

Computational Design of Nitrile Hydratase from *Pseudonocardia thermophila* JCM3095 for Improved Thermostability

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Energy mutant: -30.41kcal/mol (10mutations)			
Chain	Mutation	FoldX	Rosetta
A	I5P	-1.34	-2.40
A	T18Y	-1.51	-4.57
A	Q31L	-1.43	-2.90
A	D92H	-1.98	-2.17
B	A20P	-1.08	-2.83
B	P38L	-1.24	-2.82
B	F118W	-1.33	-2.50
B	S130Y	-1.05	-2.38
B	C189N	-1.79	-3.04
B	C218V	-2.31	-2.91

Figure S1. Snapshot from FireProt server output with mutations energy information (M10 variant).

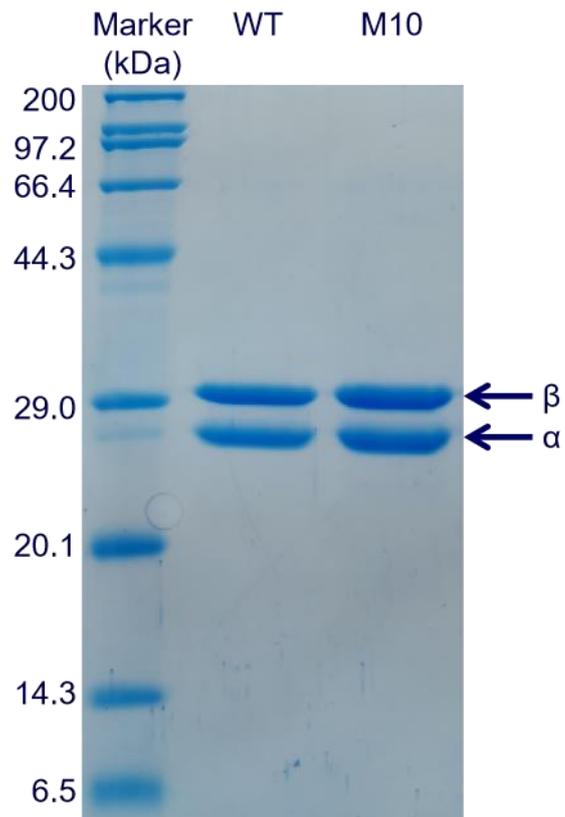


Figure S2. SDS-PAGE of the purified WT *PtiNHase* and its M10 variant.

