Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Highly efficient biosynthesis of isonicotinamide through a substrate access tunnel engineered nitrile hydratase from *Carbonactinospora thermoautotrophicus*

Junling Guo[1], *Zhongyi Cheng*[1], *Lukasz Peplowski*[2]*, *Zhemin Zhou*[1]*

[1] Key Laboratory of Industrial Biotechnology (Ministry of Education), School of Biotechnology, Jiangnan University, Wuxi, Jiangsu, China

[2] Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University in Torun, Grudziadzka 5, 87-100 Torun, Poland

*corresponding authors：

Lukasz Peplowski—Tel: +48-56-6112490; E-mail: drpepe@fizyka.umk.pl

Zhemin Zhou—Tel: +86-510-85325210; E-mail: zhmzhou@jiangnan.edu.cn

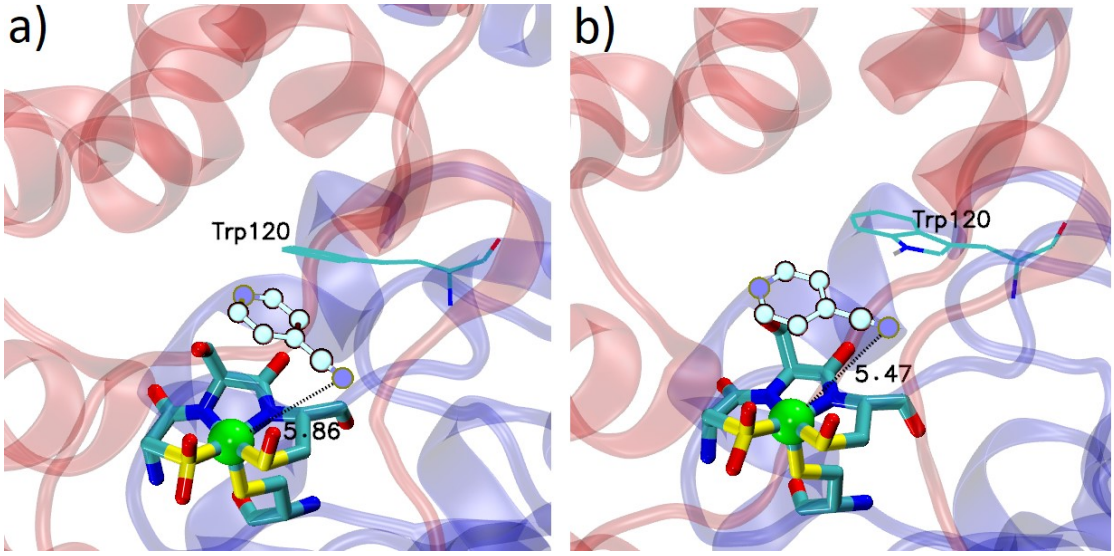

Fig. S1. Molecular docking of 4-cyanopyridine to the active site of (A) WT *C.t* NHase and its (B) βL48D mutant. Docking results to αβ2 dimer.

