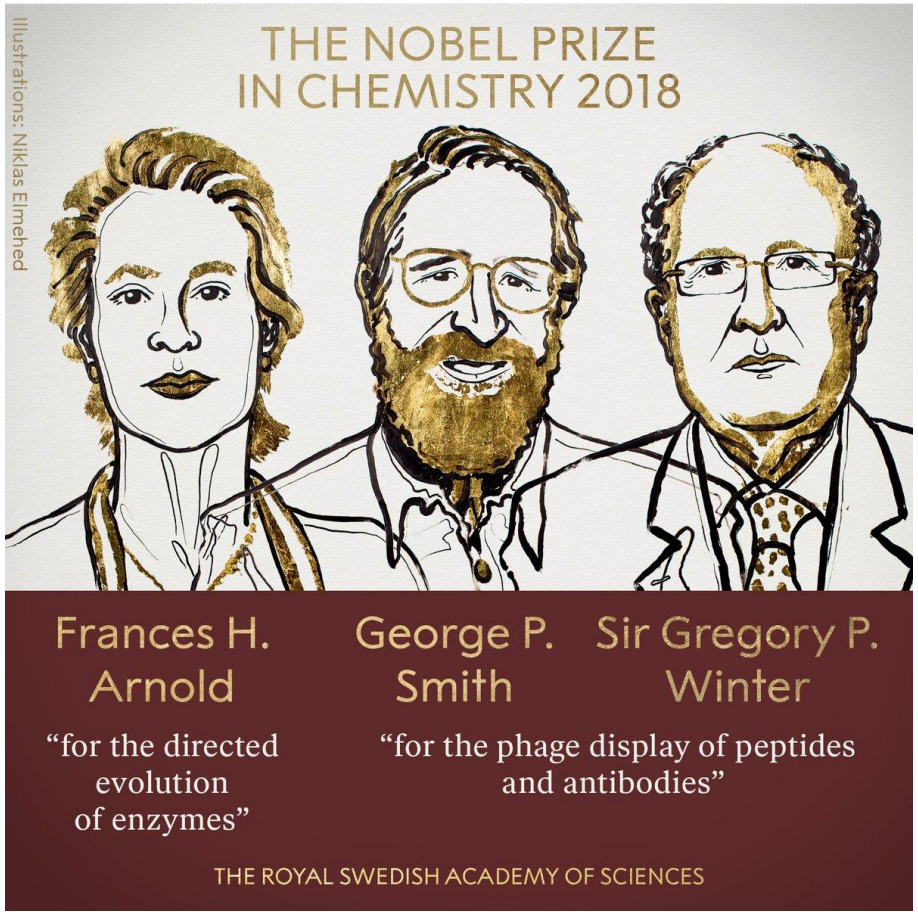
1. Introduction

1.4. Enzyme Evolution

Directed Evolution

vs

Rationally Design



2. Objective

- Determine at least one beneficial mutation for doing protein engineering to the enzyme formate dehydrogenase to allow the use of a nicotinamide biomimetic cofactor instead of the natural cofactor NADH

3. Research Method

3.1. Work Pipeline

Select the enzyme and residues for mutations

- FDH enzyme that requires a cofactor and thermostable
- Residues that interact with the cofactor

Select the biomimetic cofactor

- Binding position similar to the natural cofactor NADH
- Method: Molecular docking using SwissDock

Create mutants and determine the 3D structure

- Method: Homology modelling using SwissModel

Select the best mutant

- Interactions of each mutant with the biomimetic cofactor
- Method: Molecular docking using Autodock Vina

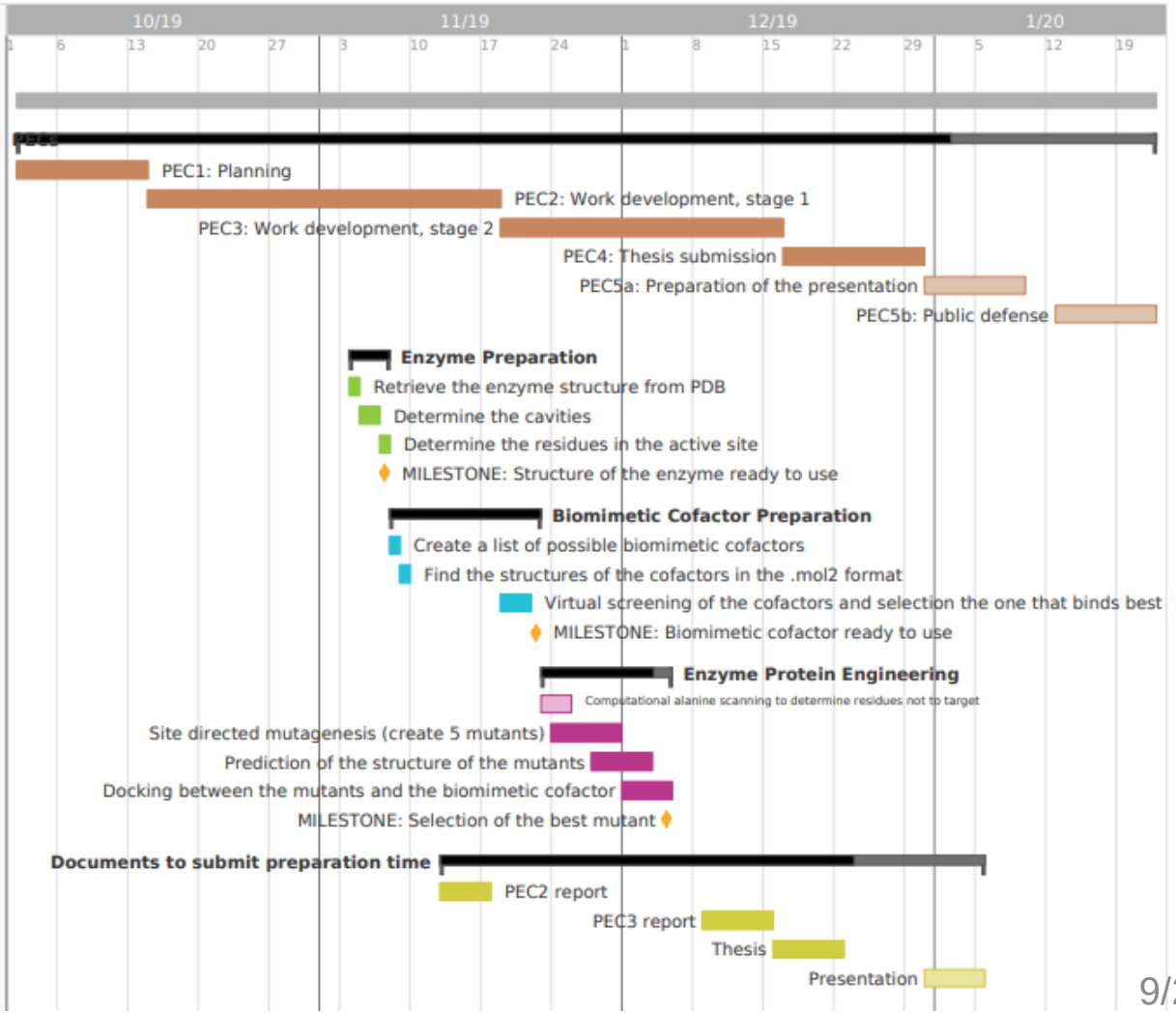
Evaluation of the viability of the mutant