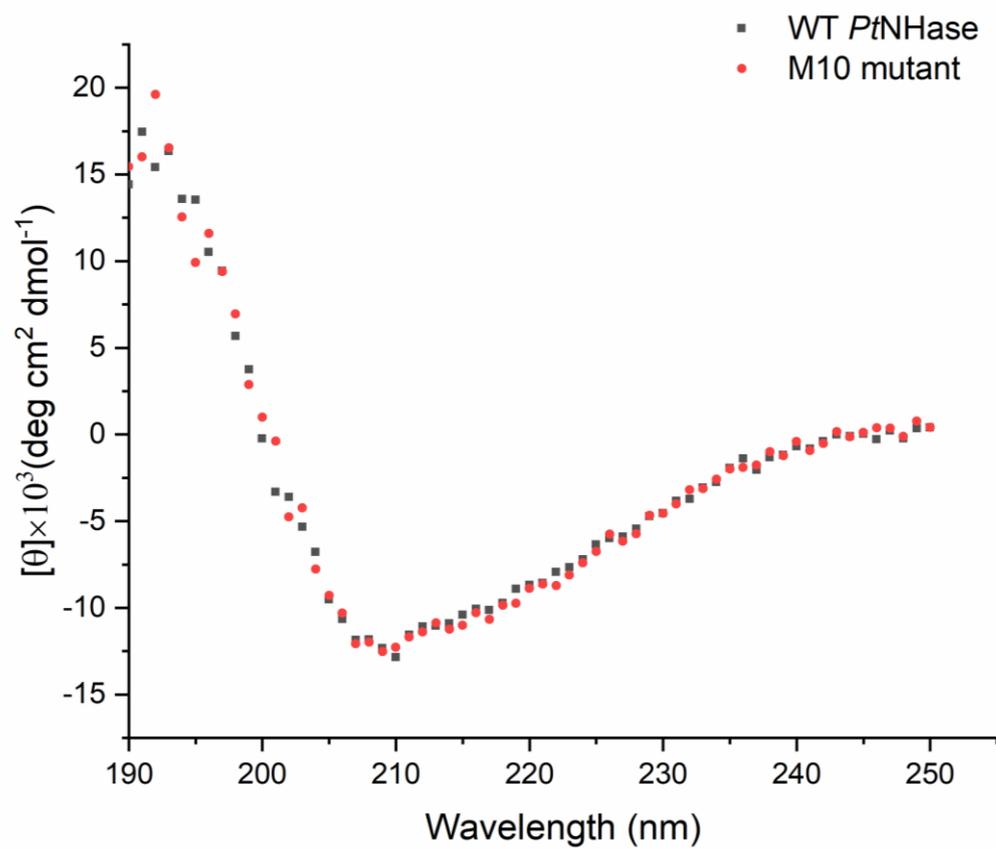


Figure S3. Circular Dichroism (CD) spectroscopy at far-UV (190 to 250 nm) of the WT *PtNHase* and its M10 mutant

