Laboratory project

Biomolecular Modelling Laboratory 2022

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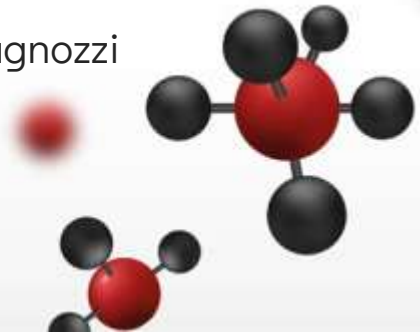


Notexin (1AE7)

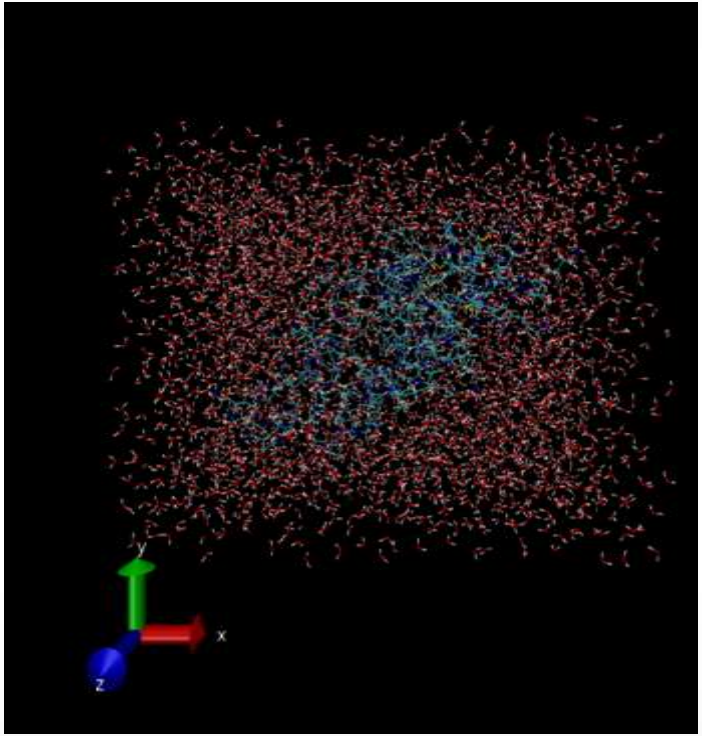
- Single-peptide chain
- 119 AAs
- 7 disulfide-bridges
- 4 main helices: αA, αB, αC and αE

SOLVATION

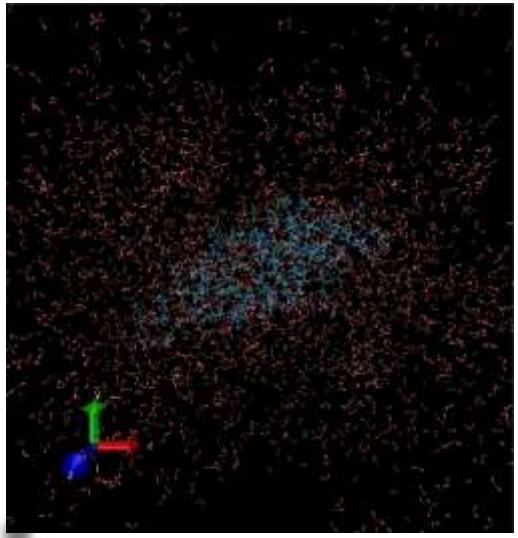
Solvate the protein in an explicit solvent

Introduce the protein in a water box with periodic boundary conditions

Create a ionized version



SIMULATION PARAMETERS



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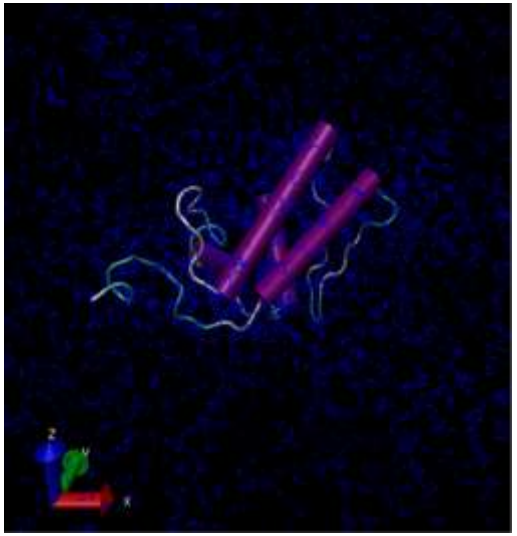


