A SPECIFIC INORGANIC TRIPHOSPHATASE FROM *NITROSOMONAS EUROPAEA*: STRUCTURE AND CATALYTIC MECHANISM

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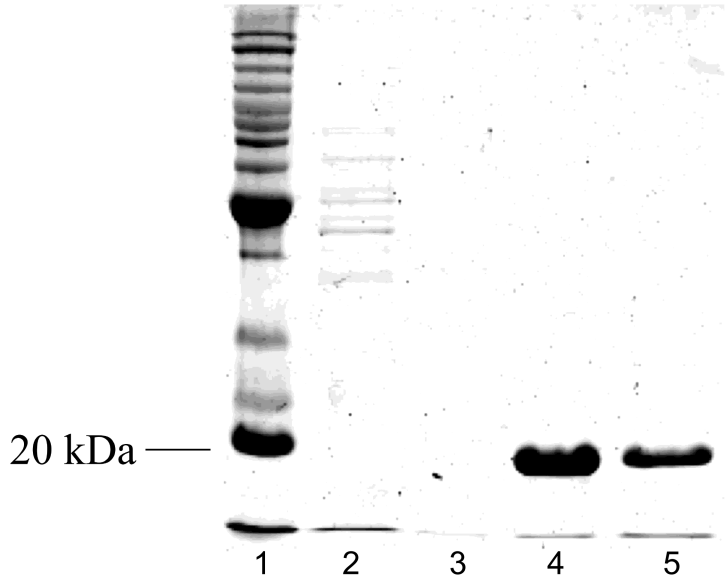
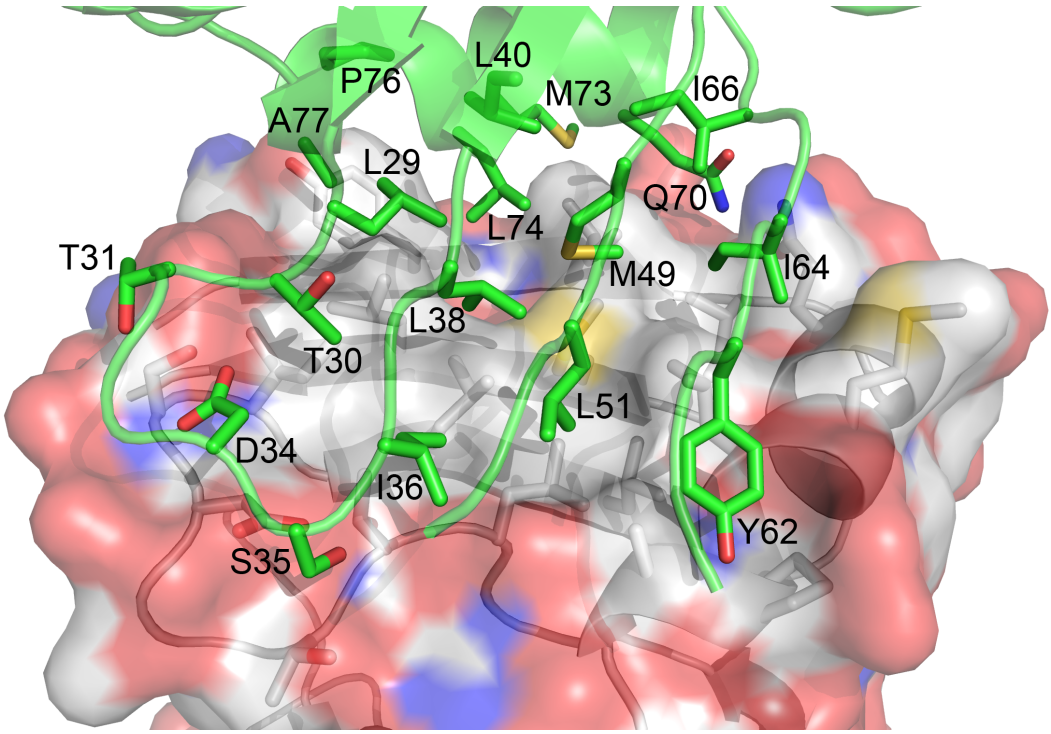
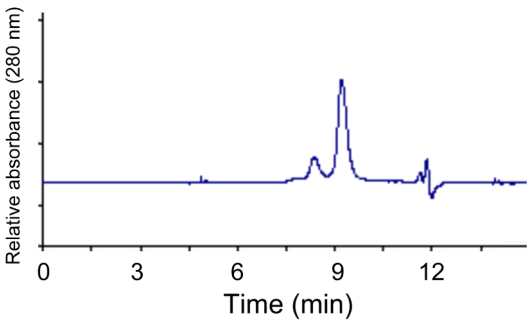
Supplemental data