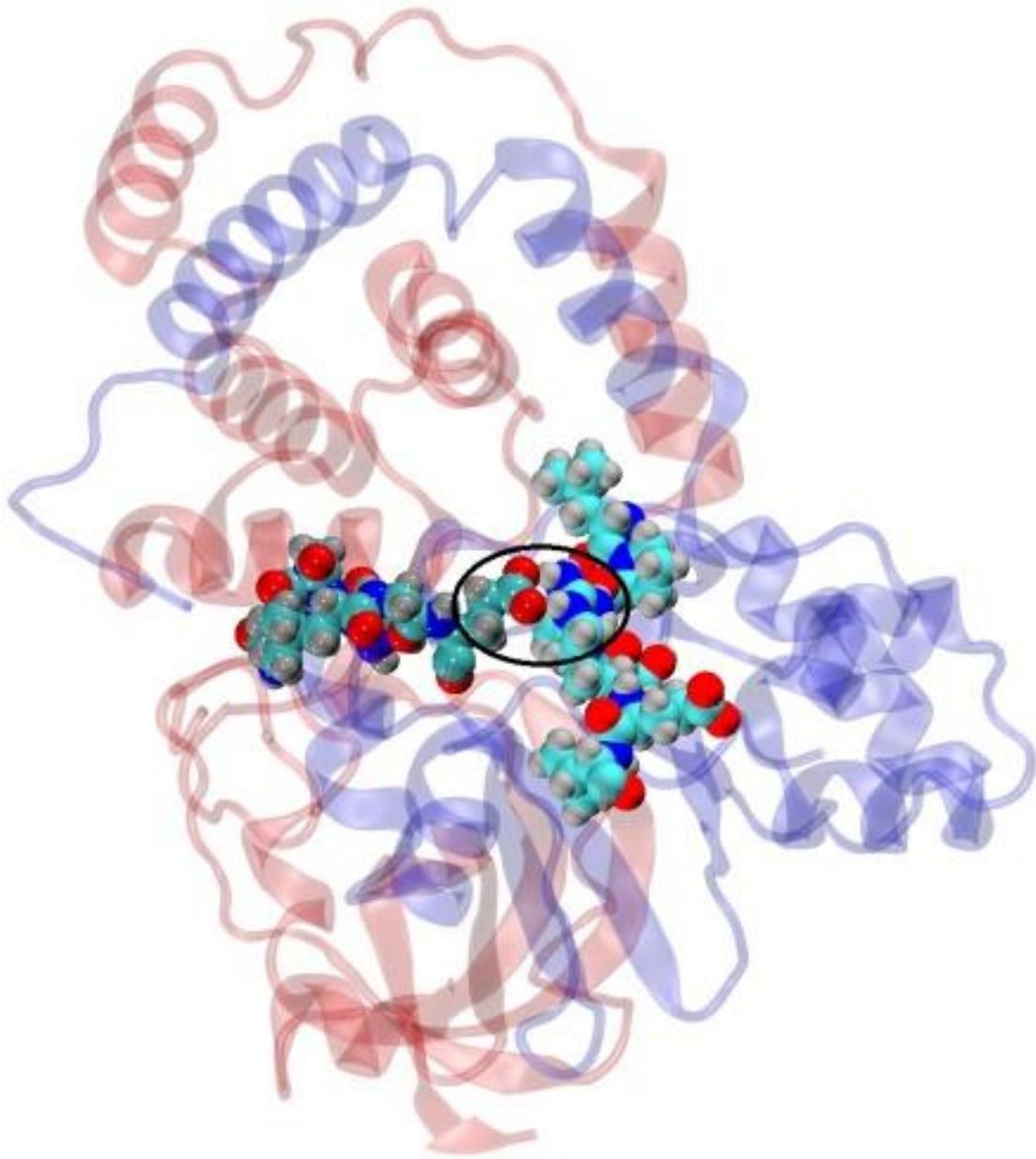


Figure S4. NHase (only $\alpha\beta 1$ domain) with highlighted fragments with high fluctuations. α Arg131- β Glu188 salt bridge is shown in black oval.