METHODOLOGY

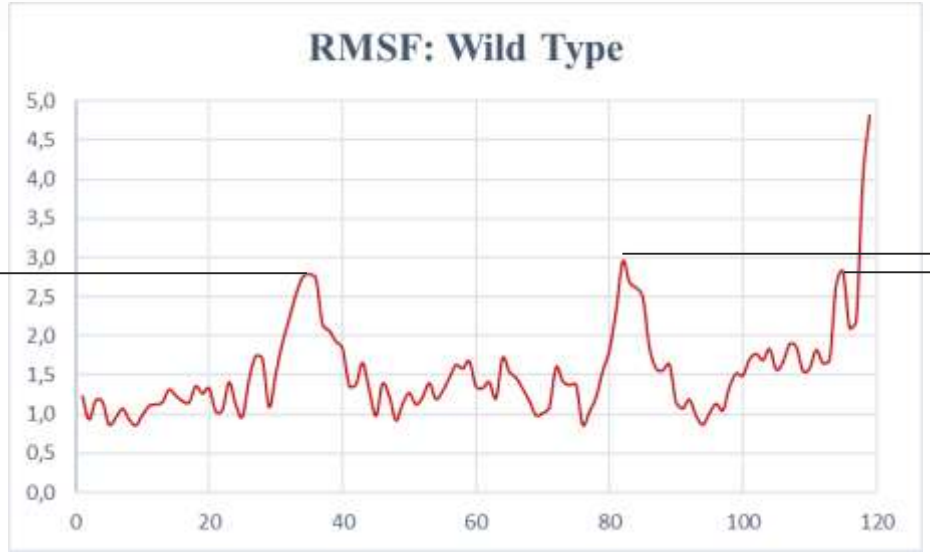
1	2	3	4	5
Two simulations of the **wild-type** protein with 4 different temperatures: 310K, 400K, 500K, 600K	Selection of the **temperature** with less native contacts	Comparison between **RMSF** and the **secondary structure** to identify the most unstable regions	Construction of protein **variants** using Foldx and choice of the best one.	Run the simulation again with the mutate protein and check the **native contacts** (Q-value)