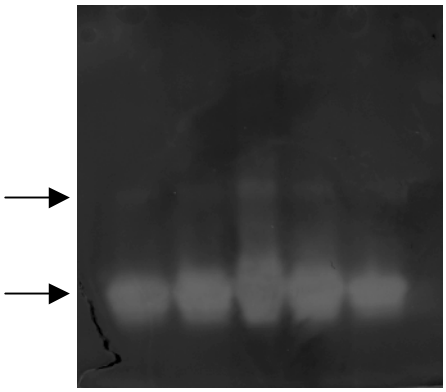
SUPPLEMENTAL FIGURE S1. **SDS-PAGE after the different purification steps of polyHis-tagged *Neu*TTM.** Lane 1, BenchMark™ Protein Ladder. Lane 2 and 3 represent the protein content of flow-through fractions that were eluted in binding buffer. Lane 4 and 5 represent the elution of His-tagged *Neu*TTM in elution buffer. It appears that almost all the protein of interest was recovered in the elution fractions and that the protein is very pure. The purification patterns of K8A, K85A, K52R and Y29F mutants were similar (not shown).



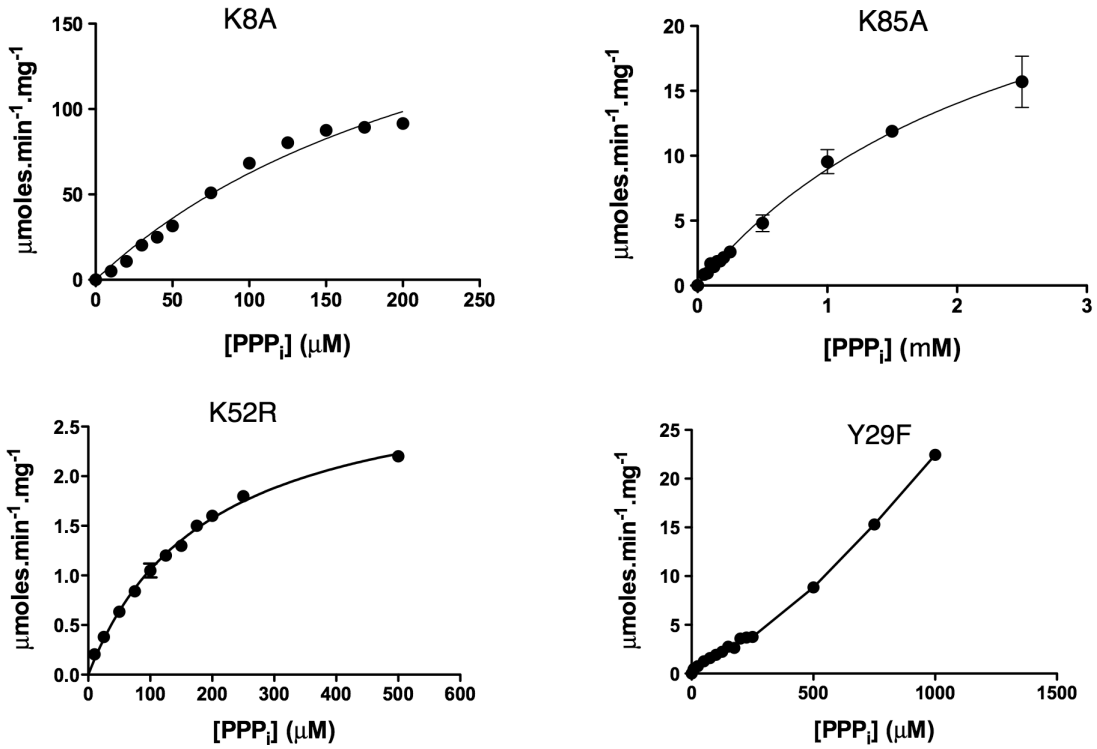
SUPPLEMENTAL FIGURE S2. **Dimerization interface of *Neu*TTM.** Monomer A is shown in cartoon representation (green ribbon, green carbon atoms), while a semitransparent molecular surface is also rendered for the monomer B (grey carbon atoms). For both monomers, oxygen atoms are colored red, nitrogen atoms blue and sulfur atoms yellow. The residues forming the dimerization interface are shown as sticks for both monomers and labeled for monomer A.