- Stability of the protein (Method: CUPSAT)
- Ligand transport (Method: Caver Web)

3. Research Method

3.2. Project Planning

TFM	start	end	83%
PECs	**10/02/19**	**01/22/20**	**82%**
PEC1: Planning	10/02	10/14	100%
PEC2: Work development, stage 1	10/15	11/18	100%
PEC3: Work development, stage 2	11/19	12/16	100%
PEC4: Thesis submission	12/17	12/30	100%
PEC5a: Preparation of the presentation	12/31	01/09	0%
PEC5b: Public defense	01/13	01/22	0%
Enzyme Preparation	**11/04/19**	**11/07/19**	**100%**
Retrieve the enzyme structure from PDB	11/04	11/04	100%
Determine the cavities	11/05	11/06	100%
Determine the residues in the active site	11/07	11/07	100%
MILESTONE: Structure of the enzyme ready to use	11/07	11/07	100%
Biomimetic Cofactor Preparation	**11/08/19**	**11/22/19**	**100%**
Create a list of possible biomimetic cofactors	11/08	11/08	100%
Find the structures of the cofactors in the .mol2 format	11/09	11/09	100%
Virtual screening of the cofactors and selection the one that binds best	11/19	11/21	100%
MILESTONE: Biomimetic cofactor ready to use	11/22	11/22	100%
Enzyme Protein Engineering	**11/23/19**	**12/05/19**	**86%**
Computational alanine scanning to determine residues not to target	11/23	11/25	0%
Site directed mutagenesis (create 5 mutants)	11/24	11/30	100%
Prediction of the structure of the mutants	11/28	12/03	100%
Docking between the mutants and the biomimetic cofactor	12/01	12/05	100%
MILESTONE: Selection of the best mutant	12/05	12/05	100%
Documents to submit preparation time	**11/13/19**	**01/05/20**	**76%**
PEC2 report	11/13	11/17	100%
PEC3 report	12/09	12/15	100%
Thesis	12/16	12/22	100%
Presentation	12/31	01/05	0%