TEMPERATURE SELECTION

Temperature [K]	Mean Native Contacts [%]
310	96
400	94
500	89
600	**86**

*Mean native contacts between the last 10 frames of the simulation

RMSF AND SECONDARY STRUCTURE



89

37

112

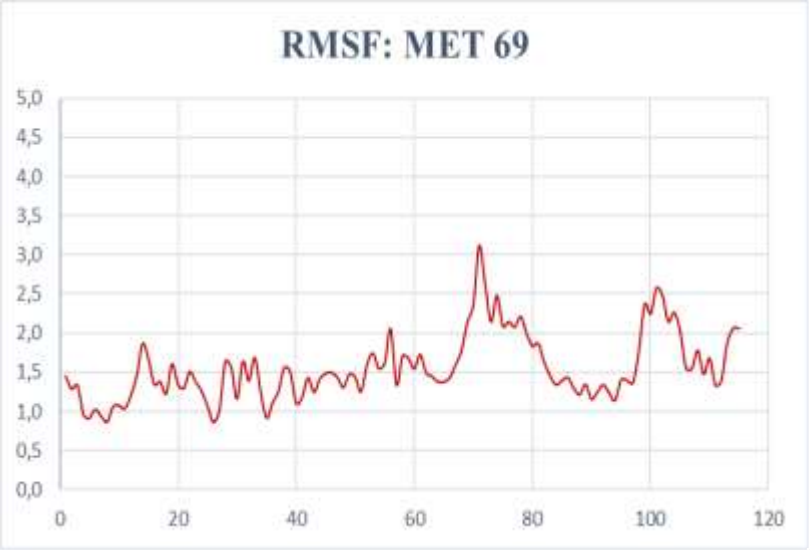
FOLDX

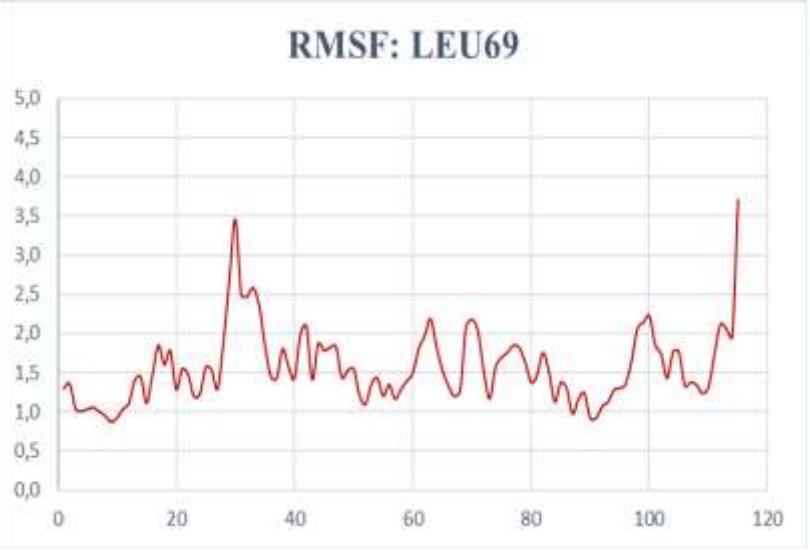
LOOP REGION	MUTATION WITH NEGATIVE VALUES	POSSIBLE MUTATIONS
14-18	✗	✗
23-29	✗	✗
30-39	✗	✗
61-74	✓	69 LYS→LEU 69 LYS→MET
85-89	✓	89 LYS→ASP
109-112	✓	112 ASP →TYR

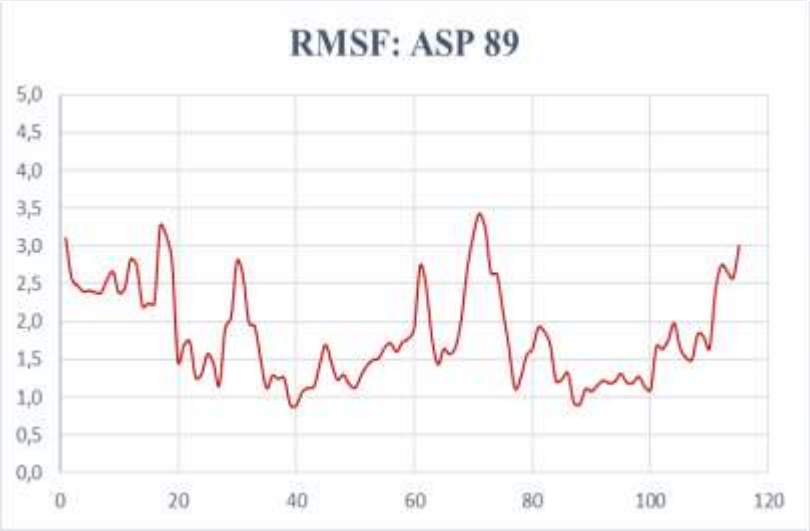
RESULTS

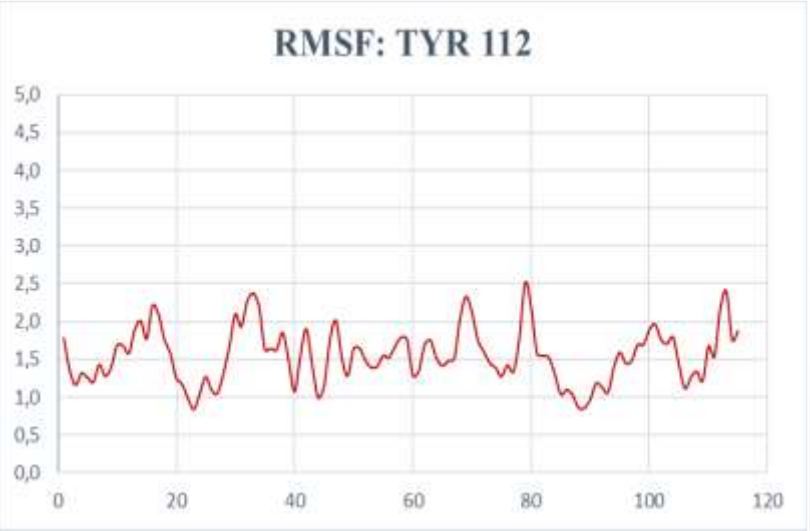
Variant	Energy output	Mean Q-value*	SD Q-value*
WT	-	0,866	0,0149
69 MET	-2,31687	0,823	0,0159
69 LEU	-2,04749	0,817	0,0088
89 ASP	-3.3399	0,821	0,0132
112 TYR	-2,1491	0,849	0,0181
69 MET & 112 TYR	-	0,811	0,0134
69 MET & 89 ASP & 112 TYR	-	0,821	0,0108