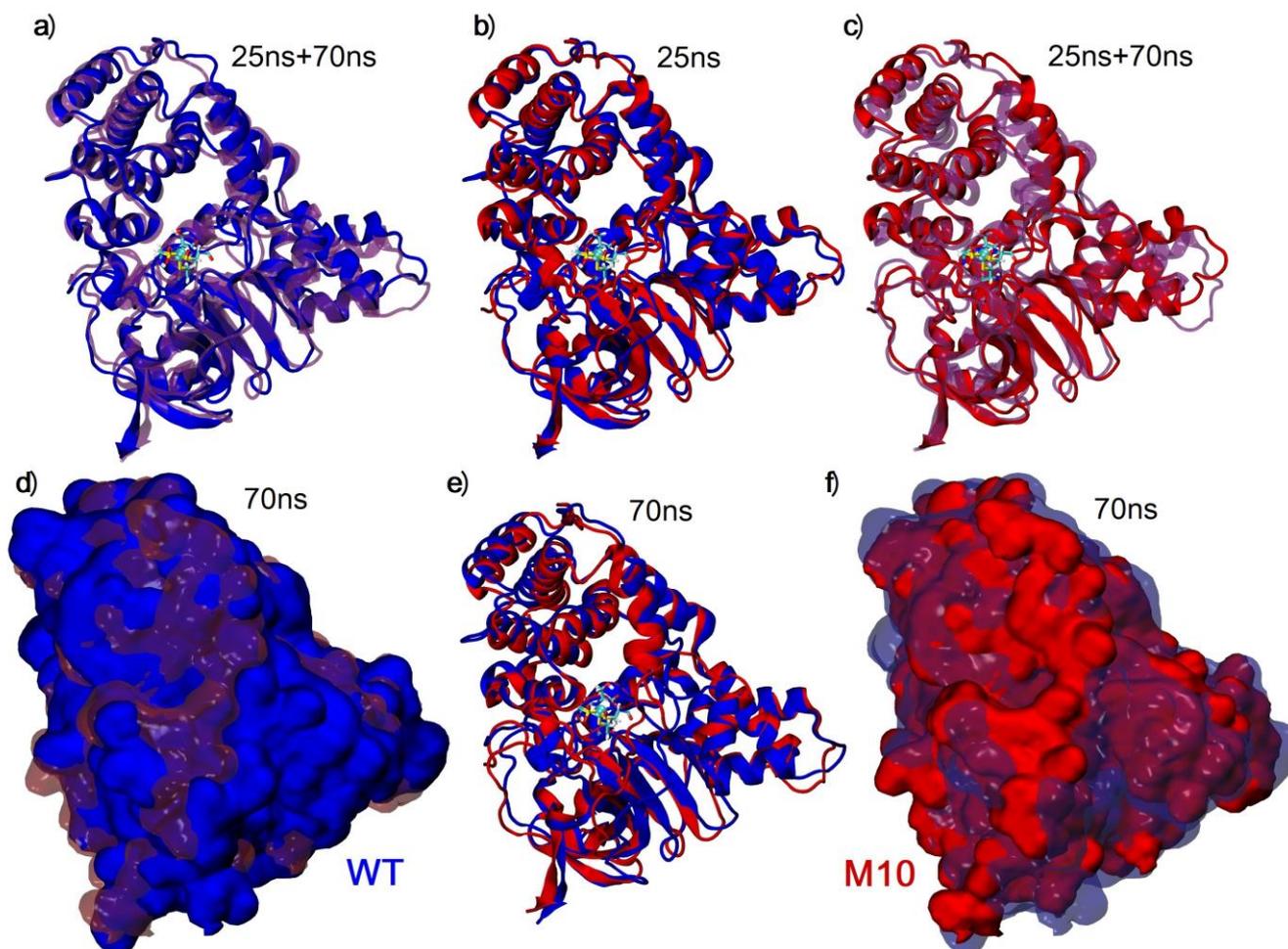


Figure S5. Superposition of $\alpha\beta 1$ structures showing changes in Rg. WT (blue) and M10 (red) NHase structures after 25ns (panel b)) and after 70 ns (panel e)) are presented. In the panel b) structures are very similar (high Rg value). In the panel e) bigger compactness of backbone for M10 NHase can be observed. In panels a) and c) structures after 25 ns are shown respectively for WT (blue) and M10 (red). In the transparent magenta color (panels a) and c)) changes of structure after 70 ns can be observed. Panels d) and f) show superposition of surfaces of WT NHase (blue) and M10 (red) after 70 ns. In the panel d) in transparent red color M10 NHase is shown. In the panel f) in transparent blue color WT NHase is shown. One can observe surface of WT NHase, and thus Rg is bigger than in case of M10 variant.

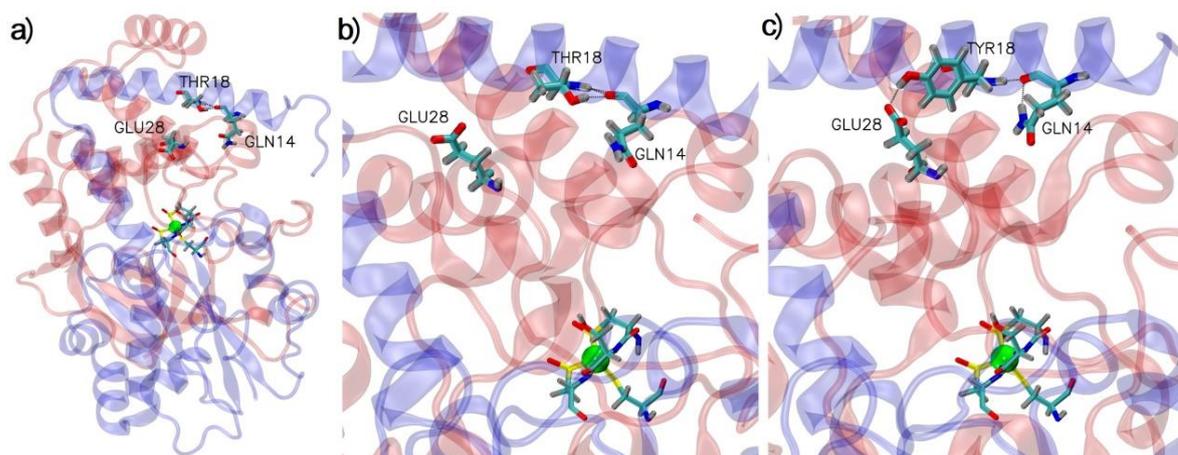


Figure S6. NHase with marked α Thr18, α Gln14 and β Glu28. $\alpha\beta$ 1 crystal structure panel (a). In blue α and β chain are shown in blue and red respectively. In the panel (b) H-Bonds between α Thr18 and α Gln14 Main chain after 100ns MD simulation is shown. In the panel (c) H-Bonds between α Tyr18 and β Glu28 after 100ns MD simulation is shown.