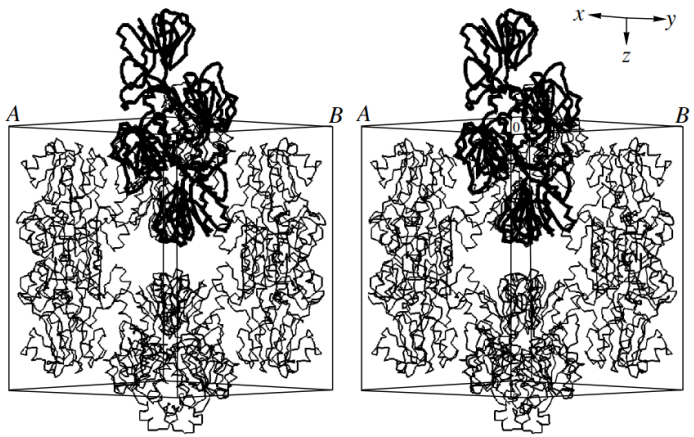


4. Results

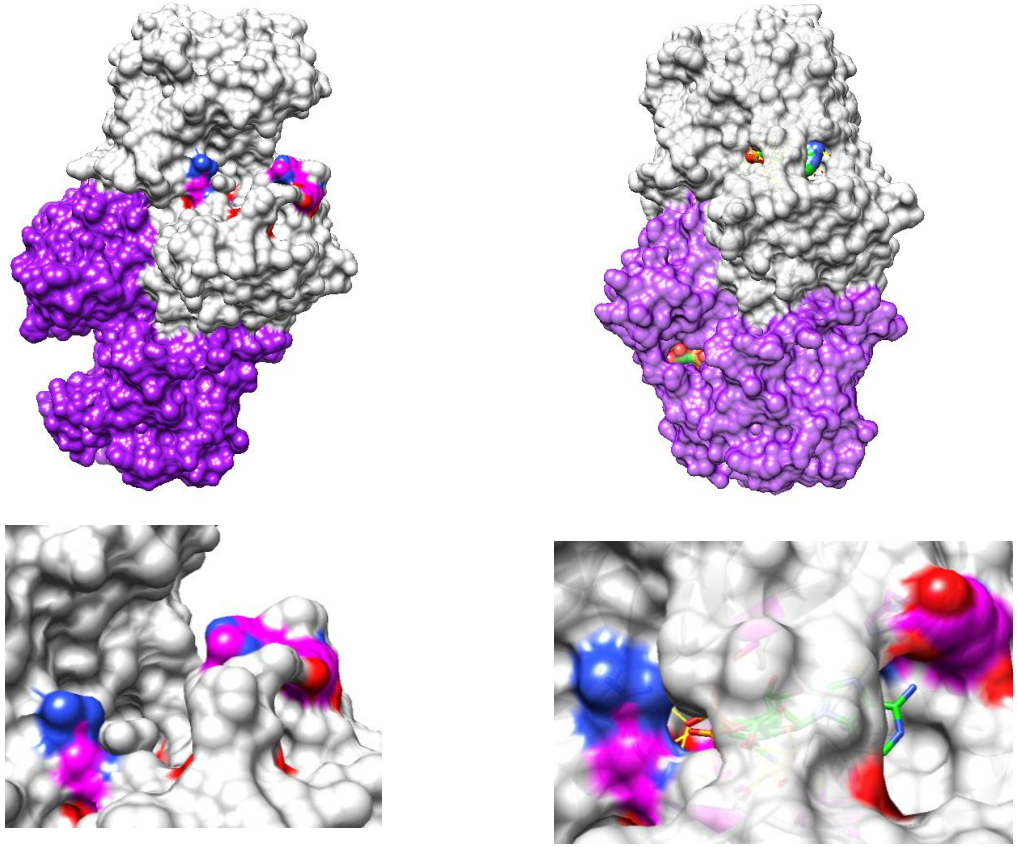
4.1. Selection of the FDH Enzyme

PsFDH (formate dehydrogenase from *Pseudomonas sp. 101*)

- Natural cofactor: NADH
- Tm: 63°C



(Filippova et al., 2005)