*Mean and SD between the last 10 frames of the simulation

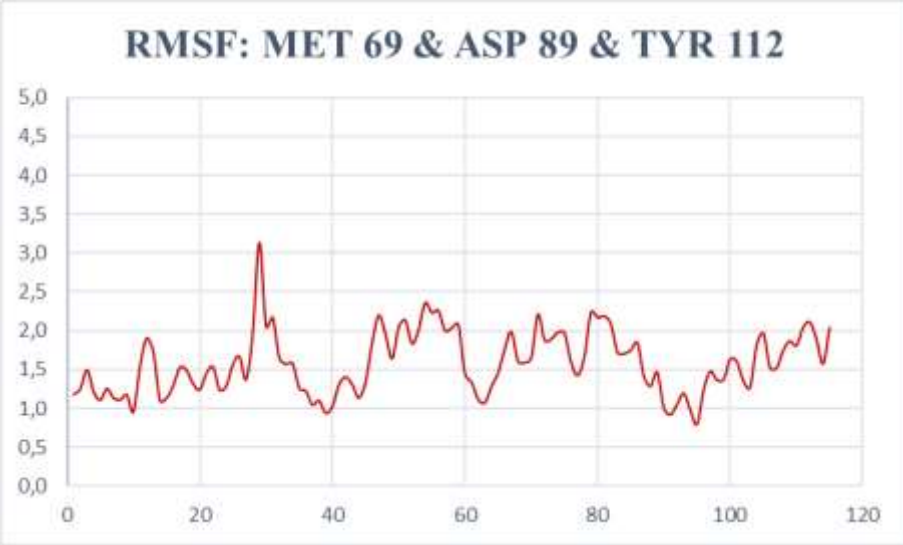

RMSF: MET 69
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100
120

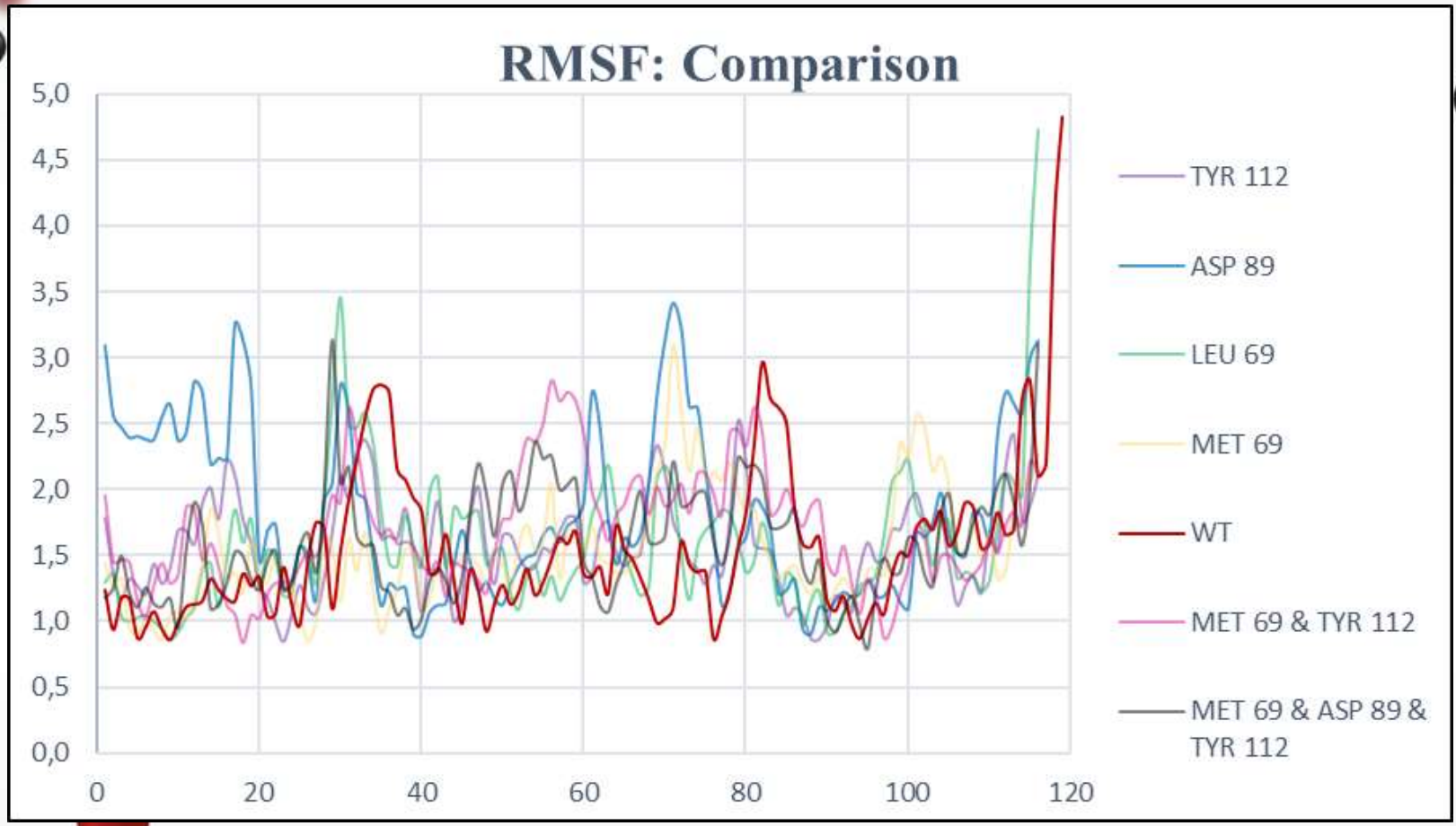

RMSF: LEU69
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100
120


RMSF: ASP 89
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100
120


RMSF: TYR 112