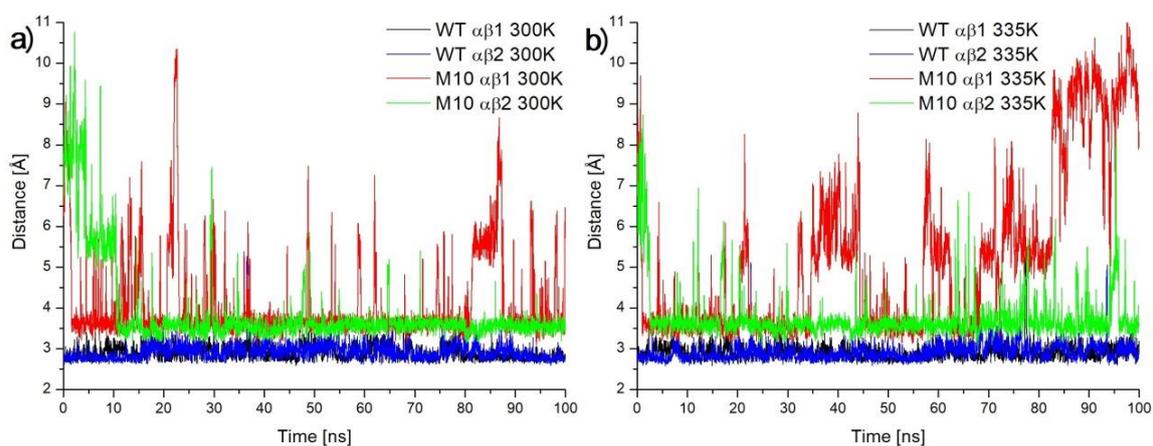


Figure S7. Distances between α Thr18 $O\gamma$ atom and α Gln14 carboxyl O atom (from main chain) (black and blue) in WT NHase. Distance below 3.5 Å indicates hydrogen bond. In red and green distance between α Tyr18 $O\delta$ atom and β Glu28 $C\delta$ atom. Distance below 4 Å indicates hydrogen bond. Panel (a) results from simulations in 300K, panel (b) results from simulations in 335K.

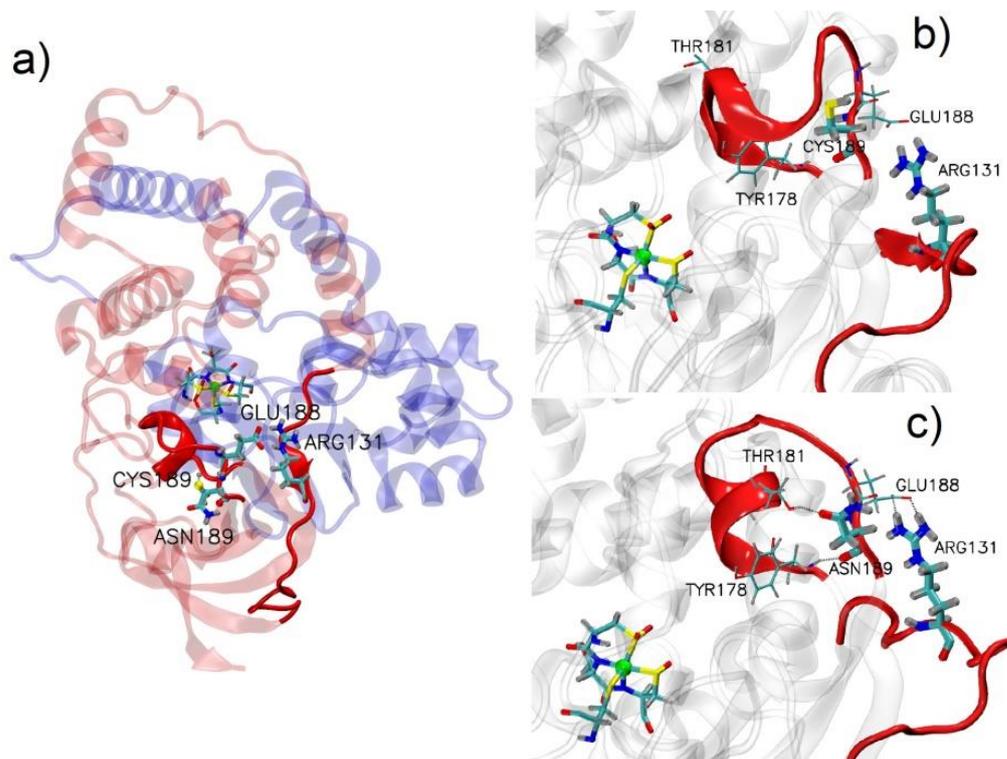


Figure S8. NHase with marked β Cys189 in WT and β Asn189 in M10. Short α -helix (residues 178 to 183) and long loop (residues 127 to 142) are marked together with salt bridge β Arg131- β Glu188 (a). The unfolding of the short helix in the case of WT is shown on panel (b). The hydrogen bond between β Asn189 and β Thr181 present in simulations of M10 is shown in the panel (c).