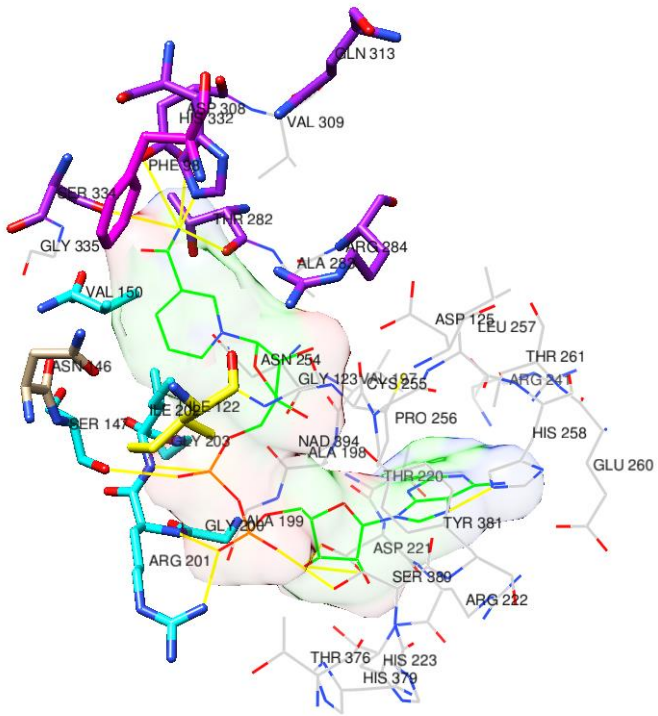
Apoenzyme
PDB: 2NAC

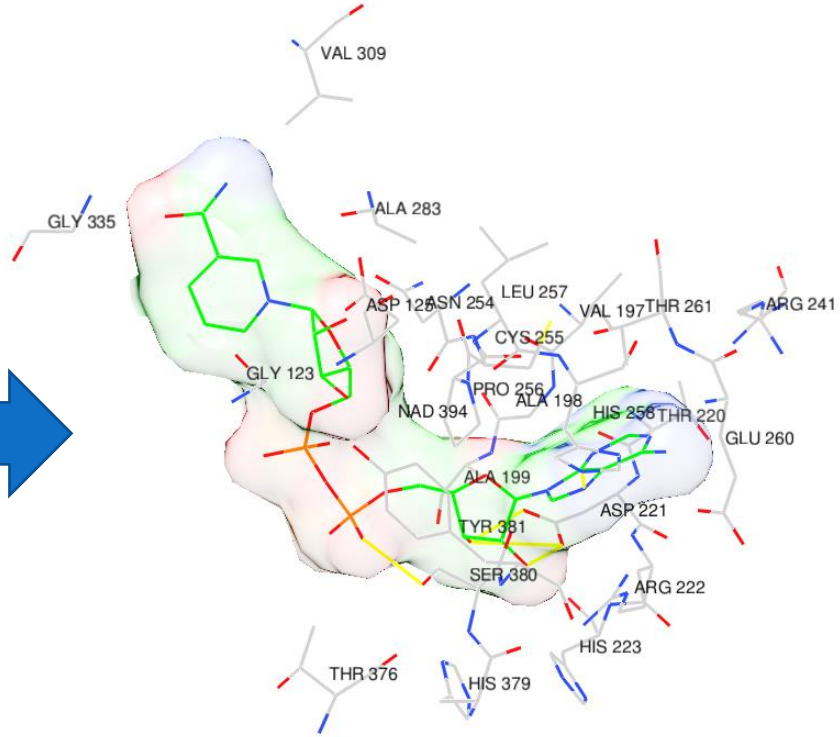
Holoenzyme
PDB: 2NAD

4. Results

4.2. Target Residues for Mutations

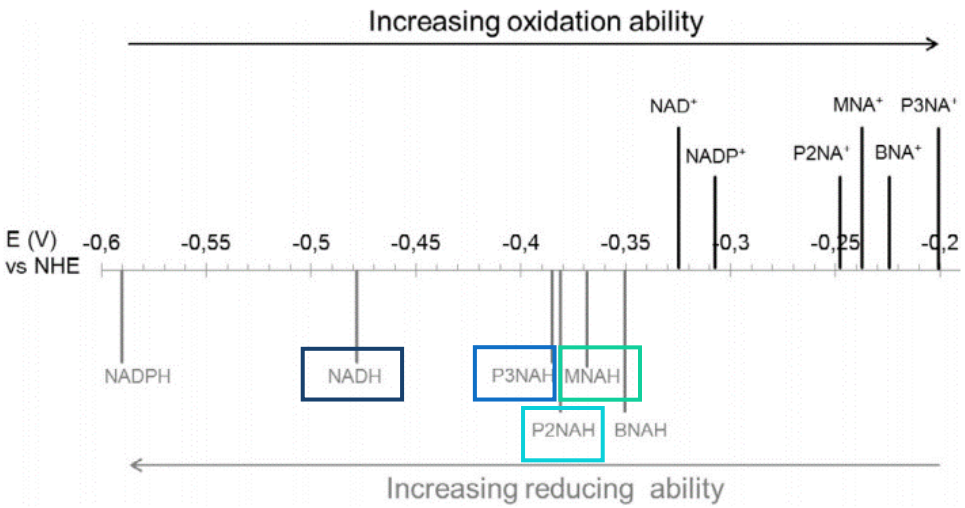
- ✘ Catalytic domain
- ✘ Reaction mechanism
- ✘ Interaction between subunits



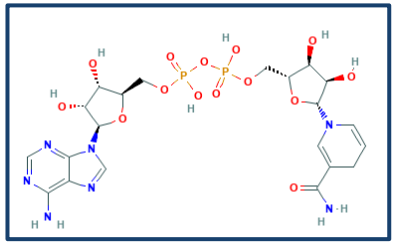


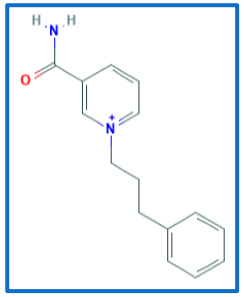
4. Results

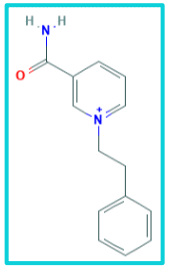
4.3. Selection of the Biomimetic Cofactor

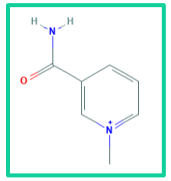


(Nowak, Pick, Csepei, et al., 2017)