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100
120


RMSF: MET 69 & TYR 112
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100
120


RMSF: MET 69 & ASP 89 & TYR 112
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100
120

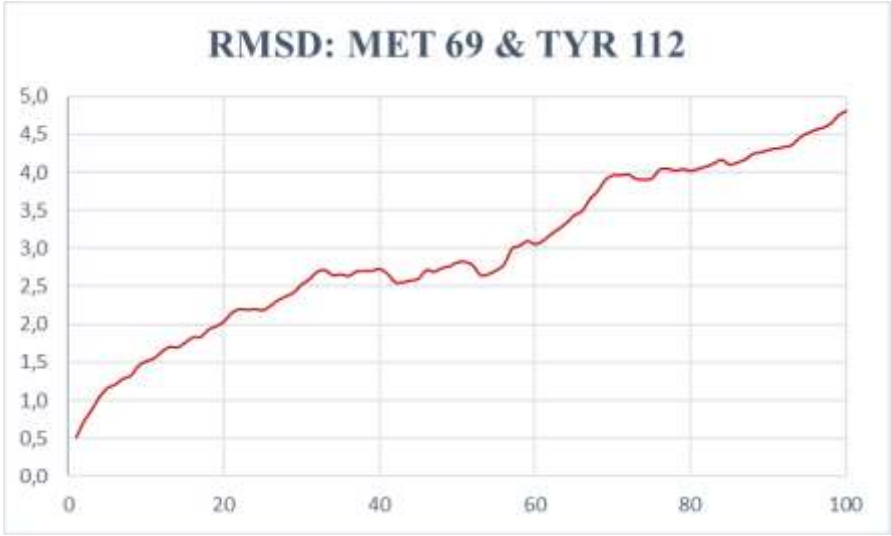

RMSF: Comparison
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100
120
TYR 112
ASP 89
LEU 69
MET 69
WT
MET 69 & TYR 112
MET 69 & ASP 89 & TYR 112