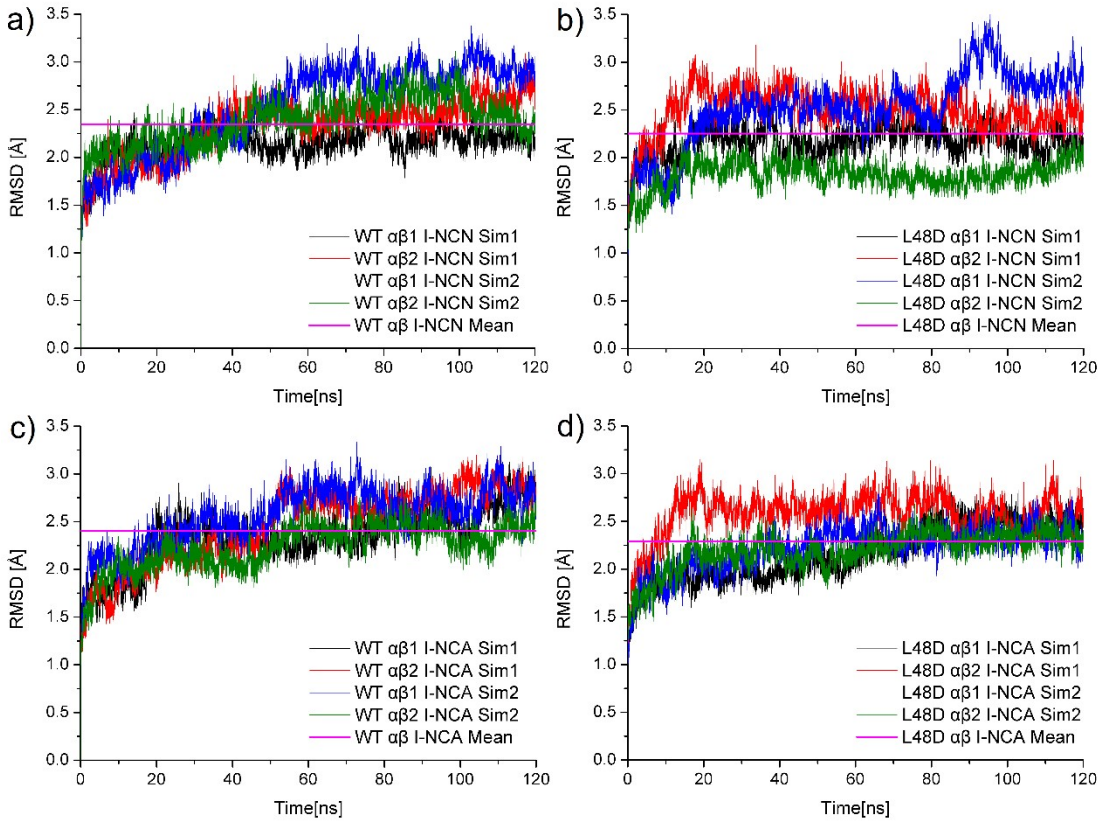

Fig. S2. RMSD Plots of WT and L48D variant of *C.t* NHase.

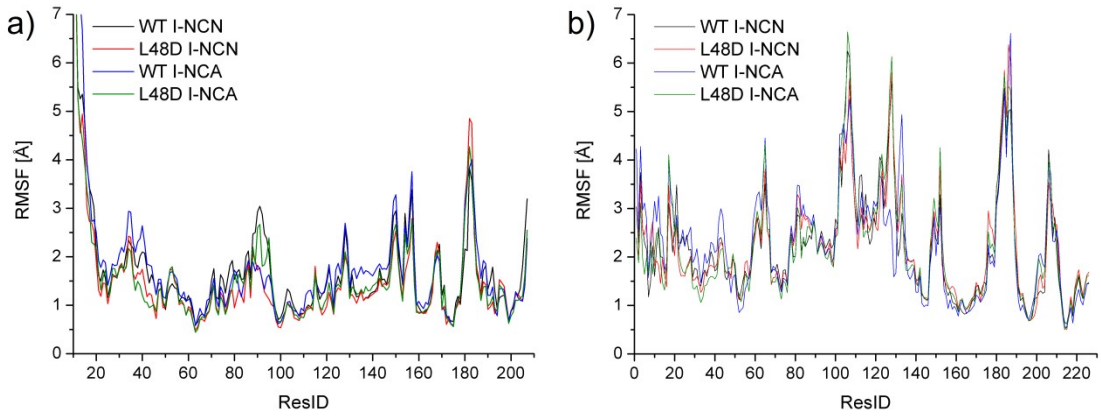

Fig. S3. RMSF Plots of WT and L48D variant of *C.t* NHase.