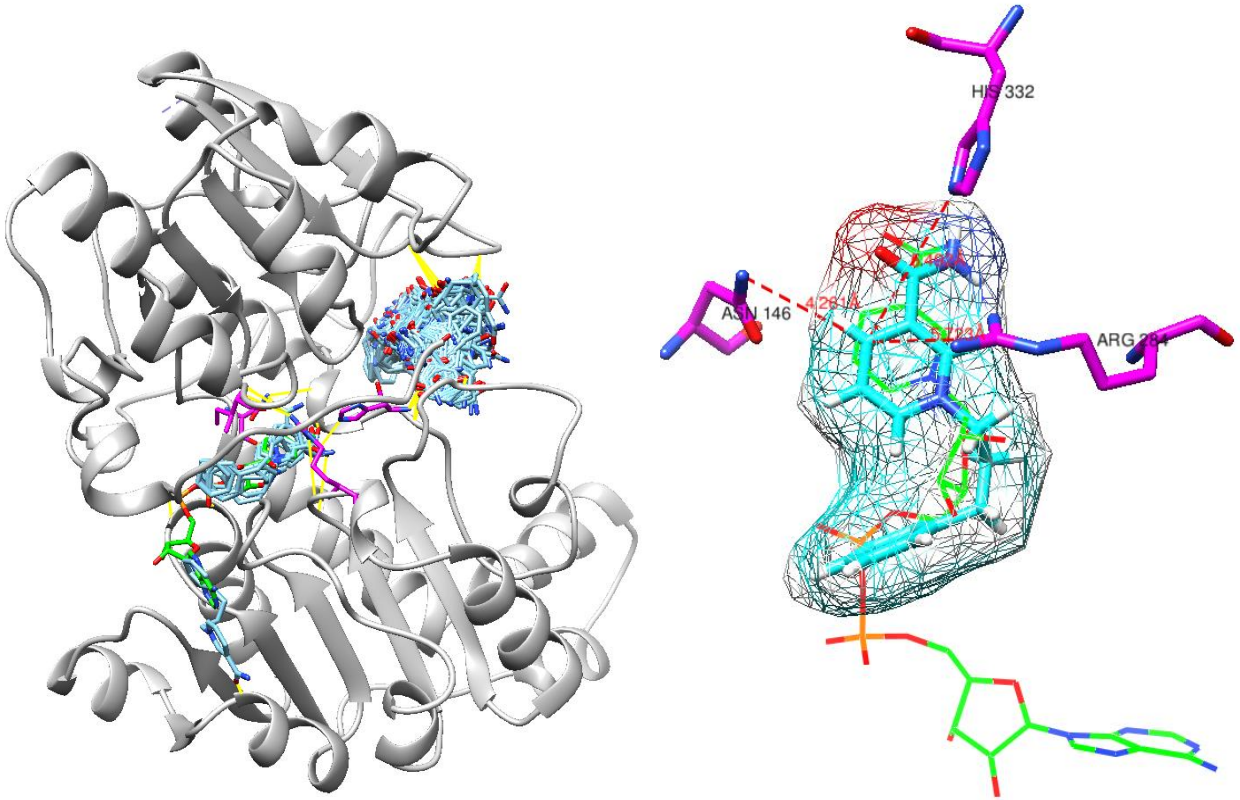


NADH

Natural cofactor

P3NAH P2NAH MNAH

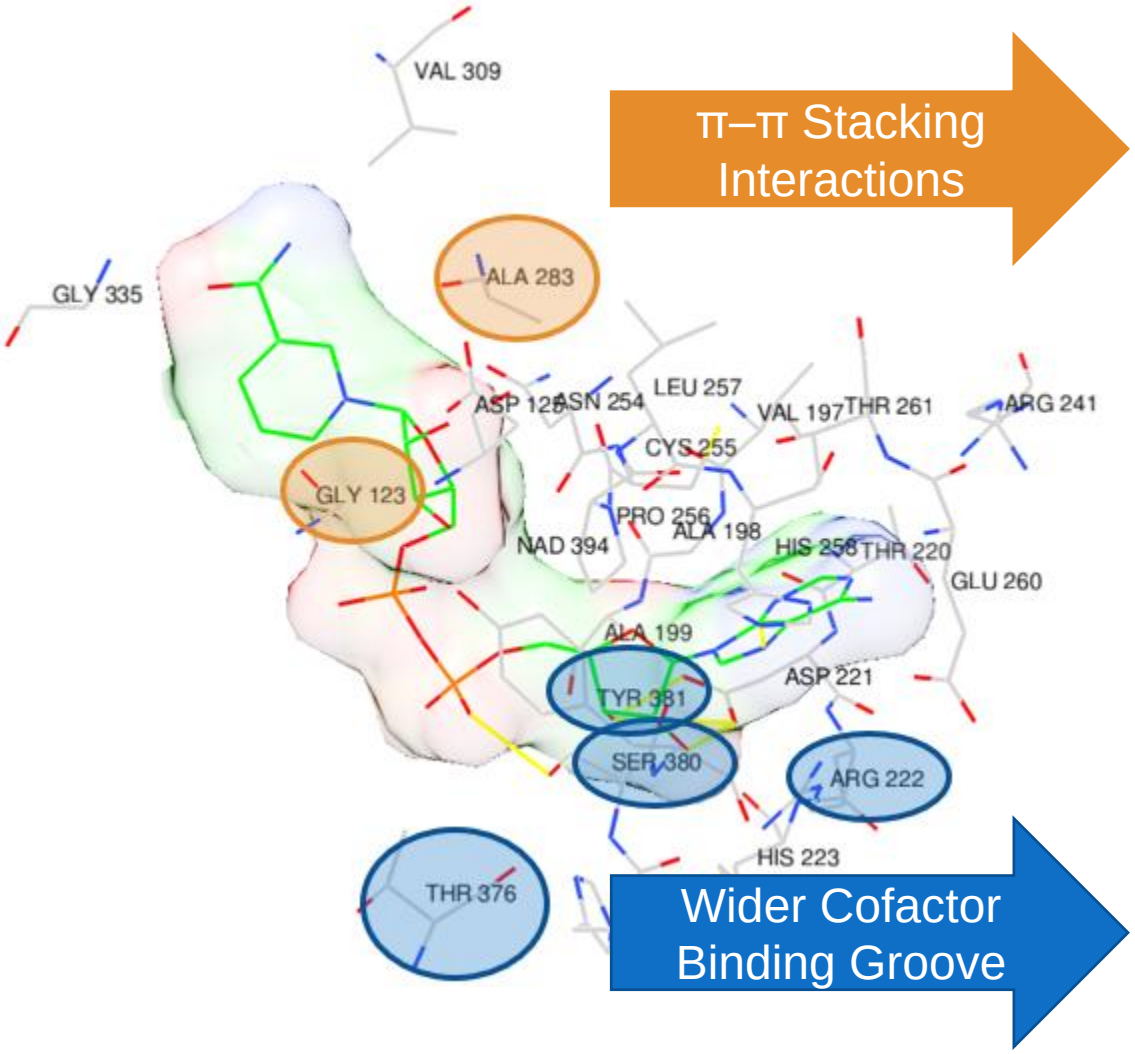
Biomimetic cofactors

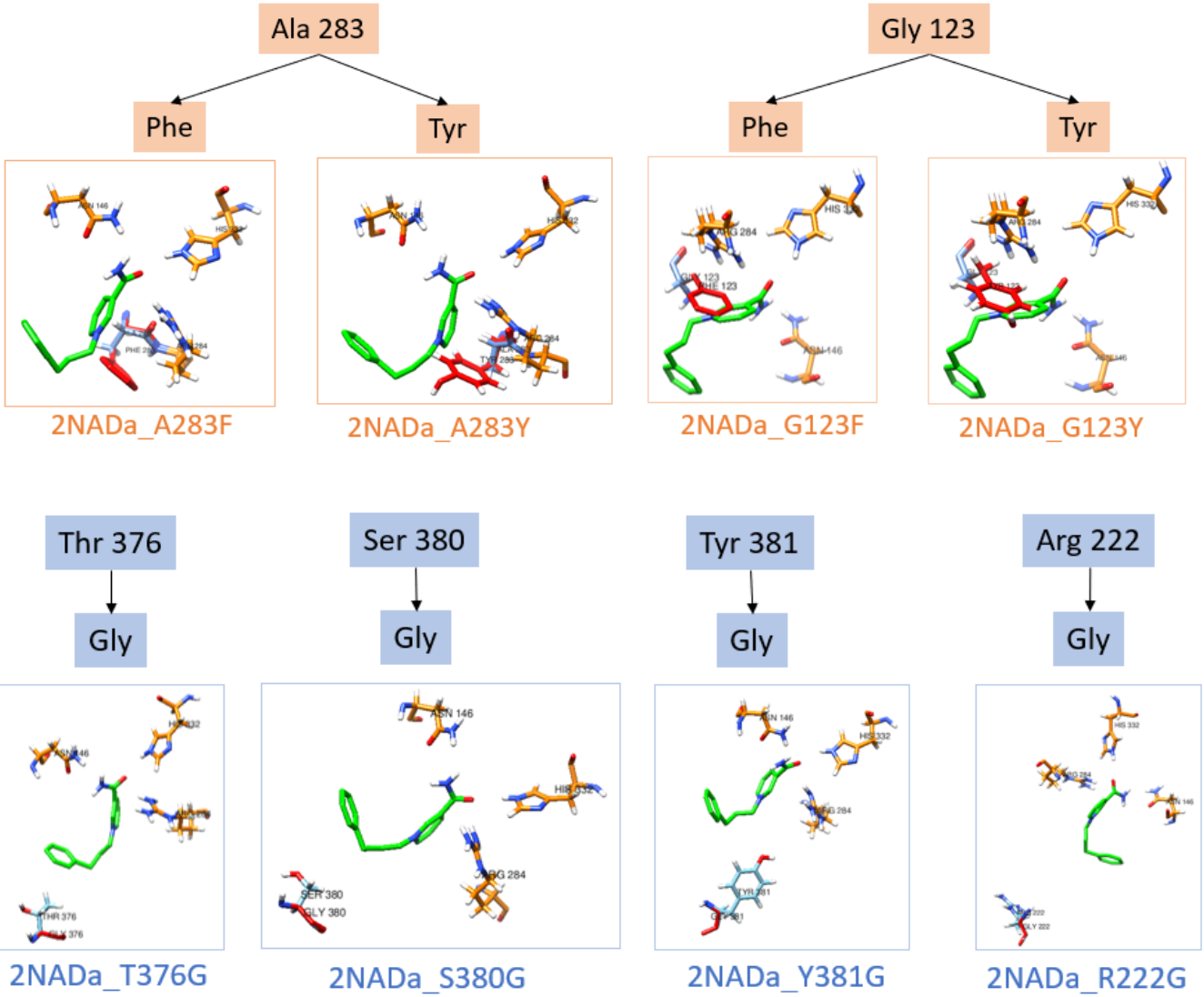


P3NAH docking in PsFDH

4. Results

4.4. Enzymes with Mutations

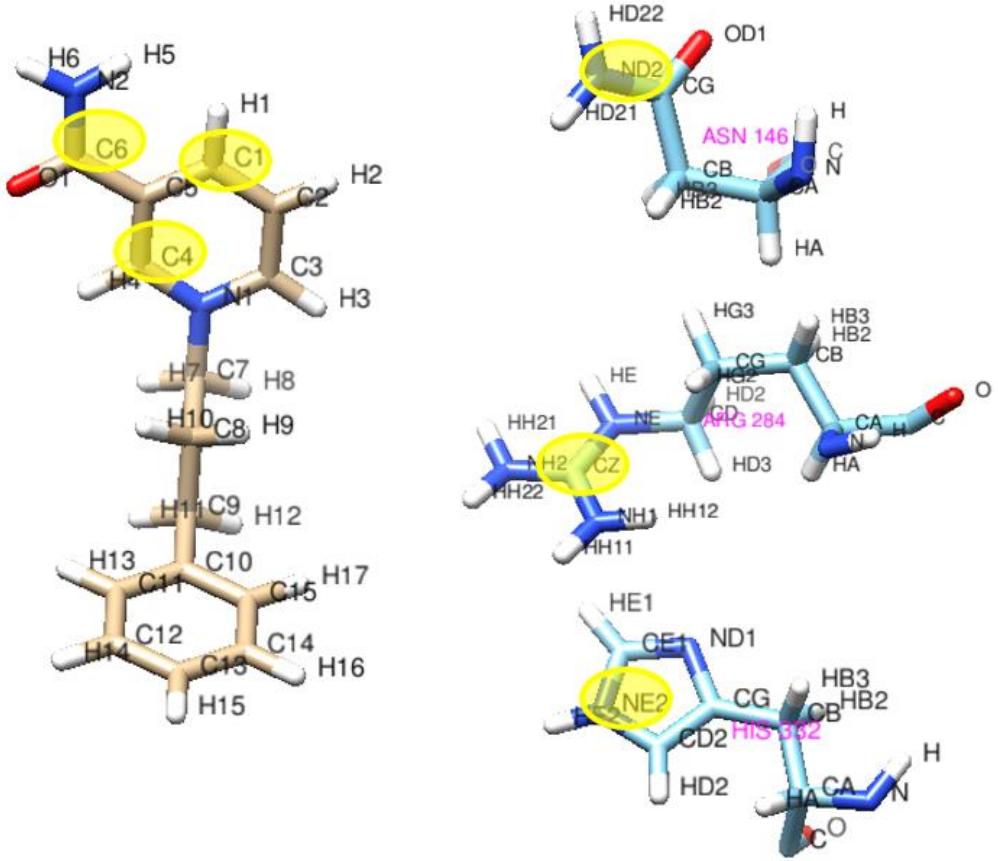




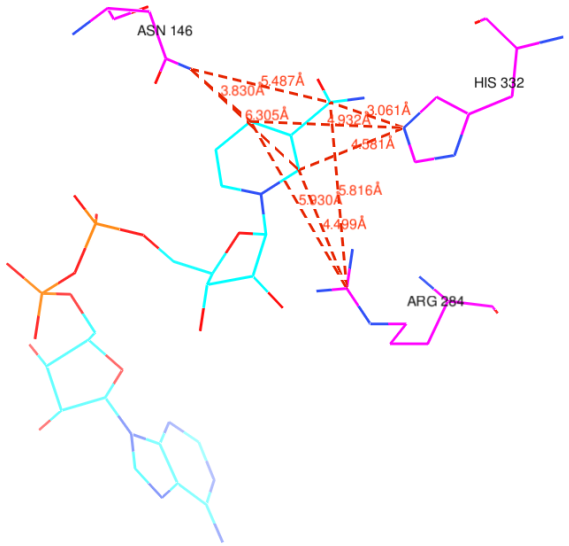