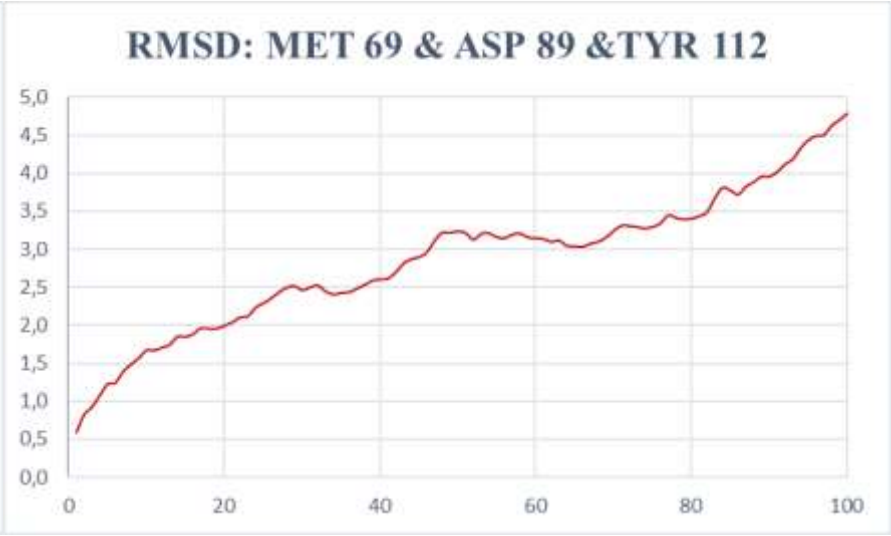

RMSD: ASP 89
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100

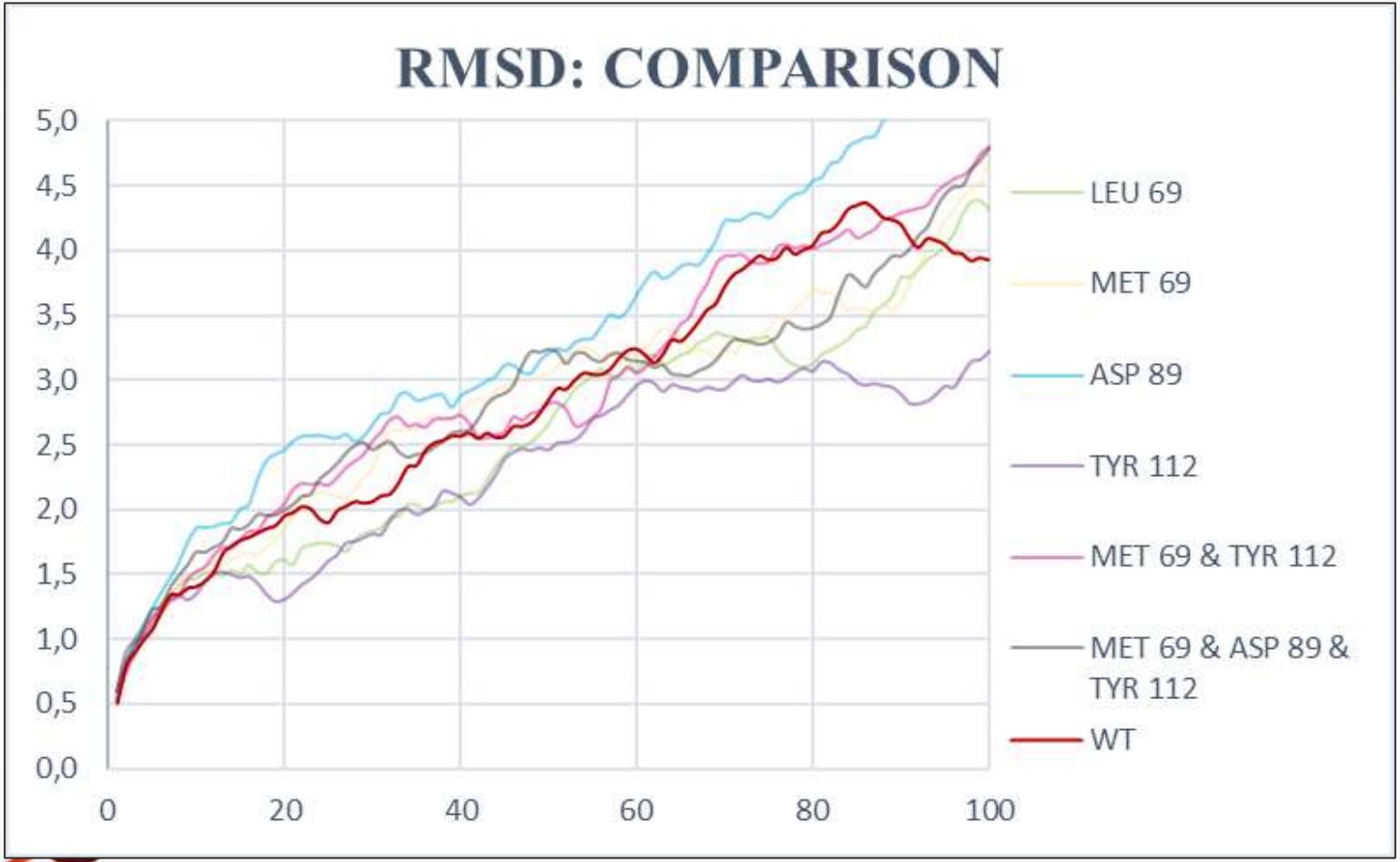

RMSD: LEU 69
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100


RMSD: MET 69
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100


RMSD: TYR 112
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100


RMSD: MET 69 & TYR 112
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100


RMSD: MET 69 & ASP 89 &TYR 112
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100