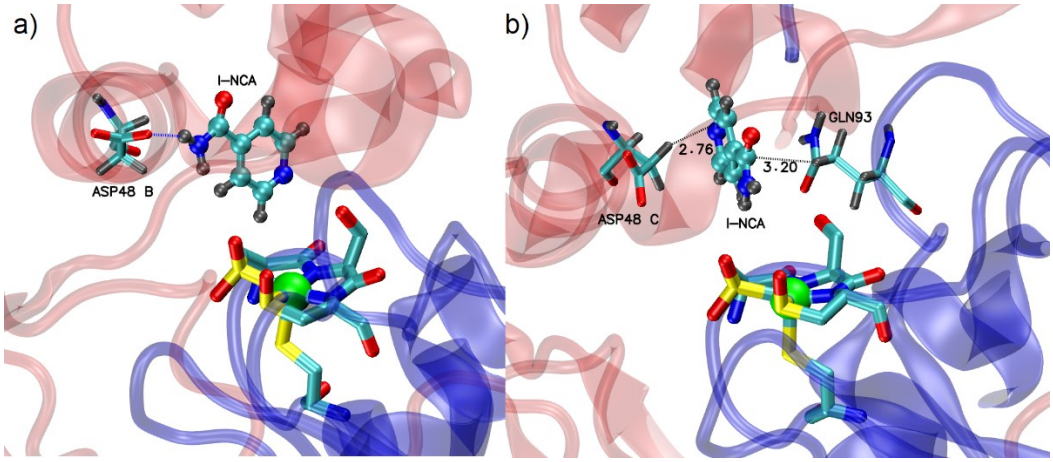

Fig. S4. βAsp48 in state B, forming hydrogen bond at the beginning of simulation a). The higher frequency of the C state in MD simulations with ligands, in comparison to simulations without ligands (see Reference 22) is forced by the passing of the reaction product between Asp48 and Gln93, when the amide is leaving the enzyme b).

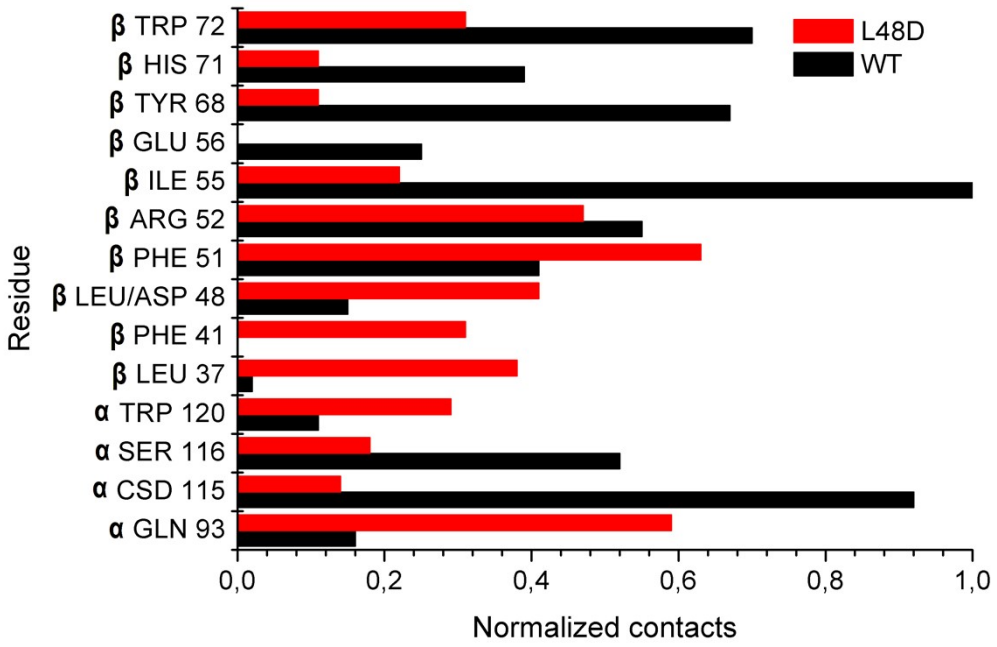

Fig. S5. Normalized contacts of isonicotinamide in simulations of WT *C.t* NHase (black bars) and βL48D variant of *C.t* NHase (red bars).