4. Results

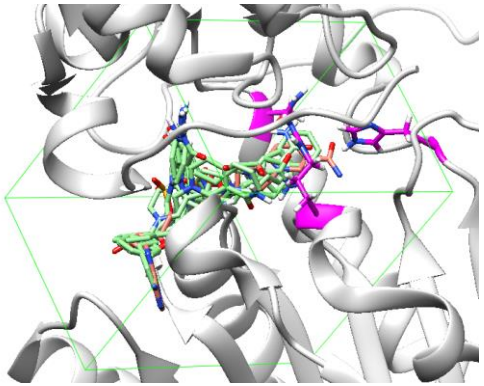
4.5. Mutants Evaluation

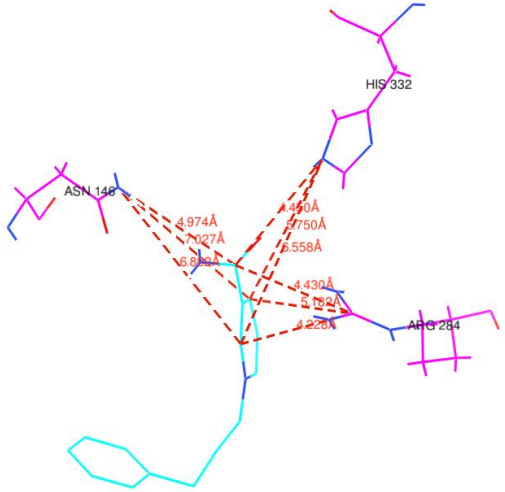
Reference atoms for measure the distance



Reference position, 0 Å (NADH)





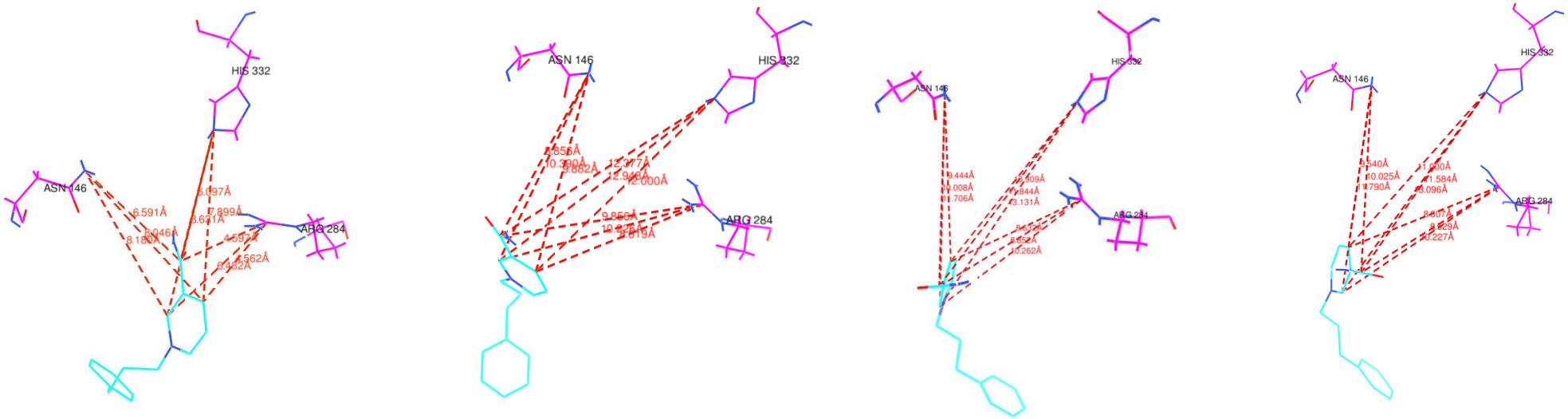


	WT
Binding Score	-7.6
H Bonds	2
Distance (Å)	1.200

P3NAH in WT