RMSD: COMPARISON
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
100
LEU 69
MET 69
ASP 89
TYR 112
MET 69 & TYR 112
MET 69 & ASP 89 & TYR 112
WT

RMSD

VARIANT	Average	SD
WT	2,853	1,022
69 MET	2,824	0,913
69 LEU	2,586	0,929
89 ASP	3,391	1,263
112 TYR	2,317	0,711
69 MET & 112 TYR	2,959	1,05
69 MET & 89 ASP & 112 TYR	2,670	0,829

STABILITY

VARIANT	TOTAL ENERGY
WT	15,79
69 MET	9,35
69 LEU	9,62
89 ASP	11,27
112 TYR	11,38
69 MET & 112 TYR	3,80
69 MET & 89 ASP & 112 TYR	**-1,10**

POSSIBLE IMPROVEMENTS

Increase simulation **time or steps**

Increase **temperature**

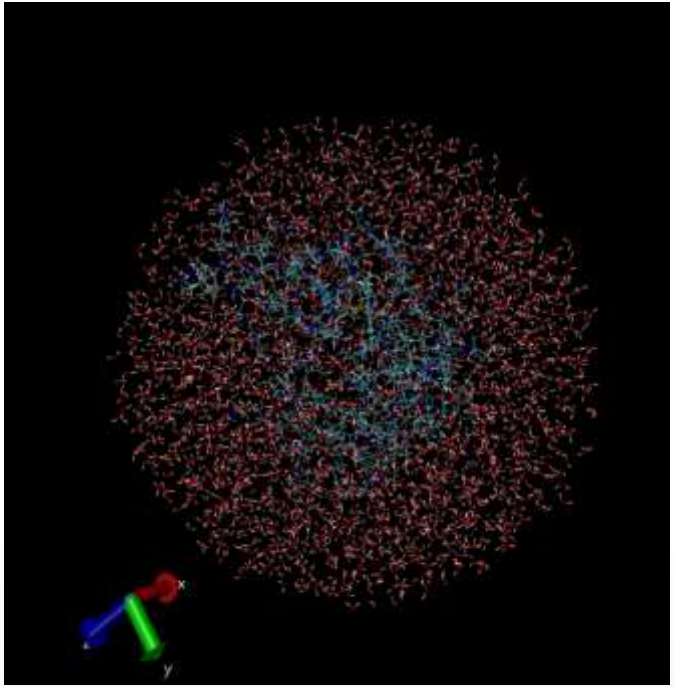
Different **solvation** method

Combination of mutations

Set **numberOfRuns** to higher numbers: optimal or trapped solution

WATER SPHERE

Variant	Mean Q-Value	SD
WT	0,84943363	0,01014880
69 & 89 & 112	0,72172545	0,03017851