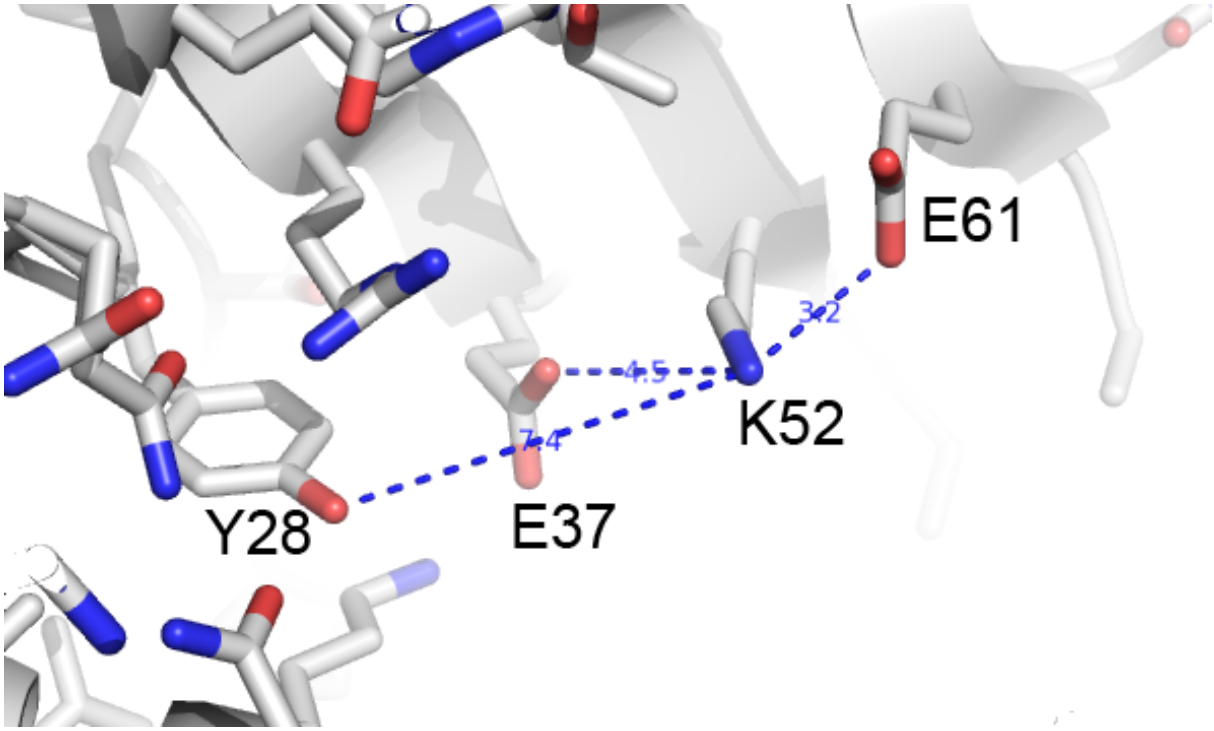
SUPPLEMENTAL FIGURE S3. **Separation of the monomer and the dimer of *Neu*TTM by size exclusion chromatography.** The TSK column (G3000SW, 30 x 0.75 cm, 7 mm) was equilibrated with 20 mM Hepes-Na (pH 6.8) and 200 mM NaCl at a flow rate of 0.5 ml/min. A volume of 20 µl of the protein solution (1.75 mg of protein / ml) were injected and the two peaks were collected for determination of enzyme activities.