4. Results

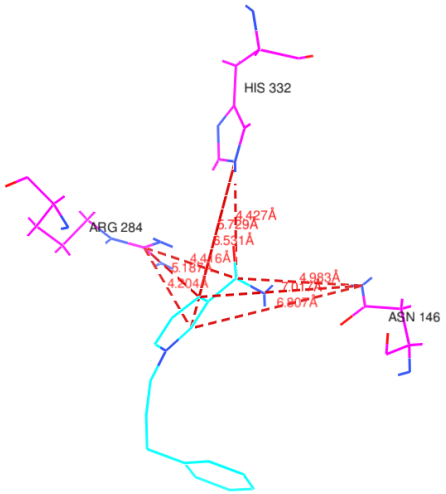
4.5. Mutants Evaluation

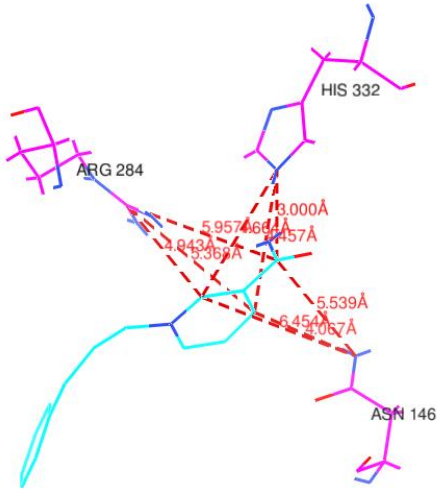


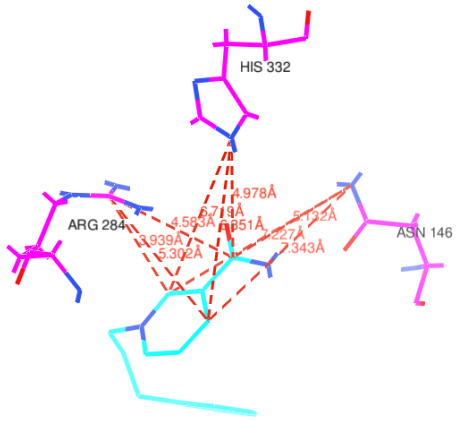
	2NADa_A283F	2NADa_A283Y	2NADa_G123F	2NADa_G123Y
QMEAN	0.27	0.29	0.09	0.11
Binding Score	-6.7	-7.4	-7.2	-7.3
H bonds	1	2	1	1
Distance (Å)	2.424	5.635	5.594	5.573