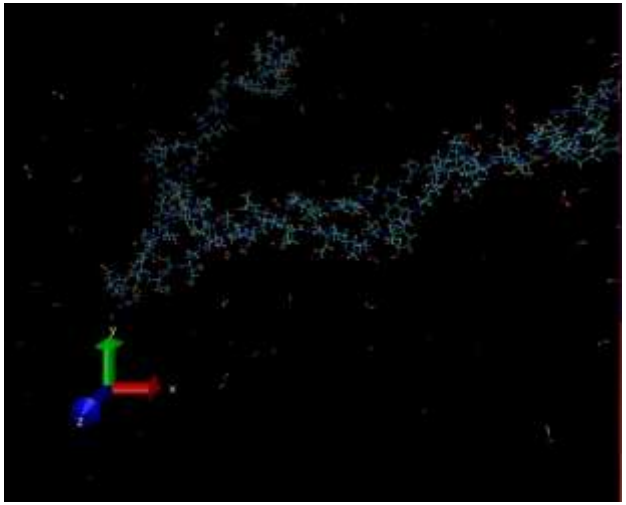


Increase simulation steps

From 10 000 to 50 000 steps

VARIANT	Mean Q-value	SD
WT	0,5313	0,00749
69 & 89 & 112	0,6016	0,00748

*Mean and SD between the last 50 frames of the simulation

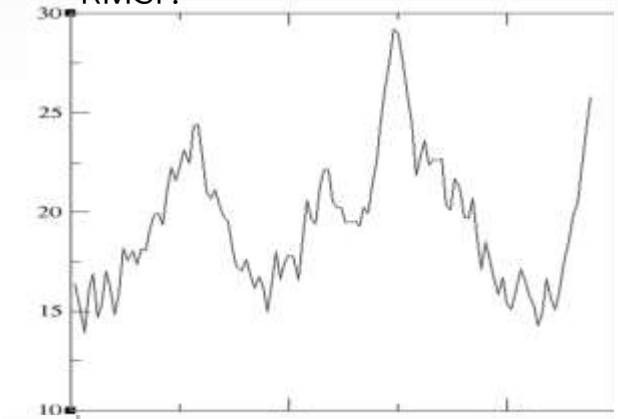


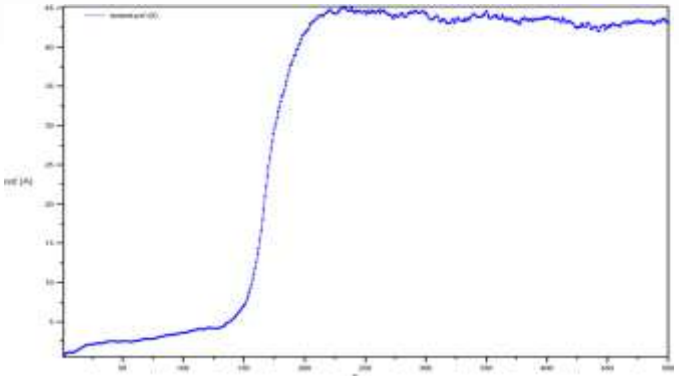
CONCLUSIONS

RMSF:

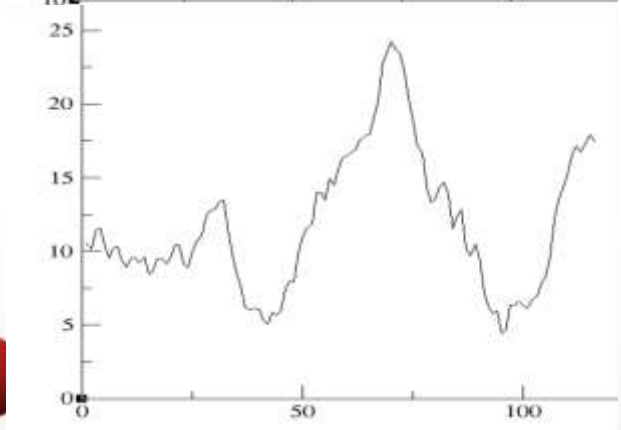
RMSD:

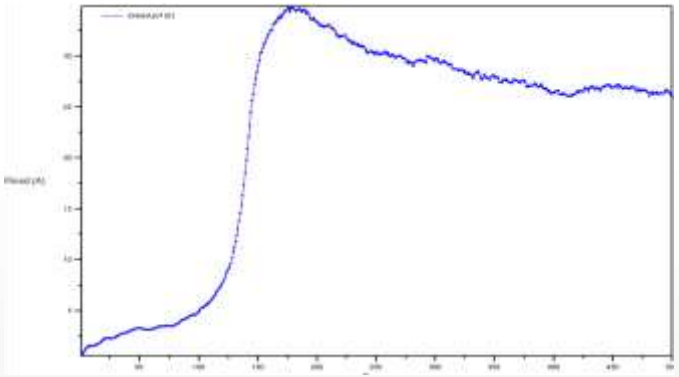
WT

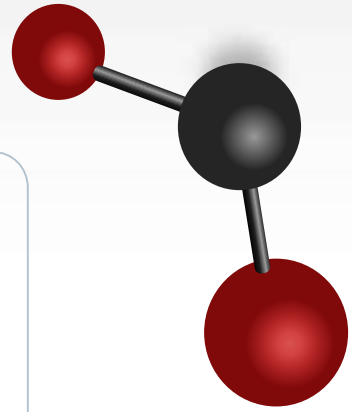




Mutate







THANK YOU FOR YOUR ATTENTION

Do you have any questions?

BIBLIOGRAPHY

- Phillips, J.,Hardy, D. (2017). NAMD TUTORIAL.Tutorials (uiuc.edu)
- Westerlund, B., Nordlund, P. (1997), Notexin, A presynaptic neurotoxic phospholipase A2 doi: 10.2210/pdb1AE7/pdb