SUPPLEMENTAL FIGURE S4. **In-gel activity determination of *Neu*TTM PPPase activity.** The active bands are colored in white. The higher dot shows the band corresponding to the dimer and the lower one to the monomer.



SUPPLEMENTAL FIGURE S5. **Substrate concentration-dependence of the PPPase activity in four *Neu*TTM mutants.** The enzymes were incubated at 37 °C in the presence of 5 mM Mg^{2+} and 50 mM Na-CHES buffer, pH 9.7. The curves were obtained by non-linear regression of the Michaelis-Menten equation (n = 3) except for Y29A where a connecting line was used (n=6).



SUPPLEMENTAL FIGURE S6. **The suggested catalytic dyad K52 and Y28 and the distances from K52NZ to the closest neighboring residues in the crystal structure.**

SUPPLEMENTAL TABLE S1

Oligonucleotides used for site-directed mutagenesis. The modified bases are in bold and underlined.

Primer	Sequence
K8A	Forward : 5'-CCGAGATCGAACGC**GC**ATTTCTCGTGGCAACTT-3' Reverse : 5'-AAGTTGCCACGAGAAAT**GC**GCGTTCGATCTCGG-3'
K85A	Forward : 5'-GCGGCGTGTAGAG**GC**AACCCGGTACAGTG-3' Reverse : 5'-CACTGTACCGGGTT**GC**CTCTACACGCCGC-3'
Y28F	Forward : 5'- CCGCTCCGTCAGGGCT**T**TCTGACCACCCCCACAG-3' Reverse : 5'- CTGTGGGGGTGGTCAGA**A**AGCCCTGACGGAGCGG-3'
K52R	Forward : 5'- GAATATTTCATGACGCTGA**G**ATCCGAGGGTGGATTA-3' Reverse : 5'- TAATCCACCCTCGGAT**C**TCAGCGTCATGAAATATTC-3'

SUPPLEMENTAL TABLE S2

PPPase activity of His-tagged K85A mutant *Neu*TTM in the presence of various concentrations of PPP_i and divalent cations. Incubations were carried out at 50 °C, pH 9.7 (n = 3-4).

$[PPP_i]$	Divalent cation	Divalent cation (free concentration)	Specific activity
mM		*mM*	$\mu mol.min^{-}.mg^{-1}$
0.5	Mg^{2+}	2	4.0 ± 0.3
	Mg^{2+}	5	4.2 ± 0.3
	Mn^{2+}	1	6.0 ± 2.5
	Mn^{2+}	4	18 ± 4
	Co^{2+}	2	0.8 ± 0.6
	Zn^{2+}	1	2.0 ± 0.5
	Zn^{2+}	4	10 ± 2
2	Mg^{2+}	2	27 ± 6
	Mg^{2+}	5	55 ± 4
	Mn^{2+}	1	80 ± 9
	Mn^{2+}	4	70 ± 6
	Co^{2+}	2	18 ± 6
	Zn^{2+}	1	22 ± 7
	Zn^{2+}	4	27 ± 5