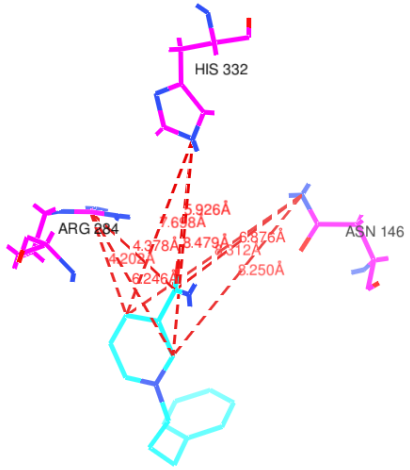
4. Results

4.5. Mutants Evaluation









	2NADa_T376G	2NADa_S380G	2NADa_Y381G	2NADa_R222G
QMEAN	0.32	0.31	0.30	0.33
Binding Score	-7.2	-7.2	-7.1	-7.5
H bonds	2	2	2	2
Distance (Å)	1.409	0.245	2.473	1.348

4. Results

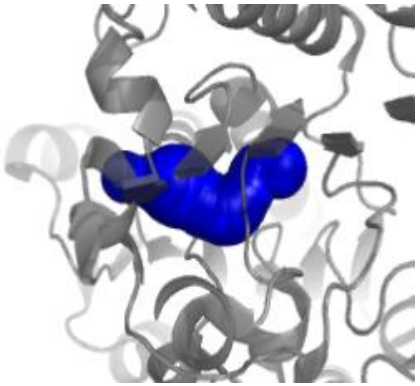
4.6. Mutant Viability Evaluation

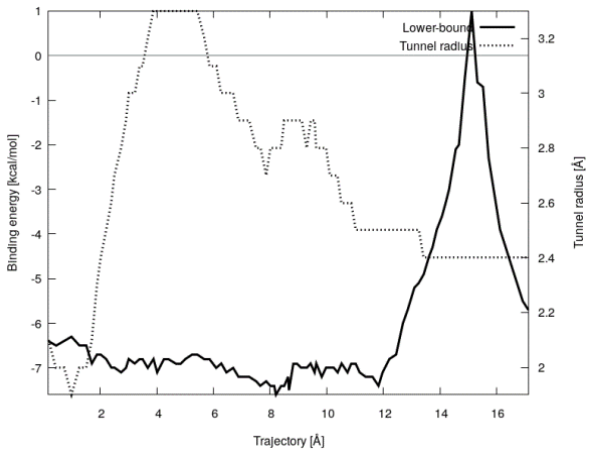
Protein Stability

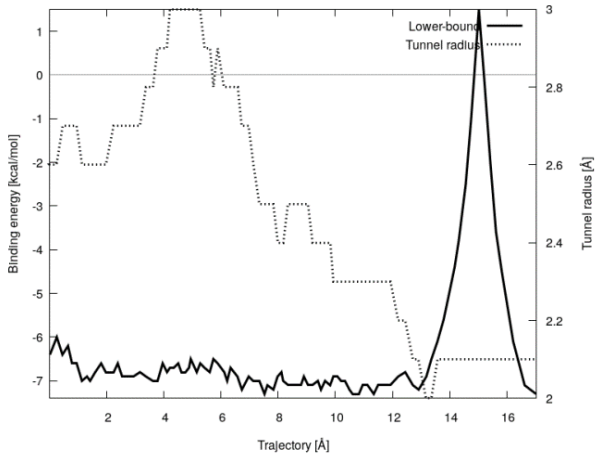
Amino Acid Mutations

Amino acid	Overall Stability	Torsion*	Predicted ΔΔG (kcal/mol)
GLY	Stabilising	Unfavourable	1.89
ALA	Stabilising	Unfavourable	3.14
VAL	Stabilising	Unfavourable	2.99
LEU	Stabilising	Favourable	1.71
ILE	Stabilising	Unfavourable	3.35
MET	Stabilising	Unfavourable	2.57
PRO	Stabilising	Unfavourable	0.04
TRP	Stabilising	Favourable	2.51
THR	Stabilising	Favourable	0.38
PHE	Stabilising	Favourable	2.72
GLN	Stabilising	Favourable	1.27
LYS	Stabilising	Favourable	0.81
TYR	Stabilising	Favourable	2.06
ASN	Stabilising	Favourable	1.33
CYS	Stabilising	Favourable	3.25
GLU	Stabilising	Unfavourable	2.01
ASP	Stabilising	Favourable	2.11
ARG	Stabilising	Unfavourable	1.23
HIS	Stabilising	Favourable	2.19

Ligand Transport







	WT	2NADa_S380G
Bottleneck radious (Å)	2.0	1.9
Length (Å)	15.7	16.4
Distance to surface (Å)	13.5	13.2
Curvature	1.2	1.2
Number of residues	40	38

5. Conclusions

- Viable mutant: 2NADa_S380G
  - Access tunnel Ser -> Gly
  - Acceptable protein stability and ligand transport
- Residues added with aromatic groups: steric hindrance
- Potential of free software for planning protein engineering

6. Limitations and Improvements

- Limitations
  - Steps are not automatically connected
  - Dependence of tools based on web servers

- With more computational power
  - Molecular dynamics
  - More mutations

Thank you for your attention!

Please, send me your questions!